# Did minerals perform prebiotic combinatorial chemistry?

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**It has long been suspected that mineral surfaces may have been important in prebiotic chemistry. The recent demonstrations of extended oligomerization of nucleotides, amino acids and carbohydrates on mineral surfaces add support to the potential prebiotic importance of minerals, but leave a number of questions unanswered.**

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The idea that chemical compounds necessary for the development of the first organisms must first have accumulated in solution can be traced back at least to Darwin's 'warm little pond' [1]:

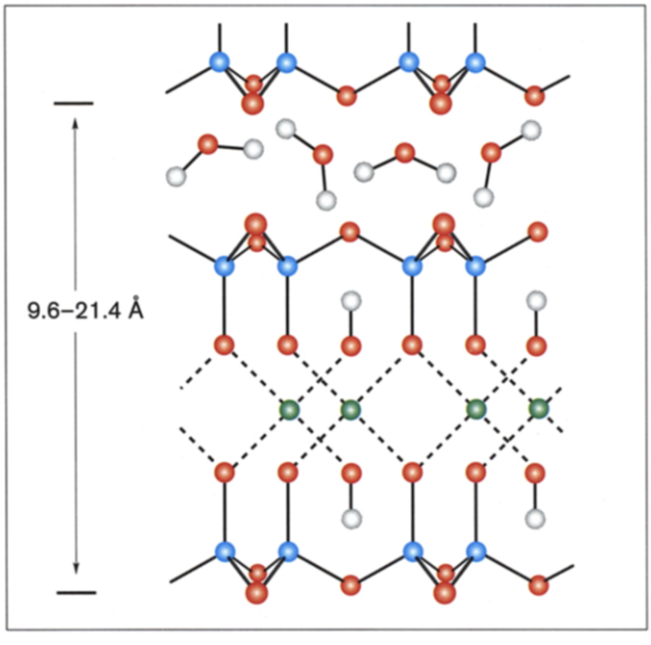
> It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc., present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed.

That any such accumulation must have been rather limited, however, is already hinted at by the fact that the entire biomass, if dissolved as organic compounds in the ocean, would constitute a solution less than $10^{-4}$ M in carbon. In his influential book 'The Physical Basis of Life', Bernal [2] suggested that clay particles could have been crucial, concentrating and protecting (from photolysis) organic molecules. Clay minerals are well-known industrial catalysts and it would not be surprising if, in addition to selectively adsorbing certain prebiotic monomers, prebiotic reactions could also be catalyzed by these materials. In spite of this expectation, and the existence of a fairly large literature on the subject, no very clear consensus seems to exist among experts in the field of prebiotic chemistry concerning the probable role of minerals. This is probably because a few of the most interesting reports that have appeared in the literature have generated some controversy in the past. Even worse, one or two rather spectacular claims have been published that, unfortunately, have never been substantiated by additional published work. Some recent results, however, demonstrating extended, mineral-catalyzed oligomerizations, seem to provide a new perspective in this complex field. There is insufficient space in this minireview to cover any but the most pertinent previous results. For broader reviews see Laszlo [3], and, in particular, Ponnamperuma *et al.* [4].

## Mineral-catalyzed oligomerization reactions

One of the first dramatic demonstrations of the selective possibilities of clay minerals in prebiotic chemistry was the report by Paecht-Horowitz *et al.* [5] that an amino-acid adenylate could be polymerized in aqueous solution in the presence of montmorillonite (Fig. 1) or illite to form adenosine-terminated chains of polypeptide. When montmorillonite was used as catalyst, alanyl adenylate polymerized to chain lengths in excess of 50 monomer units. In the absence of the mineral, hydrolysis of the adenylate was the principal reaction observed. Although there has been some discussion as to the repeatability of some

**Figure 1**




The crystal structure of montmorillonite [18], a clay mineral that has been used in a number of prebiotic chemical studies. Its formula is $Al_4(Si_4O_{10})_2(OH)_4 \cdot xH_2O$, with the atoms shown as green (Al), red (O), white (H) or blue (Si). A characteristic feature of this mineral is the expanding lattice, the spacing of which varies from 9.6 to 21.4 Å depending on the number of water molecules adsorbed between the layers (shown in random orientation).

aspects of this reaction, a series of additional publications substantiate the original observations (see references in [6]). Such a reaction must, of course, be viewed as a model rather than an example of a prebiotic reaction, as it is far from clear how amino-acid adenylates would have been produced on the primitive Earth in the necessary purity. Oligopeptides have also been produced on clay surfaces from free amino acids using wetting-drying-heating cycles, but the products are limited to short oligopeptides [7].

Another notable mineral-catalyzed reaction is that observed by Pitsch *et al.* [8], in which the mineral hydrotalcite was shown to concentrate glycolaldehyde phosphate from dilute solution and thus catalyze condensation of the carbohydrate subunits. Solutions as dilute as 20 μM were shown to result in adsorption into the mineral interlayer, with saturation depending only on the total supply of monomer. The phosphorylated carbohydrates that are produced are primarily aldotetrose-2,4-diphosphates and aldohexose-2,4,6-triphosphates [8]. This reaction, carried out at neutral pH and low concentration, is a partial extension of a potentially prebiotic pathway for the synthesis of carbohydrate phosphates developed by Eschenmoser and colleagues [9]. These results contrast strongly with the complex and highly unselective formose condensation of formaldehyde, in which glycoladehyde is the first intermediate product.

Ferris and coworkers have, in the past few years, performed an extensive investigation of the adsorption and oligomerization of activated mononucleotides on sodium montmorillonite. In one of the most interesting papers, the imidazolide of 5'-AMP (ImpA) was shown to react on the clay surface to produce oligomers up to 10 units in length and containing 85 % 3'–5' linkages [10]. This degree of regiospecificity in linkage composition is striking, although highly specific formation of the 2'–5' linkage in aqueous solution in the presence of uranyl ion has also been reported [11].

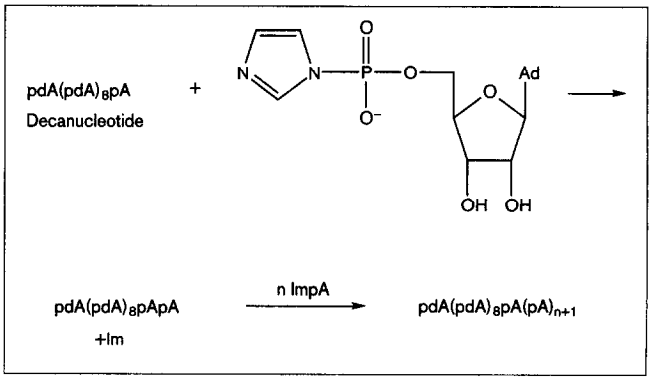
### Longer oligomers

In the reactions described above, the products are relatively short oligomers. An obvious question is, how were the first long oligomers produced? Although synthesis of long oligonucleotides of exclusively 3'–5' linkage in aqueous solution has been achieved, this has only been possible by means of the template-directed oligomerization of the 2-methylimidazolide of 5'-GMP (2-MeImpG) in the presence of (enzymatically synthesized) poly(C) [12]. Previous work on this templated reaction has demonstrated that mineral surfaces that selectively bind oligonucleotides and polynucleotides can be used to promote the ligation of short oligonucleotides on the polynucleotide template. As both the template and the short oligonucleotides that are initially formed in solution are adsorbed on a mineral such as hydroxylapatite, it is possible to reactivate the phosphate end groups and produce longer chains. Thus, Acevedo and Orgel [13] showed that the fraction of oligomers with lengths greater than 19 were increased more than 20-fold after 4 cycles of reactivation of a 'first generation' of oligo(G)s on hydroxylapatite in the presence of poly(C).

In a recent article in *Nature*, Ferris *et al.* [14] have addressed the question of the chain lengths of oligomers produced in the absence of a template. They show that short oligonucleotides as well as oligopeptides can be elongated on a suitable mineral surface by 'feeding' the reaction with fresh activated monomer. The authors started with the decanucleotide pdA(pdA)$_8$pA (where pA is adenosine-5'-phosphate and pdA is 2'-deoxyadenosine-5'-phosphate) adsorbed on sodium montmorillonite, and carried out daily additions of the activated mononucleotide ImpA (Fig. 2). For each cycle, the clay with its adsorbed oligonucleotide was washed to eliminate the shortest products, and fresh ImpA was added. After two cycles, polyadenylates containing more than 20 monomer units were observed, and after 14 cycles, polynucleotides containing more than 50 units were formed. In the latter case, the major fraction of product contained 20–40 monomer units.

Turning their attention to the oligomerization of glutamic acid, Ferris *et al.* [14] demonstrate that a second clay mineral, illite, can be used in a similar manner to promote chain elongation of oligomers of this amino acid. Using the activating agent carbonyl diimidazole, which results in the formation of an active intermediate (the cyclic *N*-carboxyanhydride of glutamic acid), a series of short oligomers were extended after 50 cycles to produce polypeptides with chain lengths in excess of 55 monomer units. In a further application of this technique, oligomers of aspartic acid, adsorbed on hydroxylapatite, were extended using cycles of exposure to the activating agent

**Figure 2**




Elongation of a decanucleotide by reaction with the activated mononucleotide ImpA [14]. Im is imidazole, pA is adenosine-5'-phosphate and pdA is 2'-deoxyadenosine-5'-phosphate.

*N*-ethyl-*N'*-dimethylaminopropyl-carbodiimide (EDAC). Under these conditions chain branching is also possible, as both carboxyl groups of the amino acid are activated, and a complex set of long oligomers was obtained.

The authors make the point that a 'library' of mineral surfaces would have been available on the primitive Earth, so a variety of chemical pathways could have been explored. This is an attractive idea, as it seems to suggest that the important stages of chemical evolution need not have occurred in bulk solution, but may have been solved by a kind of mineral-based, combinatorial chemistry. As they point out, the missing link in this scenario is the development of self-replication. Like combinatorial chemistry, in which great chemical diversity can be created in a systematic way [15], the usefulness of the mineral scenario depends on the ability to replicate one or more of the products, presumably an RNA or an RNA-like molecule. The origin of biological organization, however, does not merely depend on the presence of long oligomers; those oligomers must also be stereochemically uniform. This implies that regiospecificity must be achieved not only in the synthesis of linkages but also in the generation of homochirality.

### Achieving homochirality

At present there is no known way of achieving homochirality other than by oligomerization from a structurally homogeneous supply of monomers, which are either chirally pure or can be selected for a particular chirality with the help of a homochiral template. It is the latter possibility that seems to offer the most hope, as recent results from Orgel's laboratory suggest that the problem of enantiomeric cross-inhibition may be less severe than was originally thought. This phenomenon was encountered in attempts to use the template-directed oligomerization of 2-MeImpG as a selection mechanism to preferentially incorporate the D-isomer of the monomer when the reaction was carried out with a racemic mixture of D- and L-forms [16]. In the original experiment, it was found that the presence of the homochiral template did not prevent incorporation of the L-enantiomer at the growing ends of chains, and that this event caused premature termination of the oligomerization. It has now been found that elongation of a presynthesized homochiral (D-)oligo(G) primer in the presence of a poly(C) template permits selection of the 'correct' enantiomer to take place, although the overall reaction is still relatively inhibited. Additions of up to four D-residues to the primer have been observed (L.E. Orgel, unpublished data).

In addition (L.E. Orgel, unpublished data), preliminary results obtained using the same homochiral primer in the presence of a template consisting of 'peptide nucleic acid' (PNA), are also extremely interesting. This achiral nucleic acid analogue has a unique backbone based on a repeating amide linkage [17]. Although the reaction was less efficient than that carried out in the presence of a poly(C) template, some degree of chiral selection was still observed. PNA chains can spontaneously assume either left- or right-handed helical forms, suggesting that, in this reaction, they have conformed to the helical sense of the primer. It would seem that a short primer alone can have a significant effect on the chirality of the system. It is thus possible that conditions may exist that would permit chiral amplification of a short, randomly assembled, homochiral oligonucleotide. If, for example, a pentamer were to serve as the primer, it would have a finite chance (of $0.5^5 = 0.03$) of being assembled as a homochiral oligomer from a racemic mixture of mononucleotides.

### Limitations

This discussion should make it clear that a great many problems must be resolved on the way to a model of the prebiotic synthesis and replication of oligonucleotides. It is far from clear whether mineral surfaces can help in overcoming any of these problems. While the degree of regiospecificity in the production of short oligomers from ImpA on montmorillonite has been shown to be high [10], it is not yet clear if the 'feeding' experiment on this mineral also shows such specificity. Although adsorption on minerals seems to provide a useful solution for the problem of dilution, many of the other difficulties that plague prebiotic chemistry would still exist in the world of mineral catalysis. In the examples cited for the synthesis of oligopeptides, carbohydrate phosphates and oligonucleotides, the presence of other compounds with similar charges would probably lead to coadsorption to the mineral surface, where they may interfere in the desired reaction. Imidazolides are generally much easier to prepare and are more stable than amino-acid adenylates. Nevertheless, their availability on the primitive Earth in sufficient purity is open to question. Glycolaldehyde phosphate is a starting material for which a prebiotic synthesis has yet to be devised, and the synthesis would have to be efficient indeed to produce pure solutions of this reactive compound.

There is clearly a great deal more work to be done in this area. The few dramatic examples cited here of potential prebiotic mineral-catalyzed reactions have all been rather unexpected. Hopefully, mineral catalysis will have other surprises in store for prebiotic chemists.

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