Emerging Topics in Life Sciences (2019) **3** 459–468
https://doi.org/10.1042/ETLS20190011

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Perspective

Provisioning the origin and early evolution of life

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There is a lot of controversy in the origin and early evolution of life field, but most people agree that at the advent of genetically coded protein synthesis, cells must have had access to ribonucleotides, amino acids, lipids and some sort of energy source. However, the provenance of these materials is a contentious issue — did early life obtain its building blocks prefabricated from the environment, or did it synthesise them from feedstocks such as CO_2 and N_2? In the first case, synthesis conditions need not have been compatible with life and any kind of reaction network that furnished the building blocks — and not much else — could have provisioned the subsequent origin and early evolution of life. In the second case, synthesis must have been under life-compatible conditions, with the reaction network either along the same lines as extant biology or along different ones. On the basis of experimental evidence, we will argue in favour of prefabrication and against synthesis by life in its nascent state, especially synthesis that resembles extant biosynthesis, which we suggest would have been well-nigh impossible without biological catalysts.

Introduction

Catalysis by enzymes and RNA is one of the wonders of biology. It is thought by many that RNA played a bigger role at the dawn of life than it does now, but once genetically coded proteins arrived on the scene, biology went into overdrive. So, what would have been required for early RNA replication and the emergence of translation? This difficult question becomes easier if we only think in material terms for there can be no doubt that ribonucleotides and amino acids, or derivatives thereof, were needed. Furthermore, lipids were most likely also needed as the natural selection required to optimise catalytic activities is most easily imaginable in the context of compartmentalisation by membranes. Four nucleotides are needed to endow RNA with a wide range of functions, but about half of the amino acids look late and functional proteins are possible with restricted 'alphabets' [1,2], so let us assume that around ten amino acids were also needed. Assuming membranes composed of a single lipid species, this means that about 15 simple to moderately complex compounds would have been necessary to progress biology towards translation. These chemical entities would have had to be abundant in the environment for primitive cells to feed off, or produced by cells in high yield — a modern bacterium primed for replication has remarkably high intracellular concentrations of amino acids (∼150 mM) and nucleotides (∼50 mM) [3]. There are those who suggest that impactors could have delivered these materials [4,5], but there are several compelling arguments against this. Big impactors, which have the potential to deliver significant amounts of material, vaporise on impact and their organic cargo is largely atomised, along with a portion of the surface and atmosphere of Earth, ultimately giving species such as $NO^{\cdot}$, CO and HCN [6,7]. Even in carbonaceous chondrites, which are the richest in 'organics', nucleotides have not been detected, levels of amino acids and lipids needed for life are very low and there are a huge number, likely millions, of other compounds present [8]. Such organic complexity effectively precludes subsequent chemical assembly of macromolecules destined to become biological because there is no means of selectively conjoining canonical components in the presence of myriad chemically similar, but non-canonical, components. So, efficient synthetic chemistry that could selectively make the canonical components from simple feedstocks on early Earth is our goal.

Received: 28 May 2019
Revised: 19 June 2019
Accepted: 1 July 2019

Version of Record published:
16 July 2019

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What sort of chemistry?

We need to consider chemical feedstocks and conditions, and whether or not it is necessary to have any degree of physical separation of the various branches of the synthetic network, which makes the product tree, to enable all the products to be made. Biology's use of CHNOPS dictates the elemental composition of synthetic feedstocks needed. Water can provide some of the hydrogen and oxygen; hydrogen sulfide, the sulfur, and phosphate seems like a reasonable source of phosphorus — though solubility in the presence of certain metal ions is an issue — but carbon and nitrogen could come from quite different sources. In extant biology, the average oxidation level of carbon is around that of the carbon in formaldehyde, but nitrogen is for the most part fully reduced. Separate fixation of these elements from CO_2 and N_2, the way biology now does it, thus requires a lot of reduction, but simultaneous fixation of both elements from HCN would require less reduction. Some shy away from hydrogen cyanide as a feedstock on the grounds of its toxicity [9], but the target of cyanide, cytochrome C oxidase — responsible for electron transport to oxygen at the end of the respiratory chain — post-dates the oxygenation of the atmosphere by highly evolved life starting roughly two and a half billion years ago, at least a billion years after the origin of life. Furthermore, with respiratory chain alterations, certain microbial organisms can not only tolerate cyanide, but thrive on it as a nitrogen and carbon source [10–12].

What about the conditions under which such feedstocks could react together to produce our 15 or so simple to moderately complex building blocks? Early Earth offers a range of environments with either changing or relatively constant conditions. Precipitation and evaporation enable wet–dry cycles, geothermal and impact heating allow high temperatures to be attained, low solar luminosity allows a snowball Earth, solar UV irradiation reaches the surface — though wavelengths below ~200 nm are attenuated by CO_2 and water vapour in the atmosphere — and high pressures are possible sub-surface and in the depths of the oceans. Thus, without any constraint, there are numerous conditions and sequences of conditions possible on early Earth. However, if synthesis had to be compatible with life, then certain conditions are effectively ruled out, for example, heating above the boiling point of water, UV irradiation and so on. Yes, there are plenty of extremophiles that can cope with all sorts of hostile environments on Earth now [13], but in their cellular interiors water is liquid and penetrating UV irradiation is lethal (above a certain level). Given all of this, we now need to consider the extent to which synthesis conditions might have matched those consistent with emerging life (Figure 1). *A priori*, it is possible that synthesis conditions were completely incompatible with life, or partially, or fully, compatible. If different conditions were needed for different branches of the synthetic network, either set could have had the same degree of overlap with the conditions pertaining in nascent biological systems. One might have a gut feeling one way or the other, but surely it is worth amassing a large body of evidence from synthetic chemical investigations, so an assessment can be made *a posteriori*? Throwing such caution aside, however, and without comprehensive experimentation, some people have made their minds up that the synthetic network that furnishes the building blocks must 'resemble extant biochemistry in terms of substrates, reaction pathways, catalysts and energy coupling' [14]. Proclaiming that because biology does it this way now, it must always have done it this way, is akin to a person living in a future completely powered by renewable resources assuming that previous generations must always have lived that way. It is a denial of the possibility of adaptation. In the same way that society is beginning to wean itself off fossil fuels, biology could have gradually adapted from consuming dwindling environmental supplies of amino acids, nucleotides and lipids to making its own, *in cellulo*, from renewable resources. *Adaptation is a defining feature of life, the hydrogenation of CO_2 is not.*

To cut a long story short, organic chemists have pieced together reaction networks that make about half of the canonical amino acids, the pyrimidine and purine ribonucleotides and diacyl-glycerol-phosphate lipids [15–24]. On the basis of what is known to date, these chemists have made three conclusions that are germane to our argument:

(i) Hydrogen cyanide is a perfect feedstock to produce the palette of products necessary for the emergence of translation. It is a source of carbon and nitrogen and is constitutionally implicated in the purines (adenine is a pentamer of HCN), the amino acids (through Strecker-type syntheses), sugars and glycerol (through reductive homologation).

(ii) Certain reactions work best under anhydrous conditions, for example, nucleoside and glycerol phosphorylation, and glycosidation. Other reactions require UV irradiation or heating above the boiling point of water.

(iii) It is not possible to make everything in 'one pot' by one sequence of conditions, some degree of separation of the branches of each reaction network is necessary.

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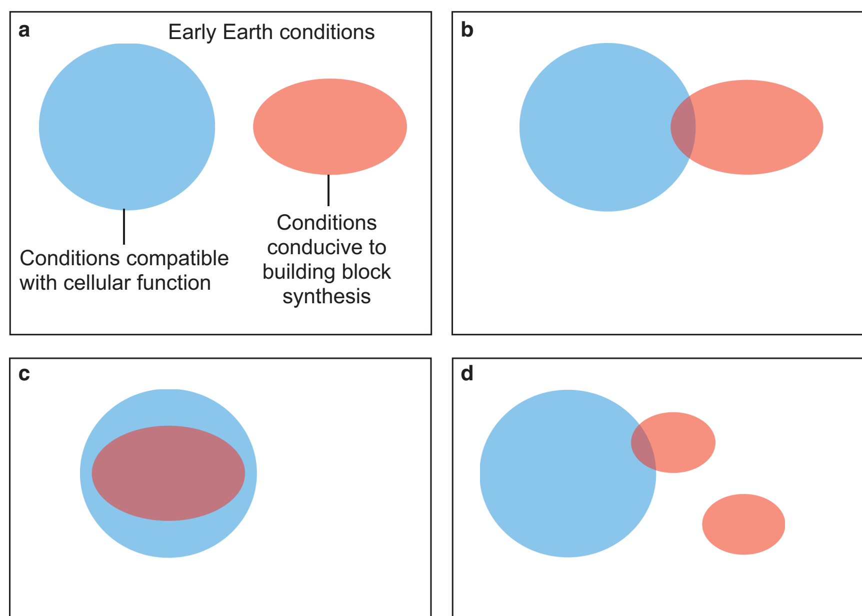

Figure 1. To what extent do life-compatible conditions overlap conditions for the synthesis of (proto)biological building blocks?

Four representative possibilities are shown: (a) no overlap, (b) limited overlap, (c) complete overlap, and (d) two different sets of conditions are needed for building block synthesis, one set partly overlaps life-compatible conditions and the other not at all.

In our contribution to this (Figure 2) [18–21,24], the pivotal importance of HCN is especially evident — every carbon and nitrogen atom of the final set of products can ultimately derive from HCN. The set of products is directed by the inherent reactivity of HCN and intermediates derived therefrom under a geochemically plausible sequence of conditions. Environmental energy in the form of heat and light supplements the chemical energy inherent in the triple bond of HCN to drive the reactions of our network. Prefabrication is thus comprehensively supported by experimentation. But it is not accepted by everyone, because it 'does not narrow the gap between prebiotic chemistry and biochemistry' [14]. Aside from the fact that the gap does not need narrowing because adaptation can traverse it, there are additional reasons to refute alternatives to prefabrication. We need to examine what would be required for early synthesis to 'resemble extant biochemistry in terms of substrates, reaction pathways, catalysts and energy coupling' [14].

According to this mantra, a reaction network — starting from CO_2 and N_2, or other inorganic nitrogen sources — of the sort shown in Figure 3 would be needed to make a similar set of products to that which we and others have made from HCN [25]. Extant biology effects these ~75 different reactions, and many others, using enzymes of extraordinarily high catalytic prowess and specificity in 'one pot' — the cell — under common conditions. Essentially, CO_2 is fixed by reduction to CO and a methyl group attached to a cofactor and these two entities are then coupled together to give, after thiolysis, acetyl-CoA. Reductive coupling of acetyl-CoA to another CO_2 then gives pyruvate, which is a major branch point. One branch therefrom leads through partial gluconeogenesis to phosphoribosyl pyrophosphate (PRPP) a key ribonucleotide precursor, another feeds into the (reductive) citric acid cycle, a third leads to leucine and valine and a fourth to alanine. Oxaloacetate, the entry point of the citric acid cycle, is also an exit point, being converted to aspartate by transamination. This amino acid is not just one of the key products, but also another branch point, precursing both the nucleobase moiety of the pyrimidine ribonucleotides and threonine. The citric acid cycle also furnishes the amino acids glycine and thence serine, glutamate, proline and arginine. Carbamoyl phosphate, formed from CO_2 and ammonia, provides the fully oxidised carbon of the pyrimidine ribonucleotides and arginine. Finally, the lipid constituents, glycerol-3-phosphate and fatty acids, are formed from the triose phosphates and acetyl-CoA, respectively. Every single reaction is associated with sophisticated and beautiful enzymology, the co-ordinated operation of which is quite simply breathtaking. But, there are people who insist that the whole network could operate without enzymes sufficiently well to provide for the needs of emerging life [14,26,27].

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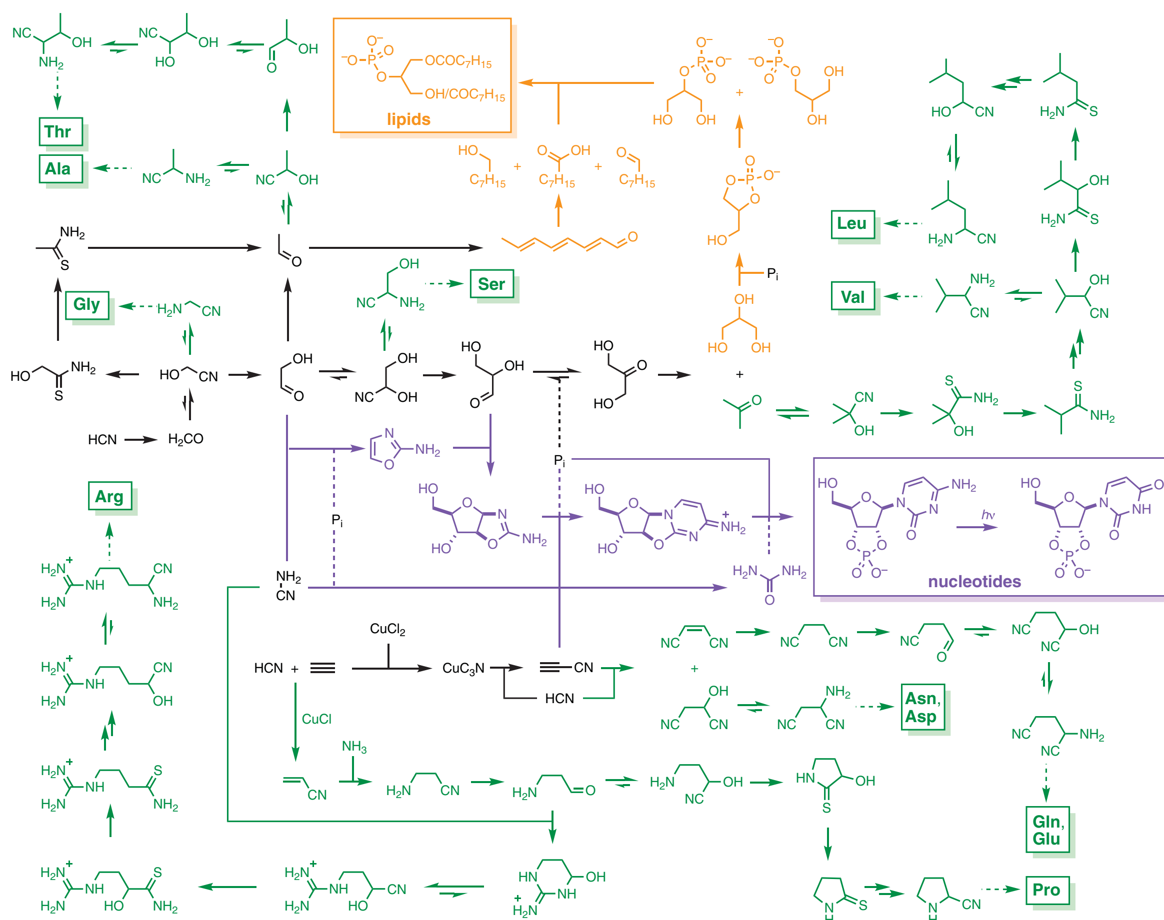

Figure 2. Cyanosulfidic protometabolic reaction network leading from HCN and derivatives thereof to pyrimidine ribonucleotides, and amino acid and lipid precursors.

Pathways, intermediates and products specific to a particular product class are shown in colour (pyrimidine ribonucleotides, purple; amino acids, green; lipids, orange) with products boxed, shared starting materials, pathways and intermediates are shown in black.

Should we believe them? In short, no, because there are eight fundamental and irredeemable problems associated with the operation of this network without enzymes:

(i) Catalysis, catalysis, catalysis. For very few enzymes is the underlying reaction rate significant — if it were, regulation would be impossible as the reaction could not be switched off as necessary. The degree of catalysis by enzymes can be remarkable, for example, orotidine monophosphate decarboxylase accelerates the uncatalysed reaction, which has a half-life of seventy eight million years, ~10^{17}-fold [28]. Without this single reaction, there would be no pyrimidine ribonucleotides produced by the (proto)biological network, no functional RNA and no prospects of translation ever emerging. Catalysis of almost all of the reactions of the network would thus be necessary for the network to operate with any degree of efficiency. For some enzymes that use cofactors (orotidine monophosphate decarboxylase does not), limited catalysis is observed for the cofactor alone [29], but the crucial organic (redox) cofactors such as nicotinamide and flavin derivatives, thiamin pyrophosphate (TPP), biotin and the corrinoids would not be present — unless they too were synthesised by the system, in which case add scores of additional, difficult reactions to the network. Thiamin pyrophosphate in particular is crucial and enzymes that deploy it as an umpolung catalyst can bring reactions with billion year uncatalysed half-lives into play in biology [28].

(ii) Negative aspects of catalysis. Metal ion cofactors catalyse deleterious as well as desired reactions. It has been known for decades that metal ions, particularly divalent ones, can catalyse certain biological reactions,

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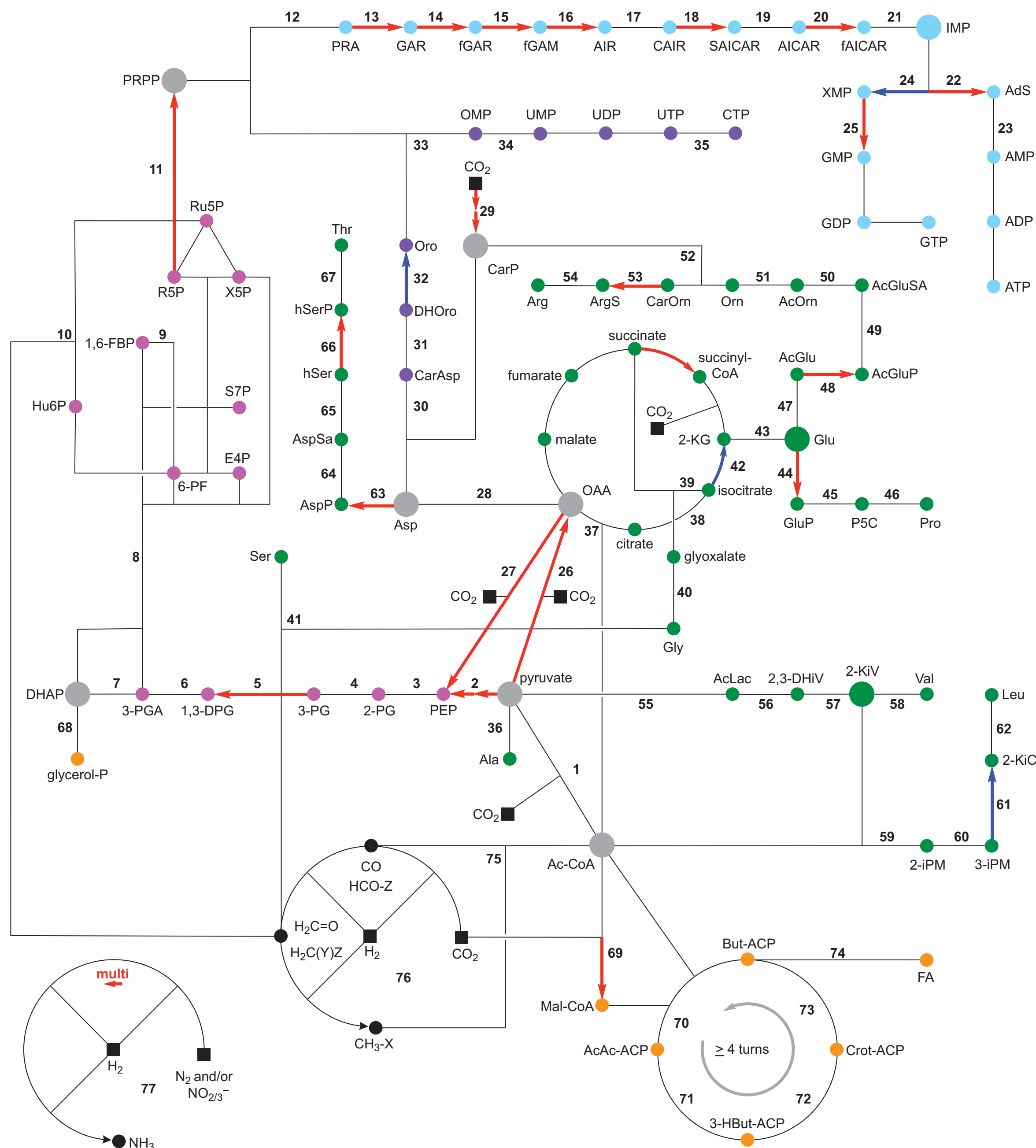

Figure 3. Minimal biosynthetic reaction network leading from CO_2 and N_2 (or $NO_{2/3}^-$) to ribonucleotides, amino acids and lipid precursors.

Hub compounds are indicated by large disks and other compounds are indicated by smaller disks coloured according to biosynthetic route/product class (partial gluconeogenesis, pink; purine ribonucleotides, light blue; pyrimidine ribonucleotides, purple; amino acids, green; lipids, orange). Feedstocks are indicated by black squares and the immediate metabolites thereof as small black disks. ATP(/GTP)-consuming reactions are shown as red arrows, the number of which matches the number of high-energy phosphate bonds consumed per reaction. Oxidation reactions are shown by blue arrows. A more complete description of each reaction is provided in the Supplementary Material, as is a list of abbreviations.

albeit modestly [30], but without shepherding by enzymes they also catalyse unwanted reactions. An essential reaction of the partial gluconeogenesis branch is the enolase-catalysed conjugate addition of water to phosphoenolpyruvate (PEP), giving 2-phosphoglycerate (2-PG), but divalent metal ions instead catalyse the

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attack of water at the phosphate group of PEP resulting in hydrolysis back to pyruvate [31]. Certain ions are particularly effective at this, the hydrolysis being 'strongly accelerated' by ferrous ions [26], for example. Likewise, the decarboxylation of oxaloacetate back to pyruvate is catalysed by divalent metal ions [32], as is the aldol dimerisation of pyruvate [33] which would compete with its conversion to acetolactate — even if that could ever be achieved without TPP — and thence valine and leucine.

(iii) Flux control from branch points and regulation. Flux through the different branches emanating from branch points has to be controlled such that all the branches are productive. Thus, if any of the reactions leading away from pyruvate is significantly faster than the others, the others suffer, yet all are crucial. This problem is compounded by the divalent metal ion problem outlined above. Biology catalyses reactions differentially such that most are brought up to a similar speed [28], thus avoiding bottlenecks, but the background reactions have widely differing rates. Thus, even if prebiotically plausible catalysts for all 75 or so reactions could be found, it would not be enough, as they would additionally need to accelerate the individual reactions in the same differential way as do the enzymes used in biology, for the network to operate with the efficiency needed.

(iv) Instability of intermediates. Evolution has resulted in biology being able to handle some extremely unstable intermediates, which decompose in the absence of enzymes. Thus, for example, phosphoribosylamine, produced by ammonolysis of PRPP, hydrolyses to ribose-5-phosphate with a half-life of 38 seconds under physiological conditions and it anomerises even faster [34]. Any non-enzymatic catalyst for the subsequent glycylation step would have to outcompete these rapid reactions or there would be no purine ribonucleotides.

Enolisation is required for the interconversion of the triose phosphates, but the elimination of phosphate from the enolate of glyceraldehyde-3-phosphate is facile, thus the triose-3-phosphates are intrinsically unstable [35]. Nature gets round this by having triose phosphate isomerase both positively catalysing enolisation and negatively catalysing the elimination. In thermophiles, the uncatalysed elimination becomes problematic with the triose phosphates only having a half-life of minutes at 80°C [36]. These microorganisms therefore have a bifunctional fructose-1,6-bisphosphate aldolase-fructose-1,6-bisphosphatase to overcome this and trap them as the more stable fructose-6-phosphate. A non-enzymatic catalyst for this combination of aldolisation and phosphate ester hydrolysis seems an extraordinarily unrealistic prospect

(v) Oxidation in a reducing environment. Biology plays redox chemistry really well, but achieving this at a network level without enzymes and redox cofactors would be extremely tricky. For sure, oxidation and reduction reactions can operate simultaneously in chemistry, but oxidising the hydroxyl group of 3-isopropylmalate, *en route* to leucine, would be challenging in the presence of other alcohols, particularly whilst simultaneously reducing dihydroxyacetone phosphate to glycerol-3-phosphate. Dehydrogenating dihydroorotate and inosine monophosphate during ribonucleotide synthesis would be similarly difficult whilst hydrogenating enoyl derivatives in fatty acid synthesis.

(vi) Substrate selectivity. By employing enzymes with concave active sites, biology is capable of discriminating between chemically similar compounds of different shapes and sizes. Without such discrimination, it would not be possible to control selectivity in the network. Thus, for example, any non-enzymatic phosphatase mimic that could hydrolyse fructose-1,6-bisphosphate, and mitigate the triose phosphate instability problem alluded to above, would be hard-pressed not to dephosphorylate any of the many monophosphates in the network, especially dihydroxyacetone phosphate. Similarly, in purine ribonucleotide synthesis any simple catalyst that happened to be able to formylate the amino group of aminoimidazolecarboxamide ribonucleotide (AICAR) would have a hard job in not formylating the more nucleophilic amino group of the closely related aminoimidazole ribonucleotide (AIR). Formylation of the latter intermediate would block purine ribonucleotide synthesis and thus prevent the formation of functional RNA.

(vii) The ammonia problem. Ammonia is needed throughout the network, but there are many intermediates which are destroyed by this potent nucleophile. Biology gets round this by storing ammonia in compounds such as glutamate and glutamine and then releasing it at the enzyme active sites [37], or at the entrances to tunnels to other active sites [38], where it is needed. Absent this trick, it would be highly difficult to reductively aminate pyruvate to alanine, for example, without ammonolysing the various thioesters and acyl phosphates of the network. Ammonolysis of acetyl-CoA to give acetamide would shut

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down the synthesis of pyruvate. Ammonolysis of acyl phosphates, or thioesters derived therefrom, would prevent the reduction of carboxylate groups to aldehydes that occurs throughout the network.

(viii) Energy coupling. Expenditure of ATP (or GTP) is required to drive many of the reactions of the network, which would otherwise be energetically unfavourable. Proponents of building block synthesis by nascent life have gradually moved away from the idea that some sort of primitive chemiosmosis could regenerate ATP (and thence GTP). They have even moved away from nucleoside triphosphates as an energy currency because of their kinetic inertness and currently seem to favour acetyl phosphate as a source of energy [14,39], even though its hydrolysis is catalysed by metal ions [40]. Granted, acetyl phosphate has the potential to phosphorylate homoserine, for example, — though it would have to be a very selective catalyst that managed to discriminate between the hydroxyl group of this alcohol and 55 M water — but what about the crucial phosphorylation of pyruvate to PEP? This latter reaction needs two high-energy phosphate bonds of ATP to be spent to drive it and the enzyme that catalyses the transformation does so using some pretty spectacular enzymology [41]. It is an almost inconceivable transformation using acetyl phosphate and prebiotically plausible non-enzymatic catalysts. Even if it was possible, it would have to compete with the divalent metal ion-catalysed hydrolysis back to pyruvate [32].

Are there any ways to escape from these eight fundamental problems? If the mantra is strictly adhered to, no. So, what is now happening is that people who insist on synthesis *in cellulo* as biology emerges are opting for reactions that are increasingly different from those in extant biology. But the eight problems are irredeemable even by this shifting of the goal posts. Thus, one cannot invoke iron-nickel sulfide catalysed synthesis of acetyl thioesters [42] at the same time and place as the ferrous–ferric iron-mediated reductive amination of pyruvate to alanine with 0.375 M ammonia [43] because of the ammonia problem. It is not possible to have a formose reaction of formaldehyde to generate pentoses [44] at the same time as ferrous iron-mediated aldolisation chemistry of pyruvate [27] because of myriad deleterious crossed aldolisations between oxoacids, formaldehyde and sugars. One cannot rely on metallic iron and hydroxylamine as a way of converting pyruvate to alanine [27] because the same conditions will destroy key intermediates in the network, for example by converting all acyl phosphates and thioesters to hydroxamates. No amount of hard selling can resurrect this dead parrot of an idea — *caveat emptor*.

Which brings us back to the idea that HCN chemistry made the 15 key products which enabled the origin and earliest evolution of life. An energy source would also have been necessary to drive energy-dissipative macromolecular assembly cycles from the monomers and maintain the system in an out of equilibrium state. Simple isonitriles are being investigated in this regard [45], they are also easily produced from HCN and can activate both phosphate and carboxylate groups as would have been essential. Provisioned and powered by the HCN product tree, early biology could have 'lived off the fat of the land'. But extant biology, does not live this way. So how could the transition to the biosynthetic network with catalysed energy coupling take place? Considering the material aspect of this problem first, as biology spread, resources would have become depleted and there would have been a need to start to make the key 15 products within cells as part of biochemistry. We cannot know for sure which were the first biosynthetic reactions, but it is easy to imagine that ribonucleotides became scarce in the environment early on. However, as a by-product of prebiotic ribonucleotide syntheses involving photochemistry [20,21], for example, the environment would have likely contained nucleobases and maybe even sugar phosphates. This immediately hints at the salvage pathways of ribonucleotide biosynthesis wherein the canonical nucleobases are ribosylated. This cuts out many steps of the *de novo* biosyntheses, in particular, the long, difficult and very energetically demanding sequence of steps from PRPP to the purine ribonucleotides. That nascent biology battled its way through this arduous sequence before it benefitted from 'homemade' purine ribonucleotides supplementing diminishing environmental supplies, simply beggars belief. It seems much more likely that a prefabricated purine was bolted on to a homemade sugar phosphate, or that both components were prefabricated and then put together IKEA-fashion in early cells. So, could a primitive ribozyme or ribonucleoprotein catalyse nucleobase ribosylation? The answer is not just a resounding yes, ribozymes can do it [46], but also that they are ideally suited to do it by a dissociative mechanism because they can use the negative charge of their backbone to stabilise developing positive charge in the nucleosidation reaction [47]. However, the ribozyme catalyst cannot stop some competing hydrolysis because ribozymes cannot envelop their substrates the way proteins can. So, there would have been an opportunity for a ribonucleoprotein based on our restricted amino acid alphabet to do even better and pull biology back from the brink. Other reactions could have been added to build up the biosynthetic network in a patchwork fashion [48] by virtue of the

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likely catalytic promiscuity of early (ribonucleo)proteins. This is the stage at which the otherwise inefficient and non-specific metal ion-catalysed reactions are brought into play with enzyme evolution restricting specificity and improving catalysis. What about energy coupling? Nucleoside triphosphates could have accumulated as by-products of nucleoside monophosphate activation then chance catalysis of ATP usage to drive a thermodynamically uphill reaction would have taken the system to another level. Depletion of ATP stocks could then have led to substrate level phosphorylation. Adaptation to the way biology now does it would have begun—the rest is history.

Summary

- Synthesising life's building blocks using the same pathways and intermediates as extant biology is a near impossible task without enzymes.
- However, the building blocks can be synthesised from hydrogen cyanide and its derivatives under prebiotically plausible conditions, but some of these conditions are incompatible with life.
- Therefore, it is proposed that the origin and early evolution of life took place in an environment containing previously synthesised building blocks.
- The pathways and intermediates of extant biology could then have been introduced in a patchwork fashion as environmental supplies of building blocks became depleted and early organisms adapted to their changing conditions.

Abbreviations

2-PG, 2-phosphoglycerate; AICAR, aminoimidazolecarboxamide ribonucleotide; AIR, aminoimidazole ribonucleotide; PEP, phosphoenolpyruvate; PRPP, phosphoribosyl pyrophosphate; TPP, thiamin pyrophosphate.

Funding

Our work was supported by the Medical Research Council [number MC_UP_A024_1009] and a grant from the Simons Foundation [number 290362 to J.D.S.].

Acknowledgements

We thank other members of the Sutherland research group for helpful discussions and suggestions.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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Supplementary Material.

Abbreviations

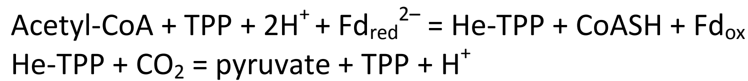
Abbreviation	Full Name	Reaction number
1,3-DPG	1,3-Diphosphoglycerate	5
1,6-FBP	Fructose-1,6-bisphosphate	8
2-iPM	2-Isopropylmalate	59
2-KG	2-Ketoglutarate	42
2-KiC	2-Ketoisocaproate	61
2-KiV	2-Ketoisovalerate	57
2-PG	2-Phosphoglycerate	3
2,3-DHiV	2,3-Dihydroxyisovalerate	56
3-HBut-ACP	3-Hydroxylbutanoyl-acyl carrier protein	71
3-iPM	3-Isopropylmalate	60
3-PG	3-Phosphoglycerate	4
3-PGA	Glyceraldehyde-3-phosphate	6
6-PF	Fructose-6-phosphate	9
AcAc-ACP	Acetoacetyl-acyl carrier protein	70
AcCoA	Acetyl coenzyme A	75
AcGlu	N-Acetylglutamate	47
AcGluP	N-Acetyl-L-glutamyl-5-phosphate	48
AcGluSa	N-Acetyl-L-glutamate-5-semialdehyde	49
AcLac	2-Acetolactate	55
AcOrn	N^2-Acetylornithine	50
AdS	Adenylosuccinate	22
AICAR	Aminoimidazole carboxamide ribonucleotide	19
AIR	5'-Phosphoribosyl-5-aminoimidazole	16
Ala	L-Alanine	36
AMP	Adenosine-5'-monophosphate	23
Arg	L-Arginine	54
ArgS	L-Argininosuccinate	53
Asp	L-Aspartate	28
AspP	L-Aspartyl-4-phosphate	63
AspSa	L-Aspartate-4-semialdehyde	64
But-ACP	Butyryl-acyl carrier protein	73
CAIR	5'-Phosphoribosyl-4-carboxy-5-aminoimidazole	17
CarASP	N-Carbamyl-L-aspartate	30
CarOrn	Citrulline	52
CarP	Carbamoyl phosphate	29
Crot-ACP	Crotonyl-acyl carrier protein	72

CTP	Cytidine-5'-triphosphate	35
DHAP	Dihydroxyacetone phosphate	7
DHOro	Dihydroorotate	31
E4P	Erythrose-4-phosphate	
FA	Fatty acid	74
fAICAR	5-Formamidoimidazole-4-carboxamide ribonucleotide	20
fGAM	Formylglycinamidine-ribonucleotide	15
fGAR	N^2-Formyl-N^1-(5-phospho-D-ribosyl)glycinamide	14
GAR	N^1-(5-phospho-D-ribosyl)glycinamide	13
Glu	L-Glutamate	43
GluP	L-Glutamyl-5-phosphate	44
Gly	Glycine	40
Glycerol-P	Glycerol-3-phosphate	68
GMP	Guanosine-5'-monophosphate	25
hSer	L-Homoserine	65
hSerP	O-Phospho-L-homoserine	66
Hu6P	3-Hexulose-6-phosphate	10
IMP	Inosine-5'-monophosphate	21
Leu	L-Leucine	62
Mal-CoA	Malonyl-CoA	69
OAA	Oxaloacetate	26, 27
OMP	Orotidine-5'-monophosphate	33
Orn	Ornithine	51
Oro	Orotate	32
P5C	Δ^1-Pyrroline-5-carboxylate	45
PEP	Phosphoenolpyruvate	2
PRA	Phosphoribosylamine	12
Pro	L-Proline	46
PRPP	Phosphoribosylpyrophosphate	11
R5P	Ribose-5-phosphate	
Ru5P	Ribulose-5-phosphate	
SAICAR	Succinyl-aminoimidazolecarboxamide ribonucleotide	18
S7P	Sedoheptulose-7-phosphate	
Ser	L-Serine	41
Thr	L-Threonine	67
UMP	Uridine-5'-monophosphate	34
Val	L-Valine	58
X5P	Xylulose-5-phosphate	
XMP	Xanthosine-5'-monophosphate	24

More detailed description of the reactions comprising the network Figure 3

1. Pyruvate ferredoxin oxidoreductase. A multistep reaction in which the acetyl group of acetyl-CoA is transferred to thiamine pyrophosphate (TPP) and reduced by ferredoxin to

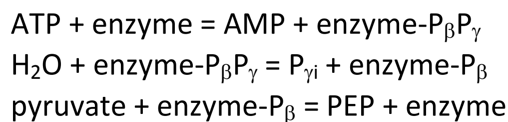
give hydroxyethyl-TPP which is then carboxylated to a new adduct which equilibrates with pyruvate and TPP:



Acetyl-CoA + TPP + $2H^+$ + Fd_{red}^{2-} = He-TPP + CoASH + Fd_{ox}
He-TPP + CO_2 = pyruvate + TPP + H^+

The electron transfer from the reduced ferredoxin is not direct but is via enzyme-bound iron-sulfur clusters. This highly complex reaction requiring umpolung is difficult to imagine without cofactors and the enzyme under reasonable conditions.

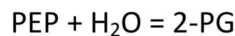
2. Phosphoenolpyruvate synthetase. Two high-energy phosphate bonds need to be spent to drive the conversion of pyruvate to phosphoenolpyruvate (PEP). This enzyme achieves this by way of both pyrophosphoryl- and phosphoryl-enzyme intermediates using some pretty spectacular enzymology [4]:



ATP + enzyme = AMP + enzyme-$P_\beta P_\gamma$
H_2O + enzyme-$P_\beta P_\gamma$ = $P_{\gamma i}$ + enzyme-P_β
pyruvate + enzyme-P_β = PEP + enzyme

One equivalent of acetyl phosphate could not drive this conversion thermodynamically and hoping to achieve it with two equivalents without an enzyme is a pipe dream. A potential alternative would go by way of oxaloacetate and reactions 26 and 27, but achieving this non-enzymatically is fraught with difficulties, *vide infra*.

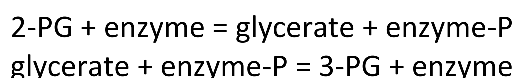
3. Enolase. Addition of the poorly nucleophilic hydroxide ion to the alkene of PEP generating an unstable carbanion which then undergoes protonation giving 2-phosphoglycerate:



PEP + H_2O = 2-PG

PEP undergoes non-enzymatic hydrolysis rather than hydration. The hydrolysis reaction is catalysed by metal ions [31], in particular being 'strongly accelerated' by ferrous ions [26].

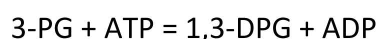
4. Phosphoglycerate mutase. The phosphate is shuttled between the 2- and 3-positions of glycerate via a phosphoryl-enzyme intermediate:



2-PG + enzyme = glycerate + enzyme-P
glycerate + enzyme-P = 3-PG + enzyme

Without an enzyme, the removal of phosphate in the first step would equate to hydrolysis to glycerate and inorganic phosphate.

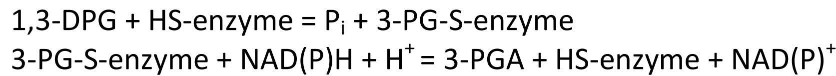
5. Phosphoglycerate kinase. Substrate-selective carboxylate phosphorylation:



3-PG + ATP = 1,3-DPG + ADP

Free 1,3-diphosphoglycerate is very unstable having a half-life for non-enzymatic hydrolysis to 3-phosphoglycerate of about 30 mins at room temperature and so it is reversibly passed to the next enzyme via an enzyme-substrate-enzyme complex [S1].

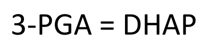
6. Glyceraldehyde-3-phosphate dehydrogenase. Covalently bound thioester intermediate and redox by nicotinamide mediated hydride transfer giving a hemithioacetal of glyceraldehyde-3-phosphate which equilibrates with the free aldehyde:



1,3-DPG + HS-enzyme = P_i + 3-PG-S-enzyme
3-PG-S-enzyme + NAD(P)H + H^+ = 3-PGA + HS-enzyme + $NAD(P)^+$

There is an enzyme that catalyses the direct oxidation of glyceraldehyde-3-phosphate to 3-phosphoglycerate, but this reaction is, to all extents and purposes, irreversible and so a non-enzymatic variant could not operate in the gluconeogenetic direction.

7. Triosephosphate isomerase. Positive catalysis of enolisation coupled to negative catalysis of phosphate elimination:



3-PGA = DHAP

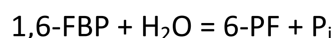
Attempted isomerisation of the triose phosphates in the absence of enzymes invariably results in elimination of phosphate by an $E1_{cB}$-irreversible mechanism and generation of methylglyoxal [35]. This elimination is extremely rapid at high temperatures [36].

8. Fructose-1,6-bisphosphate aldolase. Selective crossed aldol reaction:

DHAP + 3-PGA = 1,6-FBP

In the absence of enzyme catalysis, background aldol reaction would compete with a myriad other aldol reactions, particularly enolate hydroxymethylation in the presence of formaldehyde.

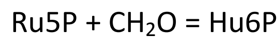
9. Fructose-1,6-bisphosphatase: Highly selective phosphatase activity that offsets the otherwise unfavourable thermodynamics of partial gluconeogenesis:



1,6-FBP + H_2O = 6-PF + P_i

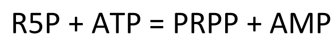
Without selective catalysis, a general background phosphatase activity would be energetically disastrous as it would deplete all phosphate monoesters. A bifunctional enzyme catalysing this reaction and the preceding one is commonly found in thermophilic autotrophic microorganisms, 'its bifunctionality ensures that heat-labile triosephosphates are quickly removed and trapped in stable fructose-6-phosphate'. The half-life of triosephosphates at 80 ^oC, pH 7, was confirmed to be 4 min. [36].

10. 3-Hexulose-6-phosphate synthase. Regioselective hydroxymethylation of ribulose-5-phosphate:


$Ru5P + CH_2O = Hu6P$

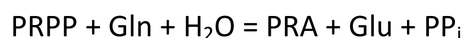
Given the large number of background enolisation reactions invoked, hoping for hydroxymethylation of just one enediolate is an appeal to magic.

11. Phosphoribosylpyrophosphate synthetase. Regioselective pyrophosphorylation of one anomer of the furanose form of ribose-5-phosphate:


R5P + ATP = PRPP + AMP

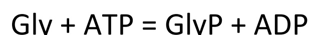
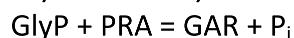
Another reaction that involves attack at the β-phosphate of ATP. Achieving the same conversion with acetylphosphate would require initial phosphorylation of one anomer of the furanose form of ribose-5-phosphate followed by regioselective phosphorylation of one of the phosphate groups of 5-phosphoribosylphosphate. This second phosphorylation would also have to be substrate-selective to avoid the phosphorylation of pretty much all other phosphate monoesters. PRPP is chemically unstable, particularly in the presence of divalent metal cations and decomposes to ribose-5-phosphate, 5-phosphoribosyl 1,2-cyclic phosphate and ribose-1,5-cyclic phosphate [S2].

12. Glutamine phosphoribosylpyrophosphate amidotransferase. Ammonolysis of PRPP to phosphoribosylamine using ammonia generated *in situ* by hydrolysis of glutamine to glutamate:


$PRPP + Gln + H_2O = PRA + Glu + PP_i$

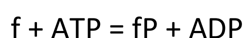
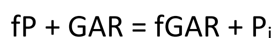
Under physiological conditions, PRA hydrolyses to ribose-5-phosphate with a half-life of 38 s and it anomerises even faster [34].

13. Glycinamide ribonucleotide synthetase. Reaction of glycine with ATP to give glycyl phosphate followed by glycylation of the β-anomer of PRA giving glycinamide ribonucleotide:


Gly + ATP = GlyP + ADP

$GlyP + PRA = GAR + P_i$

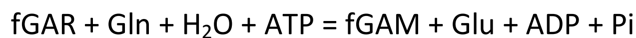
To overcome hydrolysis of PRA it is thought that it is channelled to this enzyme from the previous enzyme in the pathway [S3]. Acetyl phosphate is not sufficiently high in energy to phosphorylate glycine to a significant extent. Another transformation that would be near impossible in the absence of an enzyme.

14. Glycinamide ribonucleotide transformylase. Formylation of GAR using N10-formyltetrahydrofolate or formate and ATP - arguably the latter is simpler:


f + ATP = fP + ADP

$fP + GAR = fGAR + P_i$

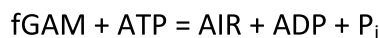
Formylphosphate is unstable undergoing hydrolysis by nucleophilic attack at both carbon and phosphorus. Synthesis of formylphosphate from formate and acetyl phosphate would be unfavourable because of the lower pK_a of formic acid compared to acetic acid.

15. Formylglycinamidine ribonucleotide synthetase. Amidination of the glycinamide amide using ammonia generated in situ by hydrolysis of glutamine to glutamate and using ATP to phosphorylate the amide oxygen such that it is converted into a better leaving group than PRA:


fGAR + Gln + H_2O + ATP = fGAM + Glu + ADP + Pi

Absent an enzyme, the glycinamide amide of fGAR is less reactive than the formyl amide. Without the phosphorylation, ammonolysis would first deformylate fGAR to GAR then cleave GAR to PRA which would hydrolyse to ribose-5-phosphate.

16. Aminoimidazole ribonucleotide synthetase. Attack of N1 of fGAM at the formyl carbon with phosphorylation of the formyl oxygen to render it a better leaving group than nitrogen:


fGAM + ATP = AIR + ADP + P_i

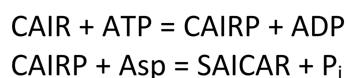
Even if the formamido group could be activated in water without an enzyme – a remote possibility – cyclisation by attack of the unsubstituted amidine nitrogen rather than N1 would be expected.

17. Aminoimidazole ribonucleotide carboxylase: Reversible formation of the carbamate by N-carboxylation of aminoimidazole ribonucleotide followed by enamine C-carboxylation:

AIR + CO_2 = CAIR

At low pCO_2, ATP is used to drive the initial N-carboxylation becoming ADP and P_i in the process. One of the only reactions in this whole scheme to proceed at an appreciable rate without deleterious side reactions in the absence of catalysis although only at high pCO_2 when the enzyme catalysed conversion is still a million times faster [S4]!

18. Succinyl-aminoimidazolecarboxamide ribonucleotide synthetase. Phosphorylation of CAIR to the acyl phosphate which is then used to acylate the amino group of aspartate:

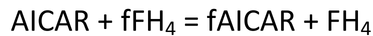

CAIR + ATP = CAIRP + ADP
CAIRP + Asp = SAICAR + P_i

Without a substrate-selective catalyst, acylation of other amino acid amino groups eg. glycine would be inevitable, this would not allow the subsequent elimination that results in overall amidation.

19. Adenylosuccinate lyase activity I. Elimination of a poor leaving group from SAICAR by concerted general acid-base catalysis by two precisely positioned histidine side chains:

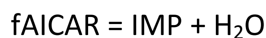
SAICAR = AICAR + fumarate

20. Aminoimidazolecarboxamide ribonucleotide transformylase. Thermodynamically uphill formylation of the poorly nucleophilic amino group of AICAR using N^{10}-formyltetrahydrofolate.

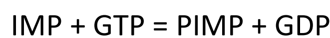

AICAR + fFH_4 = fAICAR + FH_4

Formylation of AIR, which has a much more nucleophilic amino group, would prevent N- and C- carboxylation and thereby prevent purine ribonucleotide synthesis.

21. Inosine monophosphate cyclohydrolase. Closure of the purine 6-membered ring by attack of the carboxamide nitrogen on the formyl group, possibly assisted by orbital steering, followed by proton transfers and elimination of water:


fAICAR = IMP + H_2O

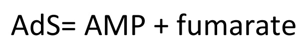
22. Adenylosuccinate synthetase. Phosphorylation of the 6-oxo group of IMP followed by attack of the imidoyl phosphate on carbon by the amino group of aspartate and displacement of inorganic phosphate:


IMP + GTP = PIMP + GDP

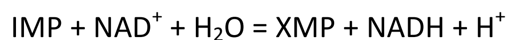
PIMP + Asp = AdS + P_i

Reaction of the amino groups of other amino acids eg. glycine, would be unpreventable in the absence of an enzyme. These inevitable side products could not undergo the subsequent elimination that results in overall amination.

23. Adenylosuccinate lyase activity II. Elimination of a poor leaving group from AdS by concerted general acid-base catalysis by two precisely positioned histidine side chains:

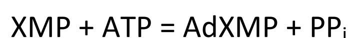
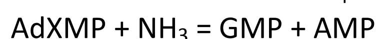

AdS= AMP + fumarate

24. Inosine monophosphate dehydrogenase. Regiospecific oxidation of C2 of IMP by NAD^+ with concomitant addition of water:


IMP + NAD^+ + H_2O = XMP + NADH + H^+

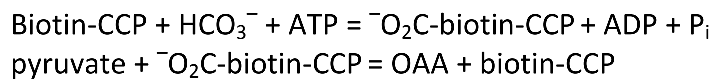
A difficult reaction in a reducing environment. Without an enzyme to control the specificity, other oxidants could oxidise the diol or imidazole ring.

25. Guanosine monophosphate synthetase. Reversible adenylation of O2 of XMP followed by aminolysis by attack of the amido nitrogen of glutamine and carboxamide hydrolysis, or less favourably by free ammonia - the latter is less complex:


XMP + ATP = AdXMP + PP_i

AdXMP + NH_3 = GMP + AMP

Another incredibly challenging transformation in water without an enzyme – O2 of XMP would have to outcompete 55 M water to react with an activating agent just to accomplish the first step.

26. Pyruvate carboxylase. This enzyme uses ATP to couple bicarbonate and biotin-carboxyl carrier protein to give N^1-carboxybiotin-CCP which then carboxylates the enolate of pyruvate to give oxaloacetate:



Biotin-CCP + HCO_3^- + ATP = ^-O_2C-biotin-CCP + ADP + P_i
pyruvate + ^-O_2C-biotin-CCP = OAA + biotin-CCP

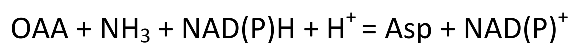
Another evolutionary triumph whereby ATP hydrolysis is used to drive an extremely thermodynamically uphill reaction – any primitive version of this reaction would have to battle the spontaneous decarboxylation of oxaloacetate which would lead to an energy draining futile cycle. The spontaneous decarboxylation is catalysed by divalent metal ions [32].

27. Phosphoenolpyruvate carboxykinase. Decarboxylation of oxaloacetate gives the enolate of pyruvate which is phosphorylated by GTP:

OAA + GTP = PEP + GDP + CO_2

Competing protonation of the enolate by solvent is prevented by a water-tight enzyme active site – how could this possibly be achieved in water without an enzyme?

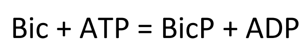
28. Transaminase or amino acid dehydrogenase. Transamination from glutamate shifts the ammonia assimilation problem so the dehydrogenase is potentially simpler:



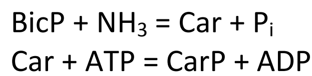
OAA + NH_3 + NAD(P)H + H^+ = Asp + $NAD(P)^+$

Reductive aminations can be achieved relatively easily without enzymes, but relatively high ammonia concentrations are necessary to allow imine reduction to the amino acid to outdo ketone reduction to the hydroxy acid. Thus, in work purporting to show that amino acids could be synthesised in deep-sea vents, a grossly unrealistic concentration of ammonia (0.375 M) was employed [43] (the concentration of ammonia in the early ocean was probably more than 5000 x lower [S5]). High concentrations of ammonia would play havoc with many of the other transformations required in this scheme. Thus, activated carboxylates would be ammonolysed to amides preventing eg. reaction 13 and 14 and thus purine ribonucleotide synthesis and converting 1,3-diphosphoglycerate to 3-phosphoglyceramide and thus preventing partial gluconeogenesis.

29. Carbamoyl phosphate synthetase. The enzyme uses ammonia from glutamate hydrolysis and two ATPs to convert bicarbonate to carbamyl phosphate. Using exogenous ammonia gives a slightly simpler process:

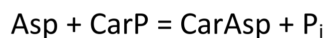


Bic + ATP = BicP + ADP


$BicP + NH_3 = Car + P_i$
$Car + ATP = CarP + ADP$

This enzyme catalyses 'one of the most remarkable reactions ever described in biological chemistry' [38]. The intermediates carboxyphosphate and carbamate are both extremely hydrolytically labile and carbamoyl phosphate can react with excess ammonia to generate urea. Unimaginable without an enzyme and no carbamoyl phosphate means no *de novo* synthesis of pyrimidine ribonucleotides and no arginine.

30. Aspartate carbamoyltransferase. Catalyses the carbamoylation of aspartate by carbamoyl phosphate:

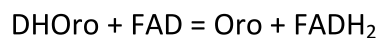

$Asp + CarP = CarAsp + P_i$

Non-enzymatically, carbamoyl phosphate equilbrates with cyanate before both are hydrolysed, a reaction that is catalysed by carbonate [S6]. Cyanate will carbamylate amines, but how can the selective carbamoylation of aspartate possibly be achieved in the presence of other amino acids and ammonia?

31. Dihydroorotase. A binuclear metal center polarises carbonyl groups and a general acid (/base) of the enzyme mediates difficult (de)protonation as carbamyl aspartate is equilibrated with dihydroorotate and water:

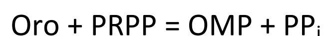
$CarAsp = DHOro + H_2O$

32. Dihydroorotate dehydrogenase. A general base deprotonates C5 of dihydroorotate as hydride is transferred from C6 to an oxidised flavin generating orotate and reduced flavin:


$DHOro + FAD = Oro + FADH_2$

A really challenging reaction without enzymes in a highly reducing environment.

33. Orotate phosphoribosyltransferase. The enzyme catalyses the stereospecific magnesium ion-dependent phosphoribosylation of orotate by dissociative nucleophilic substitution via a transition state with considerable oxocarbonium ion character [S7]. This transition state is stabilised electrostatically by precisely positioned carboxylates and water is excluded from the active site thus preventing competing hydrolysis:


$Oro + PRPP = OMP + PP_i$

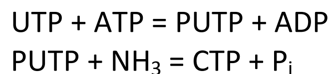
The background reaction is undetectable although non-enzymatic hydrolysis of PRPP is observed.

34. Orotidine monophosphate decarboxylase. An extraordinary decarboxylation giving uridine monophosphate:

$OMP = UMP + CO_2$

Unassisted by cofactors, this enzyme produces a huge rate enhancement (~10^{17}-fold) over the uncatalysed reaction which has a half-life of 78 million years [28]. The transformation can be accomplished photochemically with 254 nm irradiation [S8] although this seems unlikely at a deep-sea vent.

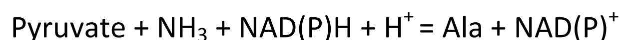
35. CTP synthetase. Phosphorylation of O4 of UTP followed by aminolysis by attack of the amido nitrogen of glutamine and carboxamide hydrolysis, or less favourably by free ammonia - the latter is less complex:



UTP + ATP = PUTP + ADP
PUTP + NH_3 = CTP + P_i

Another really challenging reaction in the absence of enzymes. Non-enzymatic phosphorylation of nucleotides is known, but occurs on phosphate or the 2',3'-diol both of which are more nucleophilic than O4. The conditions for these phosphorylation reactions – heating in formamide or molten urea – are completely incompatible with deep-sea vents and life.

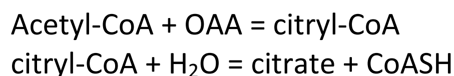
36. Transaminase or amino acid dehydrogenase.



Pyruvate + NH_3 + NAD(P)H + H^+ = Ala + $NAD(P)^+$

See 28.

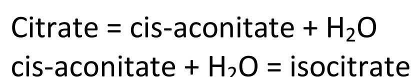
37. Citrate synthase. Claisen condensation of acetyl-CoA with oxaloacetate to form citryl-CoA followed by hydrolysis of the latter to citrate:



Acetyl-CoA + OAA = citryl-CoA
citryl-CoA + H_2O = citrate + CoASH

A suitably positioned general base generates the enolate of acetyl-CoA which the enzyme stabilises electrostatically thus favouring its formation. In water in the absence of an enzyme, a general base would simply catalyse the hydrolysis of acetyl-CoA. Even if conditions for the Claisen condensation in water could be found, selective hydrolysis of citryl-CoA in the presence of acetyl-CoA would be a daunting challenge. Oxaloacetate can be directly converted to citrate by UV irradiation [S9], but this is unlikely at a deep-sea vent.

38. Aconitase. Dehydration to cis-aconitate followed by rehydration to isocitrate:



Citrate = cis-aconitate + H_2O
cis-aconitate + H_2O = isocitrate

Coordinated general acid-base catalysis to dehydrate citrate coordinated by its hydroxyl group to an iron-sulfur cluster followed by flipping of cis-aconitate and rehydration. In the absence of an enzyme this would be a challenging transformation, particularly in the presence of better nucleophiles than water.

39. Isocitrate lyase. Retro Claisen condensation of isocitrate to give succinate and glyoxylate:

Isocitrate = succinate + glyoxylate

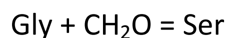
Another extraordinary piece of enzymology best appreciated by considering the reverse reaction in which the enzyme deprotonates a methylene group of succinate with a pK_a of about 25. The enzyme stabilises the aci form of the succinate carbanion by at least fifteen orders of magnitude [S10]! Doing this in water would not be possible even with a strong base which would anyway cause myriad destructive reactions of activated intermediates in the overall scheme.

40. Transaminase or amino acid dehydrogenase.

Glyoxylate + NH_3 + NAD(P)H + H^+ = Gly + $NAD(P)^+$

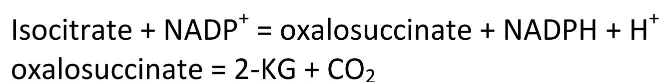
See 28.

41. Serine transhydroxymethylase. Pyridoxal phosphate dependent reaction of a stabilised glycine carbanion with formaldehyde derived from active site hydrolysis of methylene-tetrahydrofolate or, more simply free formaldehyde:


Gly + CH_2O = Ser

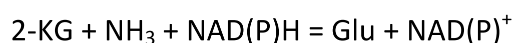
A difficult reaction without pyridoxal phosphate.

42. Isocitrate dehydrogenase. Oxidation of isocitrate to oxalosuccinate followed by decarboxylation:


Isocitrate + $NADP^+$ = oxalosuccinate + NADPH + H^+
oxalosuccinate = 2-KG + CO_2

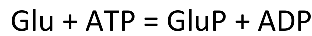
The enzyme uses a magnesium ion to facilitate both the oxidation and the decarboxylation. The decarboxylation would occur spontaneously in the absence of an enzyme if a means of oxidising the alcohol selectively in a reducing environment could be found. The spontaneous decarboxylation (like that of OAA) would be catalysed by divalent metal ions such as magnesium ions.

43. Transaminase or amino acid dehydrogenase.


2-KG + NH_3 + NAD(P)H = Glu + $NAD(P)^+$

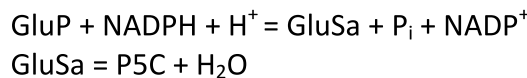
See 28.

44. Glutamyl-5-phosphate synthetase. Selective phosphorylation of the 5-carboxylate of glutamate:


Glu + ATP = GluP + ADP

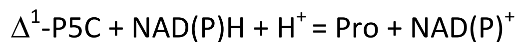
This particular acyl phosphate is more labile than most and undergoes extremely facile cyclisation to pyroglutamate. To avoid this, it is passed to the next enzyme in the sequence in a process that avoids free diffusion, probably via an enzyme-substrate-enzyme complex. In the absence of enzyme, any reagent capable of phosphorylating carboxylate groups would do so indiscriminately.

45. Glutamyl-5-phosphate reductase. Reduction of glutamyl-5-phosphate to glutamate-5-semialdehyde which spontaneously equilibrates with Δ^1-pyrroline-5-carboxylate:

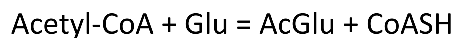

GluP + NADPH + H^+ = GluSa + P_i + $NADP^+$
GluSa = P5C + H_2O

A difficult reduction in the absence of enzymes given the propensity of glutamyl-5-phosphate to cyclise to pyroglutamate.

46. Δ^1-Pyrroline-5-carboxylate reductase. Nicotinamide mediated reduction of the iminium ion of the protonated pyrroline:

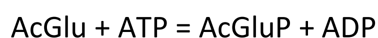

Δ^1-P5C + NAD(P)H + H^+ = Pro + $NAD(P)^+$

47. N-Acetylglutamate synthetase. Selective acetylation of the amino group of glutamate by acetyl-CoA:


Acetyl-CoA + Glu = AcGlu + CoASH

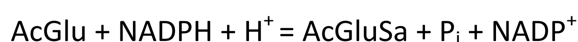
In the absence of enzymes, any form of activated acetate capable of effecting this transformation would also acetylate the amino group of other amino acids as well as other amines and ammonia.

48. N-Acetyl-glutamyl-5-phosphate synthetase. Selective phosphorylation of the 5-carboxylate of N-acetylglutamate:


AcGlu + ATP = AcGluP + ADP

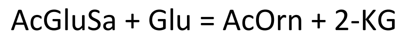
Another hydrolytically labile acyl phosphate.

49. N-Acetyl-glutamyl-5-phosphate reductase. Reduction of N-acetyl-glutamyl-5-phosphate to the corresponding semialdehyde:


AcGlu + NADPH + H^+ = AcGluSa + P_i + $NADP^+$

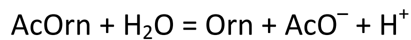
Again, a difficult reduction non-enzymatically in part due to competing hydrolysis of the acyl phosphate.

50. N^2-Acetylornithine aminotransferase. Pyridoxal phosphate dependent transamination using glutamate as amino group donor:


AcGluSa + Glu = AcOrn + 2-KG

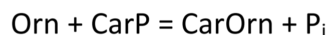
Without cofactor, a difficult transformation to achieve non-enzymatically, particularly with any substrate selectivity.

51. N^2-Acetylornithinase. Hydrolytic deacetylation by a divalent cation-dependent mechanism:


AcOrn + H_2O = Orn + AcO^- + H^+

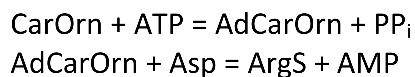
Deacetylation can be relatively easily catalysed without an enzyme, but deacetylating N^2-acetylornithine selectively without cleaving other amide bonds and more labile compounds such as thioesters and acyl phosphates is really hard to imagine. Of the other amides, the formyl amide intermediates of purine assembly would be particularly prone to competing deacylation.

52. Ornithine carbamoyltransferase. Highly substrate-selective carbamoylation using carbamoyl phosphate:


Orn + CarP = CarOrn + P_i

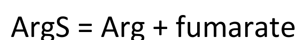
Under conditions in which the amino group of aspartate is carbamylated (reaction 30), how could the 5-amino group of ornithine possibly be carbamoylated selectively over the 2-amino group and how could this be achieved in the presence of other amino acids and ammonia?

53. Argininosuccinate synthetase. Adenylation of the ureido oxygen of citrulline followed by displacement of inorganic phosphate by the amino group of aspartate:


CarOrn + ATP = AdCarOrn + PP_i
AdCarOrn + Asp = ArgS + AMP

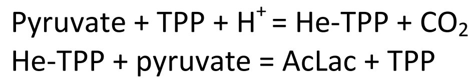
Adenylating or phosphorylating the ureido oxygen of citrulline in water in the absence of an enzyme is unbelievably challenging. Urea can be O-phosphorylated transiently in dry-state melts, but such conditions are not compatible with the other transformations of the scheme, or indeed with deep-sea vents or life.

54. Argininosuccinate lyase. Elimination of a poor leaving group from argininosuccinate by general acid-base catalysis by two precisely positioned enzyme residues:


ArgS = Arg + fumarate

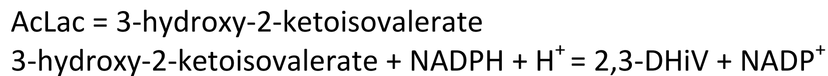
Another elimination reaction that is likely to be extremely slow in the absence of an enzyme.

55. Acetolactate synthase. Thiamine pyrophosphate mediated addition of an acetyl anion equivalent derived from one pyruvate to a second pyruvate in a reaction that also requires a divalent metal ion:


Pyruvate + TPP + H^+ = He-TPP + CO_2
He-TPP + pyruvate = AcLac + TPP

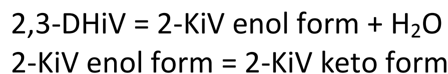
A chemically daunting transformation in the absence of an umpolung cofactor. In the absence of enzymes, divalent metal ions catalyse the unwanted aldol dimerisation of pyruvate [33].

56. Ketol-acid reductoisomerase. A hydroxide ion bridging two magnesium ions deprotonates the hydroxyl group of acetolactate and promotes a simultaneously general acid-catalysed [1,2]-sigmatropic shift of a methyl group to the ketone. The 2-ketoacid thus formed is then reduced by a nicotinamide cofactor to 2,3-dihydroxyisovalerate:


AcLac = 3-hydroxy-2-ketoisovalerate
3-hydroxy-2-ketoisovalerate + NADPH + H^+ = 2,3-DHiV + $NADP^+$

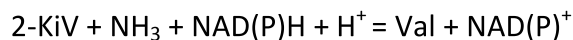
The initial isomerisation equilibrium strongly favours acetolactate so the enzyme uses precise positioning to target the reduction of the carbonyl group of 3-hydroxy-2-ketoisovalerate. In the absence of the enzyme, acetolactate is prone to decarboxylation, a reaction catalysed by divalent metal ions.

57. Dihydroxyacid dehydratase. Iron-sulfur cluster and general base-catalysed dehydration of 2,3-dihydroxyisovalerate to the enol of 2-ketoisovalerate followed by keto-enol tautomerisation:


2,3-DHiV = 2-KiV enol form + H_2O
2-KiV enol form = 2-KiV keto form

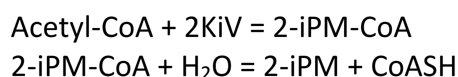
Like the elimination of water catalysed by aconitase, a demanding elimination of a poor leaving group.

58. Transaminase or amino acid dehydrogenase.


2-KiV + NH_3 + NAD(P)H + H^+ = Val + $NAD(P)^+$

See 28.

59. 2-Isopropylmalate synthase. With a mechanism similar to citrate synthase, this enzyme catalyses Claisen condensation of acetyl-CoA with 2-ketoisovalerate to form 2-isopropylmalyl-CoA followed by hydrolysis of the latter to 2-isopropylmalate:


Acetyl-CoA + 2KiV = 2-iPM-CoA
2-iPM-CoA + H_2O = 2-iPM + CoASH

See 37.

60. Isopropylmalate dehydratase. With a mechanism similar to aconitase, this enzyme catalyses the (de)hydrative equilibration of 2- and 3-isopropylmalate via 2-isopropylmaleate:

2-iPM = 2-isopropylmaleate + H_2O

2-isopropylmaleate + H_2O = 3-iPM

See 38.

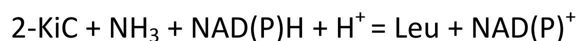
61. 3-Isopropylmalate dehydrogenase. With a mechanism similar to isocitrate dehydrogenase, this enzyme oxidises 3-isopropylmalate to 2-keto-3-carboxyisocaproate followed by decarboxylation to 2-ketoisocaproate:

3-iPM + NAD^+ = 2-keto-3-carboxyisocaproate + NADH + H^+

2-keto-3-carboxyisocaproate = 2-KiC + CO_2

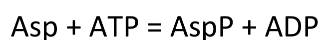
See 42.

62. Transaminase or amino acid dehydrogenase.


2-KiC + NH_3 + NAD(P)H + H^+ = Leu + $NAD(P)^+$

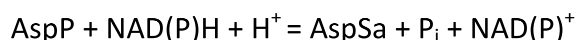
See 28.

63. Aspartyl-4-phosphate synthetase. Selective phosphorylation of the 4-carboxylate of aspartate:


Asp + ATP = AspP + ADP

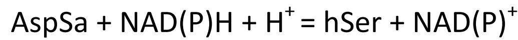
Another hydrolytically labile acyl phosphate.

64. Aspartyl-4-phosphate reductase. Reduction of aspartyl-4-phosphate to aspartate-4-semialdehyde:


AspP + NAD(P)H + H^+ = AspSa + P_i + $NAD(P)^+$

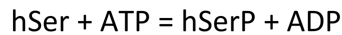
A difficult reduction in the absence of enzymes given the hydrolytic lability of aspartyl-4-phosphate. In a system in which N-acetylglutamate-5-semialdehyde is reductively aminated to N^2-acetylornithine, it would be difficult to prevent the reductive amination of aspartate semialdehyde.

65. Homoserine dehydrogenase. Nicotinamide mediated reduction of aspartate semialdehyde to homoserine:


AspSa + NAD(P)H + H^+ = hSer + $NAD(P)^+$

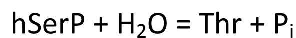
In a system in which this reduction is somehow effected non-enzymatically, it would be difficult to avoid the corresponding reduction of other aldehydes, for example N-acetylglutamate-5-semialdehyde.

66. Homoserine kinase. Substrate-selective phosphorylation of homoserine to homoserine phosphate:


hSer + ATP = hSerP + ADP

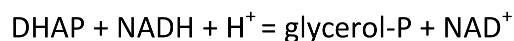
A difficult enough phosphorylation to achieve in water anyway, but without a substrate-selective catalyst, how could it be achieved selectively in the presence of other primary alcohols such as glycerol phosphate?

67. Threonine synthase. Molecular gymnastics initiated by this enzyme using pyridoxal phosphate to eliminate phosphate from homoserine phosphate giving an intermediate which is hydrated at the C3 to give, after separation from the cofactor, threonine:


hSerP + H_2O = Thr + P_i

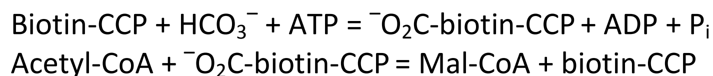
Even if the elimination of phosphate could somehow be catalysed without the enzyme and cofactor (and it looks difficult), hydration of the resultant vinylglycine would be a formidable challenge.

68. Glycerol-3-phosphate dehydrogenase. Reduction of dihydroxyacetone phosphate by a reduced nicotinamide cofactor:


DHAP + NADH + H^+ = glycerol-P + NAD^+

How could non-enzymatic reduction of a ketone be effected selectively in the presence of aldehydes such as N-acetylglutamate-5-semialdehyde?

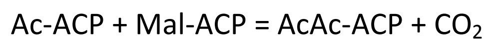
69. Acetyl-CoA carboxylase. This enzyme uses ATP to couple bicarbonate and biotin-carboxyl carrier protein to give N^1-carboxybiotin-CCP which then carboxylates the enolate of acetyl-CoA to give malonyl-CoA:


Biotin-CCP + HCO_3^- + ATP = ^-O_2C-biotin-CCP + ADP + P_i
Acetyl-CoA + ^-O_2C-biotin-CCP = Mal-CoA + biotin-CCP

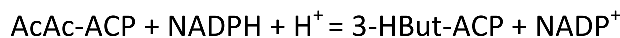
Another example of coupling ATP hydrolysis to a thermodynamically very unfavourable reaction to drive it in the synthetic direction.

70-74. Fatty acid synthetase. A molecular factory line that catalyses the stepwise assembly of fatty acids two carbons at a time. The acyl-CoA building blocks for this assembly are first attached to an acyl carrier protein (ACP) by thioester exchange which enables intermediates to be passed around the various active sites that catalyse the individual steps.

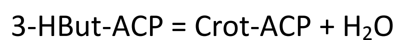
70. 3-Ketoacyl-ACP synthetase. Decarboxylation of malony-ACP generates an enolate which reacts with the acetyl thioester in a Claisen reaction giving acetoacetyl-ACP:


Ac-ACP + Mal-ACP = AcAc-ACP + CO_2

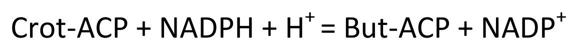
71. 3-Ketoacyl-ACP reductase. Selective reduction of the 3-keto group of acetoacetyl-ACP by nicotinamide giving 3-hydroxybutyryl-ACP:


AcAc-ACP + NADPH + H^+ = 3-HBut-ACP + $NADP^+$

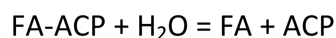
72. 3-Hydroxyacyl-ACP dehydratase. Dehydration of 3-hydroxybutyryl-ACP to give crotonyl-ACP:


3-HBut-ACP = Crot-ACP + H_2O

73. Enoyl-ACP reductase. Selective reduction of crotonyl-ACP by nicotinamide to give butyryl-ACP:


Crot-ACP + NADPH + H^+ = But-ACP + $NADP^+$

74. Thioesterase. Hydrolytic cleavage of the fatty acyl-ACP generated by elongating iterative cycles of reactions 70-73:


FA-ACP + H_2O = FA + ACP

Biology confounds the arithmetic demon associated with multistep linear synthesis by catalysing each step of this process in extremely high yield. Achieving this chemically is highly challenging – rather than Claisen reactions, thioesters exposed to base in water are simply expected to hydrolyse – but even if it could be realised with each step proceeding in 50% yield, the overall yield of, for example decanoic acid, would be $< 0.001\%$.

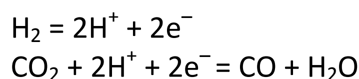
75. Acetyl-CoA synthase. In an enzymatic variant of the Monsanto acetic acid process, a methyl group from a cobalt corrinoid iron-sulfur protein (CoFeSP) is coupled at a nickel sulfide centre with carbon monoxide giving an acyl nickel species which is thiolysed by the thiol of CoASH:

Me-Co(III)FeSP + CO + CoASH = Acetyl-CoA + Co(I)FeSP

Non-enzymatically, in the presence of freshly precipitated iron and nickel sulfides, methane thiol and carbon monoxide react to give acetate via an activated form thereof as demonstrated by trapping experiments with aniline [42]. However, it has been demonstrated that methane thiol found in seafloor hydrothermal fluids, and originally thought to be of geochemical origin, is actually a product of biological processes [S11]. Prebiotically plausible non-enzymatic (activated) acetate synthesis using methyl donors other than methane thiol has not been reported.

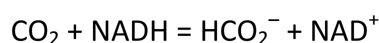
76. Carbon dioxide reduction.

In an enzymatic version of the water-gas shift reaction, hydrogenase equilibrates hydrogen with protons and electrons which are transferred to carbon monoxide dehydrogenase which catalyses the reduction of carbon dioxide to carbon monoxide:



$H_2 = 2H^+ + 2e^-$
$CO_2 + 2H^+ + 2e^- = CO + H_2O$

Alternatively, and subject to thermodynamic limitation, carbon dioxide can be reduced to formate by a dehydrogenase:



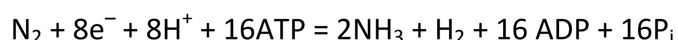
$CO_2 + NADH = HCO_2^- + NAD^+$

Difficult transformations to achieve non-enzymatically with any degree of efficiency under conditions compatible with the other reactions of the overall scheme for kinetic and thermodynamic reasons. The presence of iron-sulfur clusters in these enzymes has been interpreted as a sign of their antiquity. Speculation also has it that this implies a role for iron-sulfur minerals in catalysing non-enzymatic underlying chemistry at the origin of life. Since these clusters also bear cyanide ligands, the same line of reasoning would suggest the presence of cyanide at such a time. As cyanide is not a likely component of deep-sea vent hydrothermal fluid, this would suggest that, if such reasoning is correct, the underlying chemistry took place at a surface location. Using simulated vent conditions with precipitates of iron and nickel sulfides, the reduction of carbon dioxide to low micromolar concentrations of formate and low nanomolar concentrations of formaldehyde has been claimed. Further claims that the degree of reduction was influenced by pH gradients in the system have been refuted [S12].

Reduction of formaldehyde to methanol is plausible and mimics alcohol dehydrogeanse chemistry, but conversion of methanol to methane thiol under prebiotic conditions is highly demanding.

77. Nitrogen feedstock reduction.

Nitrogenase. The enzymes that collectively convert molecular nitrogen to ammonia do more than simply bring about the reduction as the process is coupled to ATP hydrolysis and also produces hydrogen [S13]:



$N_2 + 8e^- + 8H^+ + 16ATP = 2NH_3 + H_2 + 16\ ADP + 16P_i$

The simple reduction of nitrogen by hydrogen can be achieved by the Bosch-Haber process, but the conditions are not prebiotically plausible or compatible with the transformations of the overall scheme.

More plausibly, nitrite and nitrate produced in water under an atmosphere containing nitric oxide, due to lightning or impact shock, can be reduced to ammonia by ferrous ions. It is estimated that the concentration of ammonia in sea water would have been < 70 μM [S5],

more than five thousand times lower than the 0.375 M required to synthesise alanine by reductive amination under conditions otherwise simulating a deep-sea vent [43].

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