Self-organizing biochemical cycles

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I examine the plausibility of theories that postulate the development of complex chemical organization without requiring the replication of genetic polymers such as RNA. One conclusion is that theories that involve the organization of complex, small-molecule metabolic cycles such as the reductive citric acid cycle on mineral surfaces make unreasonable assumptions about the catalytic properties of minerals and the ability of minerals to organize sequences of disparate reactions. Another conclusion is that data in the Beilstein *Handbook of Organic Chemistry* that have been claimed to support the hypothesis that the reductive citric acid cycle originated as a self-organized cycle can more plausibly be interpreted in a different way.

metabolic cycles | reductive citric acid cycle | Beilstein

The nature of the first genetic material and the prebiotic chemistry that permitted its emergence are central themes in discussions of the origins of life. The discovery of ribozymes and the consequent rather general acceptance of the RNA world hypothesis (1) pose an inescapable question: How were ribonucleotides first formed on the primitive earth? This is a very difficult problem. Stanley Miller's synthesis of the amino acids by sparking a reducing atmosphere (2) was the paradigm for prebiotic synthesis for many years, so at first, it was natural to suppose that similar methods would meet with equal success in the nucleotide field. However, nucleotides are intrinsically more complicated than amino acids, and it is by no means obvious that they can be obtained in a few simple steps under prebiotic conditions. A remarkable synthesis of adenine (3) and more or less plausible syntheses of the pyrimidine nucleoside bases (4) have been reported, but the synthesis of ribose and the regiospecific combination of the bases, ribose, and phosphate to give β-nucleotides remain problematical. The recent work of Eschenmoser and his co-workers (5) has led to a specific synthesis of ribose 2,4-bisphosphate, but prebiotically plausible sequences of steps to the precursors of this ribose derivative and from it to the standard nucleotides are not obvious.

What are the alternatives if we tentatively agree that the direct prebiotic synthesis of standard nucleotides is implausible? Clearly, some complex chemistry must have "self-organized" on the primitive earth (or wherever else terrestrial life originated, if the panspermia hypothesis is correct) and facilitated the appearance of the RNA world. But what was it like? A popular hypothesis supposes that there was an earlier "genetic system," based on monomers that are more easily formed under prebiotic conditions than nucleotides, and that this primary genetic system "learned" to synthesize the nucleotides (1). This is the simplest form of the hypothesis, but the route to the RNA world could have passed through more than one transitional "genetic system." Possible transitional systems might have been based, for example, on pRNA, an isomer of RNA involving the pyranose form of ribose (6), or PNA, a molecule like RNA but with a peptide backbone (7).

The basis of the above hypothesis is the belief that there is a suite of monomers simple enough to form directly under primitive-earth conditions, but still able to form the self-replicating polymers of a primary genetic system. This belief has, with good reason, been challenged repeatedly. It is claimed that some other form of self-organization was needed to supply the components of the first self-replicating molecules and perhaps to catalyze their polymerization. The exact nature of the prior form of self-organization that is postulated differs from one scenario to the next, but all scenarios have one feature in common: a self-organized cycle or network of chemical reactions that does not depend directly or indirectly on a genetic polymer (8–11). In this paper, I will discuss chemical cycles with particular reference to one of the best developed of these theories, that of Wächtershäuser (9, 12).

Nonenzymatic Metabolism

The monomeric components of the primary genetic system must have been available on the primitive earth. Those who claim that direct prebiotic synthesis of a suitable suite of monomers is impossible have to explain the indirect way in which they were formed. Wächtershäuser argues that they were supplied via a complex cycle of nonenzymatic chemical reactions that took place on the surface of iron sulfide minerals, perhaps including pyrites, FeS_2 (9). Fig. 1 illustrates one of the cycles that is central to his theory, the reverse citric acid cycle. I will concentrate on this cycle because it is also the subject of a recent paper and commentary in PNAS (13, 14).

The reverse citric acid cycle is illustrated in Fig. 1 in the form adopted by Morowitz *et al.* (13). The input molecule is CO_2, while the intermediates in the cycle include relatively complicated molecules such as citric acid and oxaloacetic acid. Repeated operation of the cycle would provide an autocatalytic core mechanism for the synthesis of useful biochemicals from CO_2 and H_2 or some equivalent reducing agent (9, 13).

I begin by discussing nonenzymatic aqueous solution chemistry that might throw some light on the likelihood of complex cycles self-organizing without the help of evolved catalysts. Organic chemists interested in biochemical evolution have developed a number of self-replicating systems (15–18). They can perhaps be considered as cycles of order one, that is, cycles with a single intermediate (Fig. 2). This kind of cycle forms the natural starting point for the design of a self-replicating polymer in which the same kind of chemistry must be repeated time after time as in nucleic acid replication, but it could not be generalized to give a metabolic cycle involving several different types of reaction. It is instructive to notice how much synthetic skill is needed to develop even the simplest cycles (15–17). It will be a long time before organic chemists can match the reductive citric acid cycle in a single-pot reaction system!

The formose reaction is the only familiar chemical system that I am aware of that comes at all close to a metabolic cycle. The formation of sugars from formaldehyde under alkaline conditions in the presence of divalent metal ions is preceded by an induction period and is clearly autocatalytic. It has been studied intensively, and the complexity of the product mixture is notorious (19). Nonetheless, the core reactions clearly constitute a cycle, or more correctly, a family of interconnected cycles (20).

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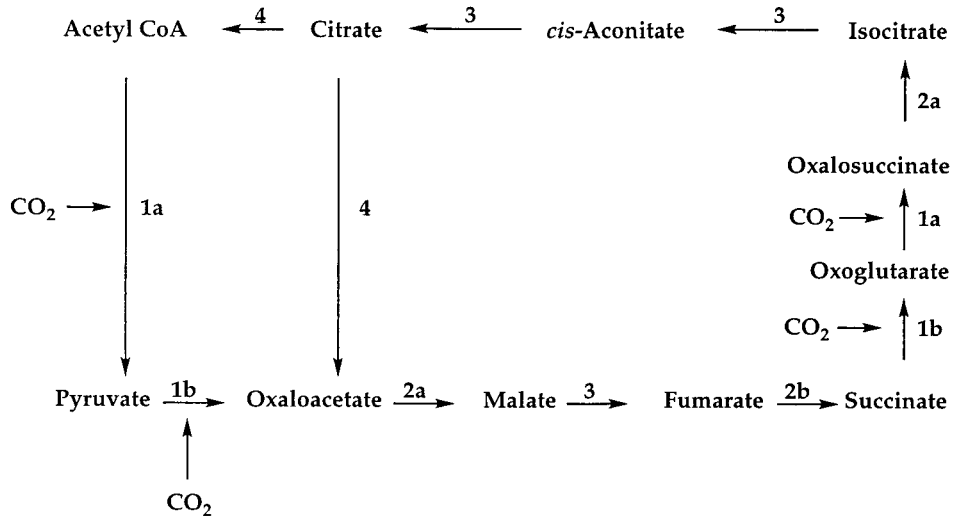


Fig. 1. The reductive (reverse) citric acid cycle adapted from the scheme in Morowitz *et al.* (13). The nature of the chemical steps is indicated as follows: 1a, introduction of CO_2 and reduction to give an α-ketoacid; 1b, introduction of CO_2 to give a β-ketoacid; 2a, reduction of a carbonyl group; 2b, reduction of a double bond; 3, reversible hydration/dehydration; and 4, cleavage of citrate to acetate and oxaloacetate.

Under the conditions of the formose reaction, redistilled formaldehyde does not yield sugars (21, 22). The addition of glycolaldehyde, glyceraldehyde, etc. initiates an autocatalytic cycle based on sequential additions of formaldehyde followed by reverse aldol reactions. An oversimplified version of the simplest of the possible cycles is illustrated in Fig. 3. The reactions illustrated in the scheme would constitute a simple autocatalytic metabolic cycle if they could be accomplished with sufficient efficiency to permit exponential growth; the input is formaldehyde, the intermediates are glycolaldehyde, glyceraldehyde, dihydroxyacetone, and tetrose sugars. A closer look at this oversimplified version of the simplest model of the formose reaction will reveal the formidable difficulties facing the development of a complex, nonenzymatic metabolic cycle in homogeneous aqueous solution.

The formose reaction does not proceed at an appreciable rate under neutral conditions, and even at high pH, is inefficient in the absence of a catalyst such as a divalent metal ion, for example Ca^{2+} or Pb^{2+} (23). My first observation then is that each step of a proposed cycle must proceed at a reasonable rate, and that this will often depend on the availability of a suitable catalyst. If one of the component reactions of a proposed cycle does not proceed spontaneously or under the influence of a plausibly prebiotic catalyst, some additional hypothesis will be needed to maintain the relevance of the cycle to the origins of life. If several of the reactions need "help," desperate measures will be called for.

One possible saving hypothesis is that the molecules that are the carriers of the cycle are also catalysts for the difficult reactions of the cycle. Unfortunately, catalytic reactions of the required kind in aqueous solution are virtually unknown; there is no reason to believe, for example, that any intermediate of the citric acid cycle would specifically catalyze any reaction of the citric acid cycle. The explanation of this is simple: noncovalent interactions between small molecules in aqueous solution are generally too weak to permit large and regiospecific catalytic accelerations. To postulate one fortuitously catalyzed reaction,

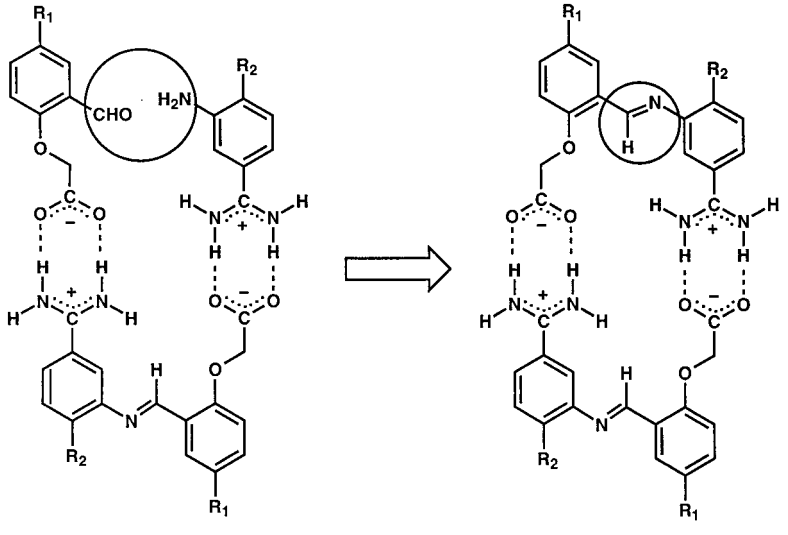
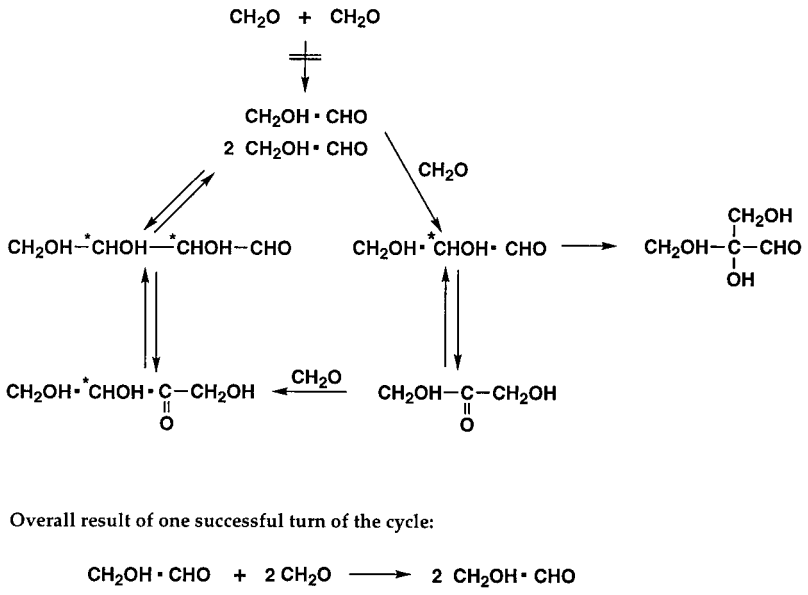


Fig. 2. A simple example of molecular replication in a nonbiological system (17).



Overall result of one successful turn of the cycle:

$$CH_2OH \cdot CHO + 2\,CH_2O \longrightarrow 2\,CH_2OH \cdot CHO$$

Fig. 3. The simplest hypothetical autocatalytic formose reaction cycle. In each turn of the cycle, a glycolaldehyde molecule facilitates the synthesis of a second glycolaldehyde molecule from two formaldehyde molecules. The direct formation of glycolaldehyde from formaldehyde does not occur. The stereochemistry of the asymmetric carbon atoms (marked with an asterisk in the diagram) is not specified. The side reaction leading to a branched-chain aldehyde is one of the many reactions that tend to complicate the cycle or divert molecules from it.

perhaps catalyzed by a metal ion, might be reasonable, but to postulate a suite of them is to appeal to magic.

The problem posed by reactions that do not proceed rapidly enough to make a cycle practical is usually the lesser of two problems for any but the simplest first-order cycles. In almost any complex cycle, alternative reactions are possible that would complicate or disrupt the cycle. In the formose reaction, for example, the tetrose sugars react readily with formaldehyde to give pentoses, each of which can dissociate to glycolaldehyde and glyceraldehyde, setting up competing cycles. Rearrangements of the sugars lead to the formation of hydroxyacids, etc., which can no longer participate in the cycle. A more detailed examination shows the simple tetrose cycle of Fig. 3 to be an oversimplification: The intermediate tetrose sugar might be either threose or erythrose, and each sugar could be represented as either the D or L enantiomer. Thus, even for a simple generic cycle like that in Fig. 3, it would not be reasonable to postulate the presence of a sufficient number of stereospecific prebiotic catalysts to make possible a particular stereospecific reaction sequence. Without the help of enzymes, or other "designed" catalysts that are unrelated to the substrates of the cycle, it seems unlikely that stereospecific cycles even as simple as that illustrated in Fig. 3 could exist.

The conclusion of this lengthy preliminary discussion is important. The idea that a complex polymerization reaction such as the formose reaction or the polymerization of hydrogen cyanide is likely to simplify to a specific cycle under the influence of autocatalysis in aqueous solution is implausible. It is not logically impossible that such an autocatalytic cycle exists, but because it seems very unlikely from what we already know about the chemistry of aqueous solutions, the burden of proof lies with the proposers of such cycles.

Wächtershäuser has put forward a very specific hypothesis that, if correct, would overcome all of the difficulties discussed above. In a scenario that he describes as "Two-Dimensional Chemi-Autotrophic Surface Metabolism in an Iron-Sulfur World" (9), he proposes that the reductive citric acid cycle and much other organized, nonenzymatic chemistry occurred on the primitive earth, but on the surface of iron sulfide minerals rather than in aqueous solution. Wächtershäuser points out that the conversion of ferrous sulfide (FeS) to pyrites (FeS_2) in the presence of hydrogen sulfide makes available reducing power equivalent to molecular hydrogen. This reducing power could be used to convert carbon dioxide to carbon-containing metabolites. Wächtershäuser also claims that the surface of iron sulfide would constrain the spatial distribution and orientation of the newly formed products of reduction in such a way as to support complex sequences of metabolic reactions.

Wächtershäuser's imaginative suggestion that ferrous sulfide in the presence of hydrogen sulfide should be an efficient reducing agent has been amply confirmed in collaborative studies with Stetter and his coworkers (24). They have shown, for example, that acetylene and mercaptoacetic acid are reduced efficiently by FeS/H_2S. Efforts to reduce CO_2 directly have so far failed, but reduction of CO has been demonstrated (12). Let us suppose that future efforts along these lines will prove successful and that one of the carrier molecules of the reductive citric acid cycle will be shown to be a prebiotic molecule derivable from CO_2 by reduction on the surface of FeS/FeS_2. Is the assumption that the complete cycle would self-organize and operate autonomously on the surface of FeS/FeS_2 then a reasonable one?

The choice of the FeS/FeS_2 system as the support for the reductive citric acid cycle is particularly appropriate as far as the reductive steps are concerned (Fig. 1, steps 2a and 2b). The experiments of Stetter and his coworkers (24) make it plausible, if not certain, that they would proceed with reasonable efficiency. However, the argument for the more general claim that the whole cycle would self-organize on the surface requires closer examination. Why should a family of disparate reactions, unrelated to oxidation–reduction, self-organize (Fig. 1, steps 1a, 1b, 3, and 4) on the surface of FeS/FeS_2, or for that matter, on the surface of any other mineral?

Wächtershäuser argues correctly that the constraints imposed on the orientation of molecules when they are adsorbed or synthesized in place on a surface should often lead to an increased specificity in their reactions. However, the very limited studies in prebiotic chemistry relevant to this hypothesis have not

uncovered any highly regiospecific reactions. Ferris and his coworkers (25, 26) have shown that montmorillonite catalyzes the oligomerization of adenosine 5′-phosphorimidazolide and leads to the formation of oligomers substantially enriched in 3′-5′ phosphodiester bonds. However, the regiospecificity is only about 80%, less than the regiospecificity in favor of 2′-5′-bonds of the uncatalyzed reaction. The corresponding reaction of the cytidine derivative on montmorillonite is less regiospecific. One could summarize the work of Ferris and colleagues by saying that montmorillonite clays catalyze the oligomerization of nucleoside 5′-phosphorimidazolides and modify the ratio of 2′-5′- to 3′-5′-phosphodiester bonds that are formed without greatly changing the regiospecificity of the reactions. The specificity of the formose reaction is not increased by catalysis on hydroxylapatite or other minerals (23). Similar conclusions can be drawn from the work of Arrhenius and his colleagues (27), who find that a number of inorganic layer hydroxides catalyze the dimerization of glycolaldehyde phosphate and modify the ratio of threose 2,4-bisphosphates to erythrose 2,4-bisphosphates in the products, but without greatly increasing the regiospecificity of the reaction.

One must expect the results of mineral catalysis to be highly idiosyncratic; most minerals will probably catalyze some reactions and many reactions will no doubt be catalyzed by some minerals. Furthermore, mineral catalysis may sometimes confine the reactions of an adsorbed substrate with high regiospecificity to one of a number of pathways that are followed in the absence of the mineral catalyst. However, in the absence of supporting experimental evidence, there is no justification for the prediction that a particular mineral will catalyze a suite of very different reactions. Nor would the situation be changed if the proposed participants in a complex cyclic reaction scheme were synthesized *in situ* on a mineral surface. If the products are mobile on the surface, the situation is identical to that for adsorbed molecules. If they are not, one must postulate a series of remarkable coincidences to conclude that all of the reactions are catalyzed on the same mineral and that each intermediate product is formed in the correct position and orientation to become the substrate of the next regiospecific reaction of the cycle. The self-organization of a complex cycle such as the reductive citric acid cycle, this time on the surface of FeS/FeS_2, although logically possible, is very unlikely. The experimental demonstration of a sequence of reactions including some of the "difficult" reactions is needed to give any credence to the theory.

In summary, it seems very likely that minerals played an important part in prebiotic chemistry, both as simple adsorbents and as catalysts. A single mineral is unlikely to have functioned as a specific catalyst for several unrelated reactions. Even if the members of a suite of minerals could each catalyze one step in a complex cycle, it does not seem likely that the cycle would self-organize on their surfaces. Any suite of minerals that included catalysts for each step of the cycle would be likely to include, in addition, catalysts for reactions that disrupt the cycle. Efficient transport of the intermediates from one catalytic mineral to another would also present severe problems. There is at present no reason to expect that multistep cycles such as the reductive citric acid cycle will self-organize on the surface of FeS/FeS_2 or some other mineral.

While it seems almost impossible that a cycle of reactions as complicated as the reductive citric acid cycle could self-organize on a mineral surface, Wächterhäuser's suggestion does raise an interesting and important question. How much self-organization is it reasonable to expect on a mineral surface in the absence of evolved, informational catalysts? Clearly, a simple surface could reasonably be expected to carry out a series of reactions of essentially the same type, say a series of aldol and reverse aldol reactions of the type involved in the formose reaction (Fig. 3), or a series of reductions of the type involved in the reductive citric acid cycle. It is not clear that any surface is likely to catalyze two or more unrelated chemical reactions, but it would be interesting to try to discover multifunctional surfaces.

The problem of stereospecificity of a sequence of similar reactions in aqueous solution or on a mineral surface is equally difficult. Repetition of some basic reaction with constant stereospecificity does not seem unlikely, because it is routinely achieved by polymer chemists. Catalysis of a sequence of reactions, each with a different defined stereospecificity, seems much less plausible. Just how far one can go in the direction of self-organization on mineral surfaces is a question for the future. One can be sure that the complete reverse citric acid cycle is out of range, but it is not obvious that some much simpler cycle relevant to the origin of life is impossible. Huber and Wächtershäuser have noted the possibility of such a cycle in a footnote added in proof to ref. 23, but it is not clear whether this cycle is intended to augment the citric acid cycle or replace it. The demonstration of a simple specific version of the formose reaction, for example, of the type illustrated in Fig. 3, would be important, but studies of the specificity of the formose reaction when catalyzed by minerals have been disappointing (23).

The Beilstein Handbook and Citric Acid Cycle

I have argued that the self-organization of the reductive citric acid cycle without the help of "informational" catalysts would be a near miracle. Recently, Morowitz *et al.* (13) have suggested that the contents of Beilstein's *Handbook of Organic Chemistry* (28, 29) strongly support the opposite point of view. Here I offer an alternative interpretation of the data presented by Morowitz *et al.* (13). Some of the points that I emphasize are mentioned in the commentary accompanying their paper (14).

The Beilstein handbook is a database of more than three million organic compounds that have been studied in adequate detail by organic chemists. The contents of the handbook do not represent a selection of organic compounds chosen at random from the set of all possible stable organic compounds up to some maximum complexity, but are strongly influenced by the interests of organic chemists. A compound is likely to appear in the handbook only if its interest at some time seemed to justify the effort needed to isolate or synthesize it. The study of molecules important in biochemistry has been from the beginning a dominant theme of organic chemistry, so any small molecule interesting to biochemists is virtually certain to appear in Beilstein.

The citric acid cycle is central to the oxidative metabolism of most living organisms. More importantly in the present context, the reductive (reverse) citric acid cycle has been proposed to provide an autocatalytic core metabolism, allowing a primitive organism to synthesize a wide range of biochemicals and precursors to biochemicals from carbon dioxide and hydrogen or some equivalent reducing agent (9, 13). In the form described by Morowitz *et al.* (13), the citric acid cycle involves 11 organic intermediates.

Morowitz *et al.* (13) draw far-reaching conclusions about the origins of life on Earth and elsewhere in the Universe from an analysis of the list of compounds in Beilstein. They are impressed that "there emerges through certain physically motivated pruning rules a small set of 153 compounds that includes all of the citric acid cycle intermediates." We have already seen that the citric acid cycle intermediates are almost obligatory entries in Beilstein, because they are important biochemicals. Their inclusion, taken alone, tells us nothing about their likely role in chemical evolution.

Should we be impressed by the pruning of the Beilstein database to 153 entries by a series of simple rules, or by the appearance of all of the citric acid cycle intermediates among those permitted by the pruning rules? The latter finding would certainly be impressive if the pruning rules had been devised

without any knowledge of the structures of the citric acid cycle intermediates. However, an examination of the pruning rules suggests that they have been devised, perhaps inadvertently, to allow the inclusion of these substances and exclude many of the other entries in Beilstein. Compounds are included only if they contain no more than six carbon atoms, contain only carbon, hydrogen, and oxygen in certain composition ranges, include a "carbonyl" group, etc. Equally plausible rules that would occur to an organic chemist seem not to have been considered. Many would either exclude citric acid cycle intermediates or permit the inclusion of a much wider range of other compounds. Why restrict the number of carbon atoms to six rather than five or seven? The choice of five would have led to the exclusion of many of the citric acid intermediates, whereas the choice of seven would have led to the inclusion of a large number of unrelated compounds. Why adopt a seemingly arbitrary rule of composition that excludes the very soluble sugar glucose ($C_6H_{12}O_6 = 6 \times CH_2O$) and numerous other sugars and related compounds, but includes acetic acid ($C_2H_4O_2 = 2 \times CH_2O$)?

We see, therefore, that all of the citric acid cycle intermediates appear in Beilstein because they are important biochemicals and of interest to organic chemists, and that they are not excluded by the pruning rules because the pruning rules are formulated in a way that allows their inclusion. The only remaining observation that requires comment is the restriction of Beilstein entries satisfying the rules to 153 items. I can see three possible reasons for the relatively small number of entries. First, the pruning rules may be so restrictive that there are only a limited number of stable compounds simultaneously satisfying them and the valency rules of organic chemistry. Second, organic chemists may not be interested in many of the compounds allowed by the rules if they are not of biological significance. Third, production of many of the nonbiological compounds by the methods of synthetic organic chemistry may be difficult. The first two explanations would not permit any connection to be made with early evolution. The third, if correct, might have some relevance to prebiotic chemistry, because it would strengthen somewhat the view that the central molecules of biochemistry are likely to be molecules for which relatively simple prebiotic syntheses are available. In summary, although it seems plausible on general grounds that the reverse citric acid cycle is an early biochemical "invention," the contents of Beilstein do not provide any new evidence to support such a conclusion, or suggest that the cycle self-organized.

Conclusions

The novel, potentially replicating polymers that have been described up to now, like the nucleic acids, are formed by joining together relatively complex monomeric units. It is hard to see how any could have accumulated on the early earth. A plausible scenario for the origin of life must, therefore, await the discovery of a genetic polymer simpler than RNA and an efficient, potentially prebiotic, synthetic route to the component monomers. The suggestion that relatively pure, complex organic molecules might be made available in large amounts via a self-organizing, autocatalytic cycle might, in principle, help to explain the origin of the component monomers. I have emphasized the implausibility of the suggestion that complicated cycles could self-organize, and the importance of learning more about the potential of surfaces to help organize simpler cycles.

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