Protocells realise their potential

How the first metabolic network was organised to power a cell remains an enigma. Now, simple iron–sulfur peptides have been used to generate a pH-gradient across a protocell membrane by catalysing hydrogen peroxide reduction. This indicates that short peptides could have fulfilled the role of redox active metalloproteins in early life.

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Evolutionary biology suggests that the last universal common ancestor of life on Earth was a highly sophisticated cell that contained a suite of biochemical (transcriptional and translational) machinery to orchestrate its metabolism through sequence-specific nucleic acid coded protein synthesis. Although there is no accurate record of the composition of simpler organisms on Earth, the core of life’s metabolic network is extraordinarily similar across all extant organisms.¹ This may indicate metabolic organisation arose early during life’s evolution. Crucially, three common energy currencies are exploited by life; proton gradients, sodium gradients, and ATP (adenosine triphosphate).² These gradients drive essential anabolic reactions that replenish a cell’s nutrients, for example membrane-bound ATP synthase harnesses an electrochemical gradient to fuel cell survival and prevent the onset of equilibria. It is clear, however, that modern translation, transcription and ATP synthase did not all arise in a single chemical step at the onset of biology; some form of metabolism must have supported the earlier stages of evolution. So what did the first metabolic network look like? How was it organised? What were its constituent parts, and what dynamic interactions were essential among its constituents?
Extant metabolism is a complex protein-regulated network, and the plexus of modern metabolic enzymes is far too complex to have self-assembled de novo prior to genetically encoded protein synthesis. However, metabolism is organised into chains and cycles of redox reactions to capture energy. For example, electrons transferred from the breakdown of nutrients—the catabolic phase of metabolism (e.g. glycolysis)—are shuttled through protein-associated nucleotide and metal cofactors, leading to an ion gradient across a biological membrane. Detailed analysis of the enzymes involved in the citrate cycle and electron transport chains reveal that iron–sulfur proteins are critical to maintaining ion gradients across biological membranes. The iron–sulfur motifs embedded within these metalloproteins are so pervasive that they may be an ancient remnant of a by-gone biological (or prebiotic) era. But if the structural complexity of the genetically encoded protein framework that adorns the iron–sulfur cores were stripped away, would the residual structure still elicit the functional properties required to establish a proton gradient within a localised environment? If so, and if this core could self-assemble de novo, it would imply that life could (initially) dispense with the imperative for large polymeric (enzyme) catalysts that are difficult to construct, select, and copy, and alleviate the pressures placed upon sequence-specific polymers (e.g. proteins or RNAs) to fulfil phenotypic differentiation in protocells.

Writing in *Nature Catalysis*, Mansy and colleagues demonstrate that simple iron–sulfur peptides catalyse electron transfer reactions (Figure 1). Although simple aqueous ferric (Fe(III)) ions oxidise NADH, the resulting acidic solution destroys the NAD⁺ that forms. Increasing the pH would prevent NAD⁺ degradation, but this leads to precipitation of inactive oxo-hydroxo iron complexes. Building on their recent work in which UV-light was shown to promote iron–sulfur cluster formation starting from a range of short cysteine peptide ligands under anoxic conditions, the present study reveals that simple cysteine peptides stabilise and solubilise Fe(III) at higher pH whilst maintaining redox activity. Fe(III)-L-glutathione was observed to furnish the most effective iron–sulfur catalysts, clearly accelerating the oxidation of NADH with respect to the uncatalysed background reaction. The reduced iron–sulfur catalyst can then be oxidised to regenerate Fe(III) by a terminal electron acceptor, hydrogen peroxide, or an intermediate electron carrier, ubiquinone. When these iron–sulfur catalysts are encapsulated in POPC (model protocell) membranes with 0.5 mM NADH, stoichiometric hydrogen peroxide reduction establishes a trans-membrane pH-gradient (40 mV), with a similar magnitude to electrochemical gradients found across biological membranes.

Establishing a trans-membrane electrochemical gradient by membrane-localised redox catalysts lays the foundations to develop simple systems that exploit these gradients to drive anabolic reactions. However, maintaining an electrochemical gradient requires, in addition to a competent catalyst, feedstock reagents (nutrients) and a well-tuned semi-permeable membrane. Simpler (fatty acid) membranes could not harness this gradient, outlining the need to simultaneously develop dynamic interactions amongst the constituent molecules of life from the outset. Functions must be balanced, and sealing a membrane to an ion gradient could, in principle, block essential nutrients (or reagents) accessing the cell. A complex charged dinucleotide, such as NADH, might not be passively
transported across a charge-impermeable membrane. This opens up several interesting questions, the first of which is whether the current system could be rendered catalytic in (membrane impermeable) NADH/NAD⁺ by a second redox couple with other (proto)metabolic processes (Figure 1). For example, could iron–sulfur redox cycling be coupled to (prebiotic) triose glycolysis,⁵ and synthesis of phosphoenol pyruvate—nature’s highest energy phosphate—to provide a proto-metabolic link between redox and phosphorylation potential? The prebiotic plausibility of the essential peptide ligand also needs to be addressed. Although a convincing prebiotic cysteine synthesis has yet to be found,⁶,⁷ the remarkable value and reactivity of thiols and sulfides in prebiotic chemistry suggests an effective synthesis awaits elucidation.⁶,⁸ Furthermore, even if short peptides, rather than long polymers, are key to function during the transition from prebiotic chemistry to metabolism, an effective synthesis of (short) peptides that tolerates a high degree of side chain functionality is urgently needed. The low information content required to code and manipulate small (information-poor) catalysts make these the obvious target at the onset of metabolic organisation and only later, once life became dependent on such catalytic function, would competitive fine-tuning of catalytic function drive information-rich polymer selection. However, current methods for (short) prebiotic peptide synthesis remain ineffective for amino acids with functional side chains such as cysteine and serine, and the pronounced nucleophilicity of thiols renders cysteine incompatible with current methods for electrophilic activation of amino acids. Nevertheless, in a broader sense, the work of Bonfio et al.⁴ suggests that the activity of simple iron–sulfur peptides could have helped to couple catabolism with anabolism during early evolution, and exemplifies value accrued by unifying catalysis with compartmentalisation.

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[References]

Figure 1. **Prebiotic iron–sulfur peptides generate a pH gradient across vesicle membranes.** Fe(III)-peptides catalyse the oxidation of membrane-bound NADH. The resultant Fe(II)-peptide is oxidised back to the active ferric state by H$_2$O$_2$ (bold arrows). The net formation of hydroxide (OH$^-$) generates a pH gradient. Ideally, NADH would be regenerated by a stoichiometric prebiotic reductant supplied to the protocell in the form of a membrane-permeable nutrient (●). The resulting oxidation product (●) can feed into other anabolic pathways to provide the protocell with building blocks required, or eliminated from the protocell (dashed arrows). L = cysteinyln peptide ligand. n = 1-4.

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