PREBIOTIC CHEMISTRY

Cyanide at the origin of metabolism

The emergence of protometabolic reactions that evolved into today's metabolic pathways is unclear. Now, evidence suggests that the chemical origin of biological carbon metabolism may have relied on the versatility of a single primitive C_1 feedstock molecule — hydrogen cyanide.

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Metabolism is the complex network of enzyme-catalysed reactions that synthesize and breakdown biomolecules. These multistep pathways can be sustained by the interconversions of the universal metabolites — acetate, pyruvate, succinate, α-ketoglutarate and oxaloacetate — produced by the tricarboxylic acid (TCA) cycle (also known as the citric acid cycle or the Krebs cycle). The oxidative TCA cycle is catabolic — that is, it breaks down metabolites into smaller units, in this case eventually to CO_2 (Figure 1a, clockwise). But it is the anabolic reverse TCA (rTCA) cycle (Figure 1b, counter-clockwise) — which reductively synthesises metabolites — that is the most appealing to those who believe that CO_2-fixing metabolic cycles were an inevitable consequence of geochemistry on early Earth.

At the heart of these proposals is that transition metals carried out the role of enzymes[1]. However, the continuous metal-mediated reduction of CO_2 — to produce the universal metabolites that organise into metabolic cycles — seems problematic because key feedstock molecules (such as pyruvate) are produced only transiently and in trace amounts[1,2]. The challenges stack up because further synthetic manipulations are needed to generate relatively complex biological molecules[3], particularly if we also insist that these multistep syntheses had to proceed (more or less) along similar lines as modern biosynthetic pathways[1,4].

Are there simpler alternatives to a chemical origin of life on early Earth? There are if we completely bypass the universal metabolites from the very beginning. Chemists have uncovered pathways that produce RNA, protein and phospholipid precursors that emanate from hydrogen cyanide (HCN) as an alternative C_1 feedstock molecule to CO_2 (refs. 3,5-7). Recently, direct links between cyanide and the universal metabolites have also begun to emerge[8-10]. Writing in *Nature Chemistry,* Ramanarayanan Krishnamurthy and colleagues now augment the role of cyanide at the origin of life with the proposal of a reductive glyoxylate pathway as a simpler precursor to the rTCA cycle[11].

The biological glyoxylate pathway (Figure 1a, red arrows) is a shortcut within the oxidative TCA cycle that evades the CO_2-generating steps [isocitrate (C_6)$\rightarrow$$\alpha$-ketoglutarate ($C_5$)$\rightarrow$succinate ($C_4$)]. The oxidative decarboxylations and α-ketoglutarate are bypassed through the splitting of isocitrate into succinate and glyoxylate. Succinate then continues in the cycle to

generate oxaloacetate, while glyoxylate is intercepted by acetyl-CoA to feed back into the cycle (after hydrolysis) as malate, which can also continue in the oxidative direction to produce more oxaloacetate. The net benefit of the glyoxylate pathway is that it is anabolic and generates larger, complex organics from acetate at a time when typical organic fuels (glucose, for example) are in short supply.

When run in reverse, a hypothetical reductive glyoxylate pathway is prebiotically appealing because it sidesteps the formidable reductive carboxylation steps of the rTCA cycle [succinate (C_4)→α-ketoglutarate (C_5)→isocitrate (C_6)] (ref. 11). Simply reversing the steps, however, immediately causes a problem because the key C–C bond-forming step to generate isocitrate is inhibited by the inaccessibility of the carbon anion of succinate for reaction with glyoxylate. Alternative ways to forge this bond are needed, and Krishnamurthy and colleagues eventually arrived at a solution by piecing together various observations made whilst evaluating cyanide as a prebiotic reductant in steps of the rTCA cycle[11].

Cyanide effectively reduced fumarate (to succinate) and oxaloacetate (to malate) under conditions where parasitic reductions[2] of pyruvate (to lactate) and α-ketoglutarate (to α-hydroxyglutarate) were not observed (Figure 1b). The reduction of oxaloacetate to malate is significant because cyanohydrin formation outcompeted the spontaneous decarboxylation of oxaloacetate to pyruvate. However, avoiding the intrinsic instability of oxaloacetate altogether would simplify matters greatly. The team get around any need for oxaloacetate by invoking reaction of malonate (an acetyl-CoA surrogate) with glyoxylate, the simplest α-oxocarboxylate available (more on where they come from later[9,10]). The product, 3-carboxymalate, was sequentially converted to malate and fumarate to complete the circumvention of oxaloacetate. The synthesis of isocitrate was found by identifying carboxysuccinate — the cyanide addition product of fumarate — as a more accessible carbon nucleophile capable of undergoing the key C–C bond forming step with glyoxylate that succinate could not. The carboxyisocitrate product was then decarboxylated to give isocitrate. This is a neat chemical solution to an ostensibly difficult abiotic reaction of succinate and glyoxylate, and this alternative pathway was made possible by cyanide.

Whilst each step is demonstrably feasible, whether all of them could organize into an abiotic cycle is a completely different matter[2]. Further innovations are needed to mitigate disruptive side reactions and to regulate each individual reaction for successive cycling. In the present case[11], the opportunity to complete the cycle was prevented because, after fragmentation of isocitrate to succinate and glyoxylate, the latter compound rapidly disproportionated to leave behind (what appeared to be) dead-end products, glycolate and oxalate (but see later[9,10]). Other steps called upon Zn^{2+} to accelerate reactions such as decarboxylation, and some dehydrations required wet-dry cycling. These steps are prime candidates for organocatalytic intervention in water, which is why RNA and peptide catalysis[7] should not be ruled out at the outset of central carbon metabolism. The progression from linear stepwise chemistry to (auto)catalytic metabolic cycles may have required

sophisticated solutions beyond the scope of prebiotic chemistry because there are some hefty demands that need to be met. Further experimentation can provide an answer to whether or not metabolic cycles that interconvert the universal metabolites are in fact just a biological phenomenon[2].

The reductive glyoxylate pathway (Figure 1b) dispenses with oxaloacetate and α-ketoglutarate, and yet these are major feedstocks for amino acid and nucleotide biosynthesis[1,3]. Glyoxylate and malonate are also major inputs. Are there prebiotic chemistries that could complement and replenish the reductive glyoxylate pathway? An affirmative answer can be traced back to cyanide yet again. A recent re-evaluation of the 'cyanosulfidic' network that provisions RNA, protein and phospholipid precursors, revealed that the corresponding nitrile hydrolysis products (lactate, malate, α-hydroxyglutarate and glycolate) underwent photochemical oxidation to pyruvate, oxaloacetate, α-ketoglutarate and glyoxylate (Figure 1c)[9]. For example, glycolate (the hydrolysis product of glycolonitrile, the first C_2 molecule from the photoredox chemistry of cyanide[5]) photooxidized to glyoxylate. Further (photo)chemistries generated acetate, citrate, succinate, isocitrate, malonate, and other useful metabolites[9]. Remarkably, all of the members of the biological (r)TCA, glyoxylate, and succinic semialdehyde pathways are accounted for by oxidative chemistry where simultaneous reductive chemistry is operating to generate RNA, protein, and phospholipid precursors[5].

In another study, the photochemical reduction of CO_2 by hydrated electrons generated glycolate and oxalate amongst a broad spectrum of organic species (Figure 1d)[10]. Besides the unprecedented quantities of CO_2 that gets 'fixed' under conditions that likely prevailed on rocky planets (such as early Earth and Mars), it was the products of the photochemically induced radical chemistry of glycolate — citrate, malate, succinate and tartrate — that stood out. So, what appeared to be dead-end or waste products of the reductive glyoxylate pathway[11] were readily interconverted to valuable metabolites and feedstock molecules capable of replenishing emerging and complementary prebiotic networks that may have been operating at the chemical origin of life on early Earth, and a single C_1 feedstock molecule may have started it all — hydrogen cyanide.

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Competing interests
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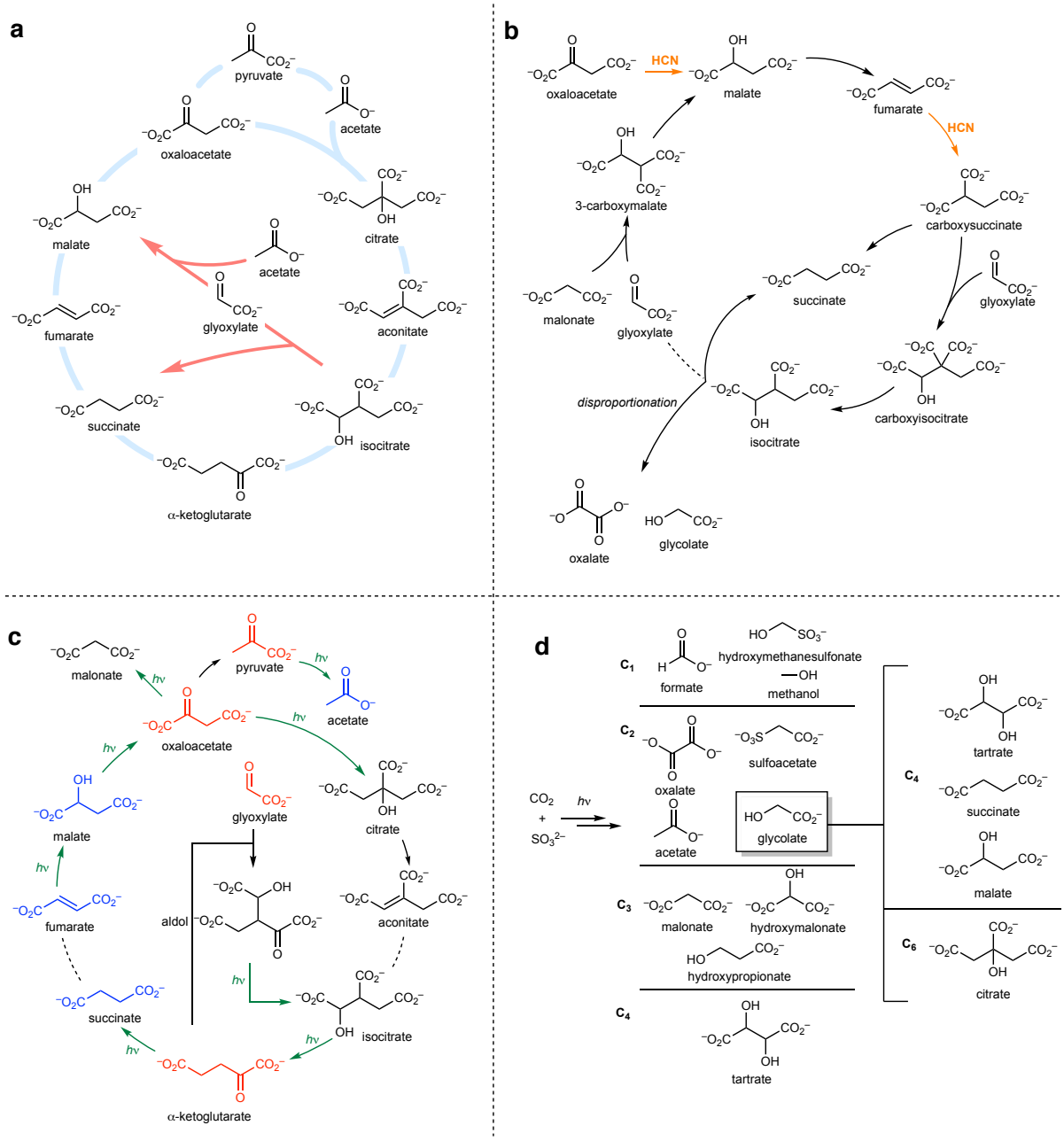

Figure 1: The centrality of cyanide in protometabolic reactions. **a.**The tricarboxylic acid cycle (blue lines) and the glyoxylate pathway (red arrows) operate clockwise in the oxidative mode. The reductive tricarboxylic acid cycle (rTCA) is essentially the reverse (counter-clockwise), and is autocatalytic because acetate generated from splitting citrate is sequentially converted to pyruvate then oxaloacetate by the epicycle[2]. Thioesters of coenzyme A (CoA) not shown. **b.** Cyanide-mediated reductions lead to a potential reductive glyoxylate pathway that bypasses oxaloacelate and α-ketoglutarate and the formidable reductive carboxylation steps of the rTCA cycle[11]. **c.** Cyanosulfidic origin of the TCA cycle, glyoxylate, and succinic semialdehyde pathways[9]. Red; oxidation products of the parent α-hydroxyacids (not shown: glycolate→glyoxylate; lactate→pyruvate; α-hydroxyglutarate→α-ketoglutarate). Blue; hydrolysis products of cyanosulfidic chemistry. Green arrows; photochemically-driven transformations. **d.** The photochemical reduction of carbon dioxide (CO_2) by hydrated electrons generated from sulfite

(SO_3^{2-}) produces a broad range of organic products including those that produce universal metabolites[10]. A key molecule is glycolate, which is also the hydrolysis product of glycolonitrile[5].