THE CHEMICAL LOGIC OF A MINIMUM PROTOCELL

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Abstract. Traditional schemes for the origin of cellular life on earth generally suppose that the chance assembly of polymer synthesis systems was the initial event, followed by incorporation into a membrane-enclosed volume to form the earliest cells. Here we discuss an alternative system consisting of replicating membrane vesicles, which we define as minimum protocells. These consist of vesicular bilayer membranes that self-assemble from relatively rare organic amphiphiles present in the prebiotic environment. If some of the amphiphiles are primitive pigment molecules asymmetrically oriented in the bilayer, light energy can be captured in the form of electrochemical ion gradients. This energy could then be used to convert relatively common precursor molecules into membrane amphiphiles, thereby providing an initial photosynthetic growth process, as well as an appropriate microenvironment for incorporation and evolution of polymer synthesis systems.

We define biogenesis as that continuum of chemical processes which, acting under the constraints of thermodynamics, led from an essentially random mixture of inorganic and organic substances in the prebiotic environment to the first assemblies recognizable as living cells. Along this continuum there must have been a minimum protocell, which we define as an entity thermodynamically separated from the environment and able to replicate using available nutrient molecules and energy sources. Replication is defined as any energy-requiring growth process in which an organized assembly of molecules produces similar assemblies over time. We do not require sequence-mediated information transfer, nor a precise doubling of the assemblies. Finally, we limit our considerations to evolutionary processes involving relatively small molecules, as distinguished from macromolecular assemblies whose size would require a degree of structural specificity improbable in the earliest cells.

There is a certain generality in the preceding reasoning. In essence there are two major structural assembly processes employed by contemporary cells: the growth of specific polymers from monomers, and the growth of membrane bilayers from amphiphiles. It follows that biogenesis could have occurred either through a polymer-synthesizing replicating system that later became packaged in a boundary

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membrane, or a replicating membrane system that later acquired the ability to synthesize polymers. These two schemes can be summarized as follows:

(I) Small molecules → macromolecules → directed synthetic system → protocell → prokaryote

(II) Amphiphiles → protocell → directed synthetic system → prokaryote

Much of the literature on biogenesis has dealt with the former scheme; we suggest here that the latter scheme offers useful insights and new experimental approaches. In particular, we note that there are fewer specificity requirements at the early stages of the second scheme, because thermodynamic phases are much less restricted in this regard than stereospecific macromolecular structures. Even today cell membranes are the least specific of all organelle components in terms of the amphiphiles that can participate in their formation.

In principle the logic of replication could be separated from the chemical constraints, but we are interested only in those logical steps for which a possible chemical embodiment exists. In order to constitute biogenesis as a physical, in contrast to a historical science (see Smith and Morowitz, 1982) we will limit the logical steps to those which can take place using chemical and physical processes that are accessible in the laboratory. The logical minimum protocell that we construct will therefore be subject to experimental demonstration or falsification.

We shall place one further constraint on a theoretical model, a restricted statement of the principle of continuity: *from any stage in biogenesis, a continuous series of plausible transitions must lead backward in time to the non-living geochemistry of the planet, and forward in time to biochemically conventional cellular life.* This is designed to deal with the problems of biogenesis using the same epistemological considerations that have been so successful in normative science. It does not disallow discontinuities, but puts the burden of proof on any theory that requires discontinuities. The principle of continuity is for biogenesis a statement of the metaphysical requirement for simplicity (Margenau 1977) which was early recognized as a guiding motif in research. It appeared first as Occam's razor: 'Non sunt entia multiplicanda practer necessitate,' Occam's words appear to have been ignored in many treatises on the origin of life.

Within this framework, early replication may be represented in an overall way by the following:

$$\text{Protocell} + \text{nutrients} + \text{energy} \rightarrow \text{protocells} + \text{waste products} + \text{heat}.$$

The existence of a protocell requires a phase separation from the environment. A heterogeneous coacervate droplet of the type suggested by Oparin (1928) was an early attempt to define such a phase separation. However, the coacervate concept developed within a paradigm of colloidal protoplasm, while we now know that contemporary cells are primarily organized by membranous compartments. The coacervate is therefore rejected by the principle of contuity. Similarly, we exclude proteinoid microspheres as protocells (Fox, 1965) in that they lack the universal barrier of a liquid crystalline, non-polar phase present in all contemporary cells.

The simplest protocell which fulfulls the principle of continuity is a bilayer vesicle made from a single species or mixture of small amphiphiles. If the membrane is relatively impermeable to certain solutes, it gives rise to a three phase system which is defined by an aqueous internal volume, the membrane phase and the aqueous external environment. If one phase represents a restricted microenvironment with respect to the total system, some of the reactions occurring therein can be thermodynamically improbable in an equilibrium system, but given an appropriate pathway can be driven energetically uphill by free energy available in the total system. Consider a protocell composed of a vesicular membrane. The external environment is a source of free energy and nutrients, while the internal volume provides a closed microenvironment in which directed chemical reactions can occur. Our primary aim here is to argue that this arrangement represents a plausible protocell which arises spontaneously from known chemical and physical principles, and is accessible in the laboratory. (See also Koch, 1985.)

We can now be more explicit in outlining the primary assumptions about a protocell whose logic arises from the principle of continuity. They are as follows:

(1) The most plausible structural components are amphiphilic compounds, and growth occurs by chemical transformation of nutrient precursors to amphiphiles.

(2) Light transduction by a primitive pigment system provides energy to drive the chemical transitions involved in growth of the protocell, and chemiosmotic proton gradients are a likely form of primary energy storage across the membrane (see Deamer, 1978, and Baltscheffsky, 1981, for reviews).

(3) Phosphate is the most probable chemical group to mediate the transition between light trapping and chemical changes in nutrient molecules that lead to growth (see Westheimer, 1987 for review). In order to activate phosphate so that it can undergo transfer reactions, we will assume that pyrophosphate is an intermediate.

One further feature is that the protocell interior is a chemical reactor where energy is constantly supplied as high energy phosphate bonds and presumably withdrawn as thermal energy. The internal chemical networks are organized both by the energy flow and by the fact that there is a single chemical form to the energy input, represented by the difference between the Gibbs free energy of pyrophosphate compared to two orthophosphates. Since metabolism universally involves a chemistry driven by phosphate group transfer, a continuous series of processes is provided from primordial chemistry to contemporary biochemistry.

We can now elaborate on these basic assumptions. The reaction product necessary for the growth of a system described in equation 1 is a bilayer-forming amphiphile. The chemical logic of equation 1 can be summarized as follows:

> Protocell + amphiphile precursors + chromophore + converter 1 + converter 2 + energy → protocells + waste products + heat.

The process described above can be decomposed into the following steps for a light-driven system:

(A) Energy input (photons) + chromophore → excited chromophore
(B) Excited chromophore + converter → electrochemically stored energy
(C) Electrochemically stored energy + converter 2 + low energy compound → high energy compounds
(D) High energy compounds + membrane precursors → membrane amphiphiles + reaction products.

The 'nutrients' for the above reactions consist of membrane precursers and low energy compounds. It is assumed that a chromophore is available in the environment, and partitions into the membrane phase. Otherwise the growth of the system would rapidly dilute the available chromophore. In a more complex system, one may imagine that the chromophore is synthesized together with the amphiphile from precursors (Heinz and Ried, 1981).

The initial protocell is a vesicle self-assembled as a bimolecular leaflet of amphiphiles and chromophores. The presence of a chromophore permits energy conversion within the lipid phase of the membrane. The low energy compounds are derived from the external environment by selective transport across the membrane, and high energy compounds are produced through photochemically coupled reactions in the internal volume. The reaction products are amphiphilic molecules that would normally be rare in the environment, but are concentrated in the membrane microenvironment through energy conversion processes. As the amphiphile is synthesized, it becomes incorporated into the existing membrane, and the area of membrane increases. At some point, the size of the membranous vesicle forming the protocell increases to the point that stabilizing forces are no longer able to maintain integrity, and the vesicle breaks down into two or more smaller vesicles (Rashevsky, 1938). This step represents replication of the protocell, and requires only that the smaller vesicles have a lower Gibbs free energy than the initial larger vesicle.

The logic outlined above is general, and follows from the principle of continuity. Attempts to impose specific chemical embodiments on the logic must necessarily be more speculative, but are essential in directing experimentation. We will first assume that randomly synthesized chromophores dissolve in the membrane in a radially asymmetric way. Although at first examination an asymmetry would seem thermodynamically implausible, in fact the difference in radius of curvature between the inner and outer halves of the bilayer imposes a steric asymmetry on vesicles of sufficiently small size. Any difference in the internal and external concentrations of ionic solutes would also impose an asymmetry by inducing differences in the electrical properties of the membrane surfaces, even though the inner and outer lipid leaflets are chemically identical.

An asymmetric distribution of chromophores permits vectorial translocation of charge, which in turn can be used as an energy source to drive thermodynamically unfavorable reactions that store the energy in chemical form. The details of how such a membrane can absorb energy and generate a transmembrane potential have been described earlier (Morowitz, 1981). We assume that chemiosmotic energy in the form of a transmembrane proton gradient represents a plausible intermediate between

light energy and chemical energy (Koch, 1985). This energy source, coupled to formation of pyrophosphate bonds, would provide a primitive converter system. The thermodynamic plausibility of generating high energy phosphate bonds has also been discussed (Morowitz, 1978).

Proton gradients are plausible energetic intermediates for two reasons. First, protons are ubiquitous in aqueous solutions, and can be produced by numerous chemical reactions, including some driven by light energy. Second, protons have been demonstrated to have specialized conductive pathways in bilayer membranes which permit a very high permeability even in the absence of specific channels (Nichols and Deamer, 1980). Conductive specificity for protons can be provided by continuous chains of hydrogen bonds existing in the membrane as a peptide strand (Nagle and Morowitz, 1978) or associated water (Nichols and Deamer, 1980; Deamer and Bramhall, 1986). As a result, proton gradients are able to generate membrane potentials (Biegel and Gould, 1981; Deamer and Nichols, 1983; Cafiso and Hubbell, 1983) and it is likely that such potentials would be available in the postulated protocell. Step B above can thus be considered to result from the interaction between an excited chromophore and a proton conductance which together produce a transmembrane chemiosmotic potential of protons.

To introduce a chemical embodiment of the vesicle, consider a mixture of alkane derivatives such as the mixtures of long chain alcohols and alkyl phosphates described by Hargreaves and Deamer (1978). This vesicle population will grow if a reaction is available that produces a membranogenic species (alkyl phosphate) from a membrane precursor (the long chain alcohol) which is available in the environment. The alcohol would then be a nutrient which partitions into the membrane phase, where some of it is converted to the phosphate ester:

$$\text{ROH} + \text{pyrophosphate} \rightarrow \text{R-PO4} + \text{Pi}.$$

The newly synthesized amphiphile would generate increased membrane area, the overall process being summarized as:

$$\text{Vesicle} + \text{fatty alcohol} + \text{orthophosphate} + \text{converter} + \text{ch romophores} \rightarrow \text{alkyl phosphate (larger vesicle)}.$$

In summary, we suggest the following logic for a minimum protocell:

(1) The initial protocell consisted of a membranous vesicle formed from a relatively rare amphiphilic material available in the prebiotic environment, together with an ubiquitous chromophore that could partition into the membrane phase.

(2) A reaction exists by which a potential amphiphile (nutrient) common in the environment could be converted into amphiphile.

(3) That reaction can be driven by a photochemical process involving the chromophore and phosphate.

(4) As a result, increased amounts of amphiphile are synthesized, and these cause growth of the membrane vesicle through addition to the existing membrane structure.

(5) At some point the vesicle reaches an unstable size, and breaks up into two or more smaller vesicles which continue the process by taking in additional nutrients and chromophore from the environment.

The logic outlined here defines several important questions, and suggests useful experimental approaches:

(1) What mechanisms are available for primitive chromophores to generate a transmembrane proton gradient? Chemiosmotic transmembrane proton gradients are plausible intermediates between light energy and chemical energy. There are several possible chromophores which can be tested for their ability to accept light energy and transduce it into a form capable of activating a converter molecule. These include the fluorescent pteridine-like and flavin-like pigments produced thermochemically from certain amino acids (Heinz *et al.*, 1979; Heinz and Ried, 1981) the phorphyrins studied by Mercer-Smith and Mauzerall (1984) and the porphyrin – carotene system reported by Seta *et al.* (1985). The fluorescent polycyclic aromatic compounds that are major constituents of the organic components of carbonaceous chondrites represent another possibility (Basile *et al.*, 1984). Meteoritic organic amphiphiles also have the capacity to self-assemble into membranous structures, and are candidates for the initial self-assembling membrane system described here (Deamer, 1985).

(2) What reactions are available for pyrophosphate synthesis under prebiotic conditions? Heat energy can be stored as chemical energy in pyrophosphate, which can be produced simply by heating/dehydration of phosphate, but it would be desirable to demonstrate a reaction occurring in soution that involves light energy and membrane-bound chromophores. An alternative to pyrophosphate for such a system comes from recent work of Saygin (1981) on nonenzymatic photophosphorylation with visible light, in which chemical energy is stored as carbamylphosphate.

(3) What reaction mechanisms are possible for phosphorylation of amphiphiles? Plausible nutrients (amphiphile precursors) include long chain alcohols. Phosphate and sulfate derivatives of such alcohols have been shown to form stable membranes, and further experimentation should be directed toward group transfer processes which would permit the formation of amphiphiles from precursor molecules.

(4) What transport processes are available for nutrient translocation across bilayer membranes? Amphiphiles readily penetrate membrane barriers, and could enter a protocell simply by partitioning into the membrane phase. However, in the scheme proposed here, phosphate also participates in amphiphile formation, and bilayer membranes are relatively impermeable to small ionic compounds like phosphate. Therefore it would be desirable to establish a transport mechanism by which phosphate could be concentrated in the protocell. It is interesting to note in this regard that phosphate is a weak acid, and like other weak acids presumably will accumulate in interior volumes which are basic with respect to the external environment. If in fact transmembrane proton gradients were established in protocells, as postulated above, and had the same vector as in contemporary

prokaryotes (active transport outward, producing an alkaline interior) phosphate would be concentrated in the interiors with some degree of specificity (see also Deamer and Oro, 1980). The efficacy of this process can be tested in a model membrane system such as liposomes.

In conclusion, we have shown here how bioenergetic principles can be used to define a minimum protocell that could have embodied energy transduction, growth and replication. Given such a microenvironment, it is less difficult to imagine how conventional modes of information transfer, enzymatic catalysis and metabolism might be incorporated at a later evolutionary stage. It is clear that bioenergetic analysis of biogenesis substantially reorders the timing of various processes leading to the origin of cellular life forms, and we consider this changed temporal perspective to be an important heuristic feature of our model.

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