

Two-dimensional life?

(origin of life/surface chemistry)

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Contributed by Christian de Duve and Stanley L. Miller, August 15, 1991

ABSTRACT A model [Wächtershäuser, G. (1988) *Microbiol. Rev.* 52, 452–484], according to which life started in the form of a monomolecular layer of interacting anionic metabolites electrostatically bound to a positively charged surface, is examined critically. The model raises a number of thermodynamic and kinetic difficulties.

Wächtershäuser (1) has proposed that life started in the form of a two-dimensional organism made of a single molecular layer developing at the interface between a positively charged mineral surface—of pyrite, for example—and hot water. This proposal, which has been further elaborated in several recent papers (2–4), has attracted considerable interest. It has been publicized by science writers (5, 6) and endorsed in a recent book (7), but its substance has not yet been subjected to critical scrutiny. In our opinion, it raises a number of questions that need to be addressed. It is the purpose of this brief article to initiate this discussion.

Prebiotic Building Blocks

Wächtershäuser opens his paper with an outright rejection of a primeval soup stocked with organic compounds produced by abiotic syntheses. “I deny,” he states (ref. 1, p. 453), “the preexistence of any arsenal of organic building blocks for life (such as amino acids).” This declaration ignores the numerous experiments that have shown the spontaneous formation of amino acids and other small organic molecules under simple, geochemically plausible conditions (8). However, these conditions include a sufficiently reducing atmosphere, which means the presence of substantial amounts of methane or of hydrogen in the case of a CO₂ or CO atmosphere. The likelihood of such reducing atmospheres occurring on the prebiotic Earth is a separate issue, but there can be no doubt that organic compounds will be synthesized under sufficiently reducing conditions. Most researchers would agree that the correlation between the molecules that can be made easily under prebiotic conditions and the components of biological macromolecules is too striking to be dismissed summarily.

A good case for prebiotic synthesis can be made on the basis of the detection in the Murchison and other meteorites of amino acid mixtures similar, both qualitatively and in quantitative proportions, to the amino acid mixtures obtained in simulation experiments (8, 9). These findings are viewed by many as a strong presumptive indication that similar materials may have formed on the prebiotic Earth or been brought to it by meteorites or cometary dust. In any case, they make it clear that conditions favorable to the synthesis of such substances must have existed on the parent body of the meteorites (presumably an asteroid). This makes plausi-

ble, but does not prove, the occurrence of similar conditions on the prebiotic Earth.

Recently, a possible abundant source of prebiotic hydrogen has been identified in the photooxidation of ferrous iron by ultraviolet irradiation at neutral pH, producing H₂ (10, 11). Another possibility, suggested by Wächtershäuser (2), also involving ferrous iron would be the oxidation of H₂S to disulfide and hydrogen, driven by the trapping of the generated disulfide by Fe²⁺ ions as insoluble FeS₂ (pyrite). Evidence that such a reaction can take place has been obtained (4), but the efficiency of the process seems low. In contrast, the UV-supported photooxidation of ferrous iron occurs with a quantum yield of at least 30%.

Wächtershäuser's rejection of “any arsenal of organic building blocks for life” is all the more surprising because many of the products of simulation experiments are carboxylic acids capable of anionically bonding to a positively charged surface, as required by his model. They also represent excellent starting points for the unfolding of a metabolic network, as has been pointed out by a number of workers (for literature, see ref. 12). In contrast, Wächtershäuser offers no explanation for the formation of the primary building blocks of his own model, beyond the possible reduction of CO₂ (CO) by the pyrite-generating oxidation of H₂S. “As a point of departure,” he writes (ref. 1, p. 470), “we assume that, in an early stage of surface-metabolic evolution, surface-bonded C₃ units (notably phospho-trioses) arise by an unknown carbon fixation mechanism through thio acid activation.” One would have liked a suggestion as to a mechanism, since it is considerably more difficult to synthesize triose phosphates than amino acids under plausible prebiotic conditions.

The Primeval Soup

In relation to a primeval soup, Wächtershäuser writes (ref. 1, p. 453): “The prebiotic broth theory has received devastating criticism for being logically paradoxical, incompatible with thermodynamics, chemically and geochemically implausible, discontinuous with biology and biochemistry, and experimentally refuted.” This categorical condemnation of a primeval soup is unfortunate. It does, however, raise a problem common to all theories that postulate an aqueous medium as the cradle for life: How did the primary biogenic reactants and, especially, their sequential products and products' products reach concentrations high enough to allow them to interact efficiently?

There is no evidence about the organic compound concentration in the oceans of the prebiotic Earth. It was pointed out by Urey (13) that if all the carbon on the Earth (3000 g·cm⁻²) were converted to organic compounds and dissolved into oceans of the present size (300 liters·cm⁻²), this would result in a 1% solution of organic compounds. Another calculation of the amino acid concentration based on production rates of HCN and aldehydes in relatively reducing atmospheres gives a value of 0.3 mM (14). Less reducing atmospheres would give lower values.

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The term "primitive ocean," which has long been used for convenience, refers not only to the ocean itself but to the shores, oceanic sediments, fresh water lakes, etc. Thus, there would be many areas where organic compounds from the ocean could be concentrated, with the sea shores and the lagoons being the most important. On drying out of sea water, the dissolved organic compounds are concentrated until they reach saturation. Thus, concentrated solutions of organic compounds could have been produced in many places on the primitive Earth, albeit in small quantities.

Admittedly, there remains the problem of the concentrations of intermediates in multistep synthetic processes, such as would be required for the spontaneous emergence of a proteinless "RNA world" (15). Even in the most contrived laboratory conditions, such processes inevitably would be thwarted by side reactions and unfavorable yields. Unless stringently channeled and contained, an aqueous protometabolism is bound to die of dilution. In living organisms, this problem is solved by the intervention of enzymes, which selectively bind reactants in close proximity one to another and guide the interactions among these reactants along specific pathways. This consideration has prompted one of us to suggest that prebiotic metabolism soon became channeled by multimeric catalysts assembled from the thioesters of primitive amino and other difunctional acids (12). A number of other ways out of the concentration quandary have been offered, including adsorption on surfaces and the participation of mineral catalysts (for literature, see ref. 12).

Wächtershäuser's solution to the problem is drastic: Nothing happens in the water phase, which is no more than a provider of inorganic nutrients for the two-dimensional surface organism and a sink for detached products of decay. These, once they are detached, are considered irretrievably lost. The organism grows and diversifies exclusively as a single molecular layer until lipid monolayers are formed that can join into bilayers and allow cellularization. This is the keystone of the model. It merits particular scrutiny.

Two-Dimensional Metabolism

According to Wächtershäuser, the primitive, two-dimensional protometabolism followed pathways very similar to those of present-day metabolism. In support of such a relationship, he cites the fact that many intermediary metabolites are anionic molecules capable of electrostatically bonding to a positively charged surface. In a similar vein, he believes that phosphate owes its major role in metabolism to its having first served as a handle in anionic bonding, rather than, as previously suggested (16, 17), as a means of preventing leakage of metabolites out of the cell.

The view that contemporary metabolic pathways derive directly from similar protometabolic pathways agrees with the model of a catalyst-guided protometabolism (12). Contrary to the latter model, however, the pathways of Wächtershäuser's model are considered entirely determined by the peculiarities and opportunities provided by anionic bonding; they end up selecting their catalysts, rather than the opposite.

Wächtershäuser has proposed a series of prebiotic syntheses that might occur on surfaces, but offers no evidence that his schemes are mechanistically plausible, or even possible. One example is the formation of thiamine pyrophosphate, which is proposed to have been synthesized from a C₅ unit, the thioamide of 3-phosphoglyceric acid, glyceraldehyde 3-phosphate, and the pyrophosphate of the thiamine pyrimidine (4-amino-5-hydroxymethyl-2-methylpyrimidine). He also has schemes for the synthesis of NAD⁺, farnesyl pyrophosphate, coenzyme A, purine ribosides, histidine, phenylalanine, tyrosine, and a number of other amino acids. In addition, there is considerable speculation about the genetic code and codon replacements based on metabolic relation-

ships. In these schemes, most of the postulated reactions involve complex and unstable precursors whose origin is neither specified nor reconciled with the author's denial of the "preexistence of any arsenal of building blocks." Furthermore, the chemistry itself hardly looks plausible within the framework of aqueous-solution organic reactions. Surfaces may alter the chemistry, but nothing that we know about surfaces suggests that they have the extraordinary organizing powers attributed to them in Wächtershäuser's theory.

Wächtershäuser agrees with those who believe that RNA molecules cannot have arisen spontaneously in the prebiotic world (18). His proposal is that RNA was preceded by a molecule he calls tribonucleic acid (TNA). In this structure, the bases are attached by pseudo-glycosidic bonds to a backbone of triose phosphates joined by hemiacetal linkages and stabilized by binding of their phosphate groups to the positively charged surface (Fig. 1). The equilibria for the formation of hemiacetal polymers are not particularly favorable in aqueous solution, but the surface may change this. However, this raises its own set of problems. If polymer formation is favorable and rapid on the surface, then, by the principle of microscopic reversibility, the decomposition will also be rapid enough to "dissolve away" any genetic information in the polymer. Thus, there needs to be some way to stabilize the TNA kinetically as well as thermodynamically after it has been formed.

Even if the processes envisaged by Wächtershäuser should prove mechanistically feasible, their occurrence under primitive Earth conditions raises two additional problems—one kinetic, the other thermodynamic.

Kinetic Aspects

According to Wächtershäuser's model, metabolites are bound more or less strongly to the pyrite surface by purely electrostatic interactions and with little or no specificity. The model relies solely on thermal agitation and on chance molecular impacts to bring two neighboring molecules in some reciprocal configuration that allows them to join or to exchange groupings or to undergo some other interaction. This is random chemistry in two dimensions, distinctly more constrained than random chemistry in three dimensions but essentially still random in nature, very far from the kind of specifically oriented binding of substrates within the sphere of action of a catalytic center, as is accomplished by enzymes. It is difficult to visualize the development, even in two dimensions, of a complex metabolic network such as is proposed in the model without more diverse and catalytically active binding surfaces than those provided by a simple, charged plate.

The author does bring in catalytic help in the form of anionically bonded molecules that serve catalytically in some reactions—for example, as electron or group carriers—and at the same time participate in a reaction cycle involved in their own production. These molecules are seen as precursors of the principal coenzymes, and their autocatalytic reproduction is pictured as the first form of hereditary memory.

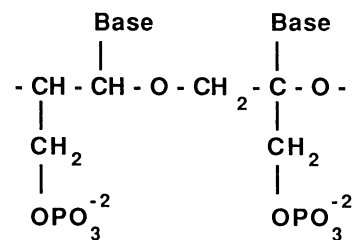


FIG. 1. Backbone of "tribonucleic acid" (TNA) according to ref. 1.

According to the author, the emergence of novel autocatalytic cycles represents the main driving force of diversification. As already mentioned, these abstractions are fleshed out by a number of actual reaction schemes, which, however, are entirely hypothetical and highly complex chemically. Whether they could ever be realized solely with the help of enforced proximity is a debatable point.

Thermodynamic Aspects

Wächtershäuser's model is even weaker on thermodynamic grounds. It offers only two sources of free energy to drive the metabolism of the surface biont: sulfide oxidation and anionic bonding. The first source serves simply to supply appropriate reducing equivalents for some primary reductions, such as that of CO₂ to formic acid. Because of the lack of a coupling system, the free energy of sulfide oxidation cannot be used to drive other processes, such as assembly reactions.

This leaves only the strength of anionic bonding as the driving force. In the case of a polymerization reaction, for example, this condition means that adsorption at each step should be able to overcome the free-energy barrier that opposes elongation. The following calculation shows this to be unlikely.

Consider monomer M and its oligomers M_n. M can be an amino acid, nucleotide, or other suitable monomer. Let ΔG_n be the Gibbs free energy of addition of a monomer to an oligomer M_n in aqueous solution:

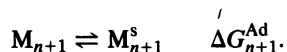
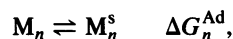


and let us assume that ΔG_n is independent of n. ΔG_n is about +5 kcal·mol⁻¹ unfavorable for both amino acids and nucleotides.

Consider the reaction on the surface:



where the superscript s refers to the surface-adsorbed molecule. Writing the equations for the adsorptions gives:



It follows that:

$$\Delta G_n^s = \Delta G_n - \Delta G_1^{Ad} + \Delta G_{n+1}^{Ad} - \Delta G_n^{Ad}.$$

For the polymer chain growth to be favorable, the ΔG₁^{Ad} should be small, and the increment in adsorption free energy of the n + 1 mer over the n mer must be greater than the ΔG_n of peptide or internucleotide phosphodiester bond formation in aqueous solution. With ΔG_n equal to +5 kcal·mol⁻¹, this means a difference between ΔG_n^{Ad} and ΔG_{n+1}^{Ad} of the order of 5 kcal·mol⁻¹. Oligomers of 20 units would have a ΔG_n^{Ad} of 100 kcal/mol or an equilibrium constant of 10⁺⁷³ for adsorption. This is an unlikely value. It is recognized that large molecules bind more strongly than small molecules, but incremental binding free energies for the n + 1 mer of 5 kcal·mol⁻¹ do not seem reasonable.

Irrespective of actual quantitative values, one does not see what could prevent the organism, assuming it ever came into being, from evolving into a dead end, in which the surface is covered by molecules so tightly bonded that they cannot be further displaced. The model does not provide for the con-

tinuing free-energy flux that is indispensable for growth and development of the system, or even for its maintenance.

Philosophical Aspects

In a number of places (ref. 1, pp. 453, 459, 460, 473, and 480), Wächtershäuser invokes conformity with the philosophy of Karl Popper as an argument in support of the validity of his model. However, philosophical conformity does not constitute a scientific reason for anything. Surely a theory is not automatically right for being formulated in a manner that satisfies given theoretical principles, however pertinent these may be.

The author also accuses certain current philosophical trends of having hindered research into the evolution of metabolism. He does so in no uncertain words (ref. 1, p. 453): "That the stark peculiarity of these [biochemical] pathways has resisted evolutionary explanation for so long is due, I believe, to our entanglement in three of the most vicious snares of science: inductivism, reductionism, and determinism." It may be worth pointing out that the great advances of biochemistry, cell biology, and molecular biology have been accomplished in spite of these "vicious snares."

Unfortunately, Wächtershäuser is somewhat ambiguous regarding the philosophical features of his own model. Some of his comments suggest a highly deterministic sequence of events, similar to that postulated in ref. 12. For example, he writes (ref. 1, p. 457): "The preexistence of such autocatalytic cycles is dependent on special chemical constellations, and it is this paucity of possibilities for surface-bonded autocatalysis which establishes the compositional uniformity, uniqueness, and identity of the surface metabolists." Environmental conditions being right, he expects the surface bionts to arise reproducibly, even today (ref. 1, p. 480). His references to "virtual pathways" as somehow guiding chemical evolution are also suggestive (ref. 1, pp. 460 and 473). On the other hand, the evolution of his system is also depicted as open-ended, exploratory, opportunistic, awaiting the chance emergence of a novelty that becomes locked into place by its autocatalytic character, somewhat in the manner of Prigogine's "dissipative structures" (19). Witness the following quotation from Wächtershäuser's paper (ref. 1, p. 457): "metabolic novelties . . . arise after a more or less protracted induction period as sudden saltations from one unique and distinct stage of the surface metabolist to another."

Conclusion

Wächtershäuser's theory is imaginative and original, but none of it is plausible in the framework of aqueous solution chemistry. If things are different on surfaces, then this should be demonstrated experimentally. From the theoretical point of view, the model seems to us to have several serious flaws of mechanistic, kinetic, and, especially, thermodynamic nature. The essential point is whether the proposed chemistry can work at all, especially under geological conditions. In our opinion, surfaces are not likely to have the properties that Wächtershäuser assumes in order to solve the problems of the origins of life. Striking results might force us to change our opinion. The time is now ripe for the demonstration of one or two of the many provocative surface-catalyzed chemical syntheses proposed by Wächtershäuser.

We wish to thank Neil Patterson, Sherri Foster, and Morgan Ryan for their useful editorial comments and suggestions. This work has been partly supported by National Aeronautics and Space Administration Grant NAGW 20 (to S.L.M.).

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