

Inorganic Complexes Enabled the Onset of Life and Oxygenic Photosynthesis

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Abstract Mackinawite ($[\text{Fe} \gg \text{Ni}]_2\text{S}$), greigite ($\text{NiS}_2[\text{Fe}_4\text{S}_4]\text{S}_2\text{Fe}$) and a tunnel manganite (CaMn_4O_8) similar in structure to hollandite were minerals that enabled the onset of chemosynthesis and, later, of oxygenic photosynthesis – the two events to make the greatest impact at the surface of our planet. The inorganic complexes contributing to the growth of such minerals – ($[\text{FeS}_2\text{Fe}]_4\text{H}_2\text{O}$; $[\text{Fe}_4\text{S}_4]^{2+/1+}$; $[\text{Fe}_3\text{S}_4]^{+1/0}$; NiFe_5S_8 , CaMn_4O_8 as well as $\text{HP}_2\text{O}_7^{3-}$) – were later sequestered by small organic molecules (initially polypeptides or carboxylate groups) to become active centres of the enzyme precursors that initially catalyzed the primary reactions of energy conversion and nutrient cycling. Examples of such adventitious cooptions were to produce (i) pyrophosphate ‘eggs’ in successive main chain NH peptide nests; (ii) protoferredoxins as thiolated metal sulfide eggs in peptide nests; (iii) precursors to carbon monoxide dehydrogenase

(CODH)/acetyl CoA synthetase (ACS) as a Ni-peptide and a thiolated egg in a peptide nest and (iv) the precursor to the active centre of the OEC by periplasmic carboxylates and hydroxyls adjacent to RC II in a protocyanobacterium.

Keywords Greigite, hollandite, mackinawite, origin of life, oxygen evolving complex

Introduction

Autogenic life emerged as the most effective way to discharge geochemical energy on the early Earth. The steepest chemical and electrochemical gradients obtained where alkaline hydrothermal spring waters at $\leq 120^\circ\text{C}$, issued into a cool Hadean ocean. This is where CO_2 in the acidulous ocean reacted with hydrothermal hydrogen and ammonia in reactions catalysed by pyrophosphate and trace metal sulfides. The strong chemical and physical gradients focussed at the surfaces of the hydrothermal mound thereby drove the acetyl-CoA pathway into existence. Acetate (joined later by methane) was the effluent produced during biosynthesis. These first microbes were to evolve into the acetogenic

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bacteria and the methanoarchaea (Martin and Russell 2007).

Ocean floor spreading was eventually to convey the prokaryotes (now including secondary heterotrophs) that had expanded from the mound and occupied the ocean floor, to the photic zone where some bacteria evolved to exploit solar energy along the ocean shore. Here much of the protection from hard UV would have been provided initially by fine-grained manganite and manganate photoprecipitates. We investigate here how minerals and transition metal-ion clusters first set the scene for the onset of both chemosynthesis and oxygenic photosynthesis.

Mineral precipitates enabling life's emergence

Although a strong disequilibrium obtained between hydrothermal H_2 and the atmospheric CO_2 dissolved in the early ocean, activation energies and catalysis were required to conquer the kinetic barriers to reaction. A submarine mound produced at a hydrothermal alkaline Mg-bearing submarine spring presents itself as a potential reaction site. In part comprised of sulfides of iron and minor nickel and phosphates, the mound could act as a catalytic flow reactor, the margins of which supported strong redox and pH gradients (Russell and Hall 2006). These gradients supplied the activation energies required for reaction. Acetate or methane and other organic molecules were the products (Martin and Russell 2007). The 'ready-made' catalyst enabling the reactions were freshly precipitated mackinawite ($[Fe \gg Ni]S$) and greigite ($NiFe_5S_8$). Comprised of transition metals and sulfur—elements of variable valences—these minerals could transfer electrons, act as protohydrogenases, and catalyse reductions of carbon oxides to carboxylic and amino acids (Heinen and Lauwers 1996; Huber and Wächtershäuser 1997, 2003).

Low temperature pyrophosphates such as kanonerovite ($MnNa_3P_3O_{10} \cdot 12H_2O$) are more rare (Popova et al. 2002). However, pyrophosphate has bond energies similar to those in ATP and thus may have acted as a condensing agent in emerging

life. The pyrophosphate could have been produced both by the proton gradient acting across the freshly precipitated inorganic barrier at the interface between the acidulous ocean and the alkaline hydrothermal fluid (Russell and Hall 2006) as well as by the generation of acetate through the acetyl CoA pathway (Fuchs 1989; de Zwart et al. 2004).

Peptide takeover

While the iron-nickel sulfides and the pyrophosphates may have been moderate catalysts or condensing agents, they were variously prone to dissolution, hydration and oxidation. On the other hand, were the inorganic clusters of what otherwise went to make up the mineral forms of the phosphates and the iron sulfides, to have been sequestered via the partial positive charges of the NH groups of short peptides (cf. Huber and Wächtershäuser 1998), these would have then acted as uncoded proto-enzymes or coenzymes. Thus $[Fe_4S_4](RS)_4^{2-/3-}$ or $[Fe_3S_4](RS)_4^{3-/4-}$ peptide complexes may have acted as simple ferredoxins (Milner-White and Russell 2005) (Fig. 1a). A similar arrangement involving a thiolated cubane juxtaposed to a nickel-diamine of a peptide may have produced an uncoded precursor to a bifunctional CODH/ACS. And a nitrogenase may have developed in a comparable fashion (Table 1) (Russell and Hall 2006). That phosphate anions were ligated in such a manner (Fig. 1b) is suggested by the fact that to this day the commonest phosphate binding

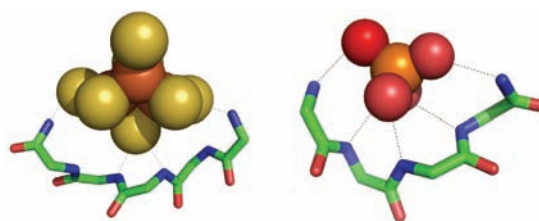


Fig. 1 Stick pictures showing early nests of short peptides produced by main chain atoms of polypeptides: (a) (left) $[Fe_3S_4](RS)_4^{3-/4-}$ bound to RLRLR nest (cf. a ferredoxin), (b) (right) phosphate bound to LRLR nest (cf. the P loop) (carbon, green; oxygen, red; iron, rust; nitrogen blue; phosphorus, orange; sulfur, yellow) (From Milner-White and Russell 2008)

Table 1 Suggested inorganic complexes and alternative pathways either to mineral structures or inorganic "ready-made" protoenzyme clusters and their likely first chelators

Mineral	Complex	Enzyme cluster	Chelator
Kanonerovite MnNa ₃ P ₃ O ₁₀ ·12H ₂ O	2HPO ₄ ²⁻ + H ⁺ → HP ₂ O ₇ ³⁻ + H ₂ O	Pyrophosphate HP ₂ O ₇ ³⁻	Successive main chain NH ⁺ peptide nests
Mackinawite (Fe>>Ni)S	[FeS ₂ Fe]4H ₂ O [FeS ₂ Ni]4H ₂ O	(Rieske protein) Hydrogenase e ⁻	Thiolated egg in peptide nests?
Greigite cuboidal moiety	2[FeS ₂ Fe] → [Fe ₄ S ₄] ²⁺ + 2e ⁻	Ferredoxins [Fe ₄ S ₄] ^{10/+} , [Fe ₃ S ₄] ⁺²⁺	Thiolated egg in peptide nests?
Greigite as NiS ₂ [Fe ₄ S ₄]S ₂ Fe	NiFe ₅ S ₈	CODH/ACS NiFe ₄ S ₅ and [Fe ₄ S ₄]cys-Ni-cys ₂ -Ni	Ni-peptide and thiolated egg in peptide nests
Greigite twin as [Fe ₄ S ₃]S ₄ [S ₃ Fe ₄]	[Fe ₄ S ₄] ^{2+/+} and MoS ₄ ²⁻	Nitrogenase [Fe ₄ S ₃]NS ₃ [S ₃ Fe ₃ Mo]	Peptide nests + carboxyl group?
Hollandite or tunnel manganite	Ba(Mn ⁴⁺ ,Mn ²⁺) ₈ O ₁₆ or CaMn ₄ O ₈	O ₂ evolving complex CaMn ₄ O ₅ (aa) ₈ ± 2H ₂ O	Periplasmic carboxyl groups

motif is the 'P' or phosphate loop comprising five or six amino acid residues that invariably include at least one glycine. This achiral amino acid lacks a carbon-containing side chain which gives it a wide range of bond angles and freedom in the articulation of the peptide chain.

Mineral precipitate enabling oxygenic photosynthesis

The process of oxygenic photosynthesis entails the extraction of four electrons and four protons from two water molecules through the activities of another metalloenzyme, the oxygen evolving complex (OEC) located in PS II. PS II feeds electrons to PS I. In strong contrast to the iron and nickel-iron-sulfide complexes that enabled the onset of life, at the heart of the OEC lies a calcium-manganese-oxide cluster. Below we explore how such a complex might have been first incorporated.

Allen (2005) has argued that evolution to cyanobacteria was via a proto-cyanobacterium that had the two reaction centres, RC I and RC II, either of which could function depending on the available substrates. RC I and RC II were to evolve into PS I and PS II. RC I is similar to the reaction centre housed in *Chlorobium* that extracts electrons linearly from hydrogen sulfide to energize biosynthesis, and operates at low reduction potential. RC II now occurs in *Rhodospseudomonas* spp. and

Chloroflexus spp. Allen (2005) suggests that RC II evolved from the ancestral complex RC I within a single anaerobic lineage to oxidize organic detritus at a higher redox potential as the H₂S supply occasionally waned. In times of extreme dearth, and if the potential were high enough, we imagine H₂O could have supplied electrons through RC II directly to RC I in place of CH₂O_{detritus} if the putative redox switch, which prevented both centres from operating at the same time, were to have been disabled by mutation (Allen 2005). However, for water to be oxidized by a modified RC II required a cooptable 'ready-made' manganese-bearing redox cluster. And for RC II to be transformed into PS II required such a redox catalyst to have a potential high enough to oxidize water.

In the anaerobic conditions obtaining on the early Earth micromolar concentrations of manganese would have been present in surface waters, wherefrom manganate and manganite minerals would have been precipitated on photooxidation. Anaerobic photosynthetic bacteria may have used these photolytic manganese oxide-bearing minerals (and their soluble clusters) as electron donors, acceptors and UV shields. We imagine hydrated manganese clusters within a shallow aqueous environment and adsorbed onto the cell wall of a photosynthetic bacterium, adventitiously losing electrons to RC II during dehydrogenation, while ferrous iron and hydrogen sulfide were oxidized. Before considering the likely makeup of the

original manganese cluster affecting the oxidation of water we need to understand the nature of the present-day active centre.

The metal oxide complex at the heart of the OEC comprises an isosceles triangle with calcium at the apex 3.4 Å from the two manganese atoms, themselves 2.8 Å apart at its base. One of these manganese lies ~2.7 Å from a third manganese which itself lies ~2.7 Å from a distal manganese (Yano et al. 2006). The OEC does not change topology on the loss of four electrons in the oxidation cycle. This open structure contrasts to the cubanes comprising the ferredoxins associated with CODH/ACS which only require the transfer of one or two electrons at a time before recharge. Recognising that a manganese-only OEC does not work, we consider structures comprising various manganese compounds or minerals (and thereby the likely mineral clusters) with comparable stoichiometries or at least formula that might inform us as to the nature and origin of the 'ready-made' active centre to the OEC.

Because of its somewhat similar stoichiometry to the OEC we originally considered a ranciéite cluster ($3\text{H}_2\text{O}\cdot\text{CaMn}_4^{\text{IV}}\text{O}_9$) to have been the likely precursor (Russell and Hall 2006). However, Sauer and Yachandra (2004) have shown that the layered conformation of ranciéite make this unlikely. Nevertheless it is instructive to know that, while the comparable all-manganese mineral birnessite ($3\text{H}_2\text{O}\cdot\text{Mn}^{\text{III}}\text{Mn}_4^{\text{IV}}\text{O}_9$) may be reduced to the cubane-bearing spinel mineral hausmannite ($\text{Mn}_2^{\text{II}}\text{O}_4[\text{Mn}_4^{\text{III}}\text{O}_4]$) – ranciéite cannot. This is paralleled by the fact that the would-be calcium spinel ($\text{Ca}_2\text{O}_4[\text{Mn}_4^{\text{III}}\text{O}_4]$) does not exist (Post 1999). We have argued that it is the relatively low ionic potential of calcium that disallows the formation of a CaMn_3O_4 cubane. This may be one explanation for why an all-manganese OEC does not function, for if a cubane were to form on reduction, the kinetic energy required to open it again would be too great to allow the speedy delivery of the four electrons required for biosynthesis during the Kok reaction cycle (cf. Lind 1988). Another implication is that models involving a cubane at the heart of OEC cannot be correct. Furthermore, Chen et al. (1995) demon-

strate that calcium encourages the photoassembly of $(\text{Mn})_4$ -clusters.

In their explanation for the origin of the OEC, Sauer and Yachandra (2004) note that the bond types and lengths of the manganese atoms in the tunnel mineral hollandite ($\text{Ba}(\text{Mn}^{4+}, \text{Mn}^{2+})_8\text{O}_{16}$) are directly comparable to those in the OEC (Table 1). We assume here, given the likely high calcium:barium ratios in littoral waters, that a calcium ion could take the place of barium in a hollandite structure to give a mixed valence cluster with the formula CaMn_4O_8 . In this vein we note that an open structure mixed valence tunnel calcium manganite (CaMn_4O_8) has recently been dry-synthesized (Table 1) (Barrier et al. 2005). Judging from the OEC's present placement, we suggest that such a tunnel manganite structure was first sequestered by periplasmic carboxylate and hydroxyl ions. Such a sequestered 'ready-made' cluster would have been available for cooption by Allen's protocyano bacterium, perhaps near subaerial sulfurous and manganiferous springs (Russell and Hall 2006) (Table 1). Once the genetic machinery evolved to place the CaMn_4 -complex reliably at the peripheral end of what became PS II, this part of the membrane was invaginated. Thus, although the OEC is presently situated in the lumen side of the thylakoid membrane, it is topologically still on the cell's exterior (Fig. 2).

In contrast to this thesis, Sauer (2007) now suggests that the development of oxygenic photosynthesis developed in steps, the first involving only two manganese atoms (initially as Mn^{III} and Mn^{2+}) that oxidized two water molecules to hydrogen peroxide, so releasing two protons and two electrons to RC II (cf Blankenship and Hartman 1998; Barynin et al. 2001). Catalase activity is held to have seen to the production of dioxygen, though ambient ferrous iron may have been the direct and initial recipient.

Conclusions

Without an inorganic start, life could not have come into being. And without a ready-made inorganic

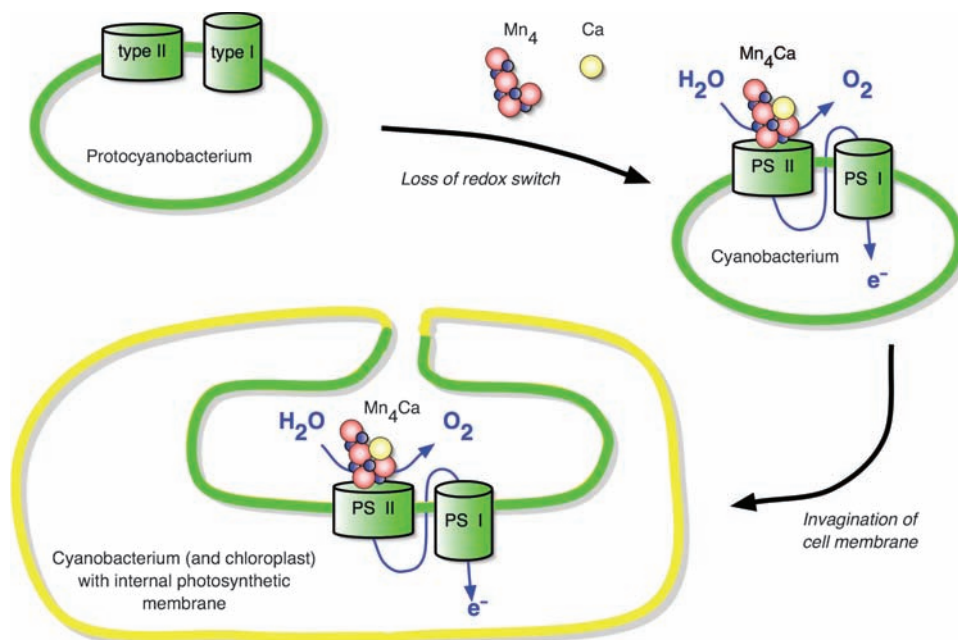


Fig. 2 Adventitious acquisition of a mineral, water-oxidising, Mn₄Ca catalyst at the external, donor side of a membrane-intrinsic Type II photosynthetic reaction centre; connection of type I and type II centres in oxygenic photosynthesis; and internalisation of the water-oxidising catalyst with the evolving topology of photosynthetic membranes

redox catalyst, oxygenic photosynthesis could not have emerged. Life is grounded in autotrophy and its chief representatives at the base of the food chain are the acetogenic bacteria, the methanarchaea and the oxygenic cyanobacteria. Enabling the emergence of these three stalwarts of life were variously pyrophosphate, nickel-iron sulfides and eventually calcium-manganese oxide photo-precipitates, as well as the clusters from which the minerals were built. In time, these same clusters were spontaneously coopted; the phosphates and the thiolated sulfides by the main chains of achiral peptides, whereas the open MnCaMn₃ oxide cluster was sequestered by extracellular carboxylates of side chains and C-termini of proteins.

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