

# Catalysis in prebiotic chemistry: application to the synthesis of RNA oligomers

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## Abstract

The role of catalysis in prebiotic synthesis is illustrated using the montmorillonite clay catalyzed synthesis of RNA oligomers. Reaction of activated monomers of nucleotides in pH 8 aqueous solution containing  $Mg^{2+}$  in the presence of montmorillonite yields 6–14 mers. The reaction takes place at the clay interlayer. The catalyst controls the synthesis of RNA oligomers in that a limited number of sequences are formed, there is control on the selectivity of phosphodiester bond formation and there is homochiral selectivity. Oligomers of length greater than 40 mers can be formed in reactions where the activated monomer is added daily to a primer. The need for catalysis in prebiotic synthesis is discussed.

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## 1. Introduction

A basic question concerning the origin of life is the source(s) of the organic compounds on the primitive earth that eventually resulted in the formation of the first life. Three potential sources have been identified (1) carbonaceous meteorites and/or comets (2), reactions of the reduced compounds present in the atmosphere of the primitive Earth and (3) hydrothermal systems resulting from plate tectonics on the primitive Earth. Potential sources (1) and (2) are a source of complex mixtures of simple compounds as well as, in the case of carbonaceous meteorites, some complex, kerogen-like structures. In the case of hydrothermal systems the simple organics are formed from more oxidized organics because of the reducing capabilities of the minerals present but no complex structures have been produced to date. No biopolymers have been identified in any of the three basic sources of organics so it is likely that these were formed by the subsequent fractionation and reaction of these initial organics on the primitive Earth.

The investigation of the prebiotic synthesis of biopolymers is an objective of this research program. It is proposed that catalysis had a central role in the formation of biopolymers. The additional beneficial aspects of catalysis in controlling the course of the polymerizations reactions will be demonstrated in our investigation of polymer formation using a montmorillonite clay catalyst.

### 1.1. Catalysis and prebiotic chemistry

Catalysts may have been important for the origins of life because they tend to direct the reaction along a few reaction pathways so that a limited array of products is obtained. Catalysts bind specific types of compounds to their surfaces and then convert them to a limited number of products. This can be accomplished in the presence of a large amount of water (55 M) where the concentration of reactants is  $10^3$  or greater times lower than that of water. In the absence of catalysis these compounds often hydrolyze faster than they polymerize.

The proposal that clay minerals could have served to concentrate organics on their surfaces and catalyze their subsequent polymerization was first made in 1947 by Bernal in a lecture (Bernal, 1949). Experimental data

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will be provided for a clay mineral that fulfills the requisites proposed by Bernal for a catalytic mineral.

### 1.2. Proposed reaction conditions on the primitive Earth

The reactions that led to the formation of biopolymers probably took place in the presence of water since there is no evidence for the presence of other solvents. The oceans today have a pH of about 8.2 but there is some evidence suggesting that pH of the primitive ocean may have been in the pH 5–6 range (Walker, 1985; Grotzinger and Kasting, 1993). It is probably safe to assume a primitive ocean pH of  $7 \pm 3$ .

Since liquid water exists in the 0–100 °C ranges at an atmospheric pressure of one atmosphere it is reasonable to believe that the temperature of the primitive oceans and lakes were also in that temperature range. Our bias is for the formation of biopolymers near the lower temperature limit since the oligomers and their precursors are more sensitive to hydrolysis at the higher temperatures. In addition, the weak interactions essential in biological systems today, like hydrogen bonding, are too weak to be effective at higher temperatures.

As noted previously, water is a difficult solvent in which to carry out the synthesis of biopolymers because RNA oligomers and the activated monomers from which they are made are hydrolyzed by water. Consequently it is essential to enhance the rate of oligomer formation from activated monomers. Studies of the reaction of activated monomers in water in the absence of catalysts have shown that the principal reaction is the conversion of the activated monomers to non-activated ones by water hydrolysis. A minor reaction pathway, even with  $\sim 1$  M concentrations of activated monomers, is the formation of dimers in yields of 12–27% and only trace amounts of longer oligomers (Kanavarioti, 1998). Consequently it is essential to have catalysts to enhance the rate of polymer formation over to that of hydrolysis of the activated monomer.

### 1.3. Prebiotic catalysts

Three general types of catalysts have been proposed to enhance the rates of prebiotic organic reactions; minerals, metal ions and polypeptides. The main emphasis in the area of mineral catalysis has been clays and these will be discussed later. Metal ions that have been

catalysts include  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{UO}_2^{2-}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$ . Short peptides that formed spontaneously on the primitive Earth have also been suggested as catalysts (Barbier and Brack, 1992). They could have been formed by a process that would have synthesized the same polypeptide repetitively since the chance of forming appreciable amounts of the same polypeptide by random synthetic processes is unlikely (Ferris, 2002a). Alternatively they may have been formed by peptide replication (Saghatelian et al., 2001).

### 1.4. The RNA world

The focus of this investigation is the role of catalysis in the prebiotic synthesis of RNA oligomers that may have initiated the first life on Earth. This first life had RNA or a related information-storing polymer as its central biopolymer. This polymer not only stored genetic information but also served as a catalyst in this initial primitive life (Cech et al., 1981; Guerrier-Takada et al., 1983).

Evidence for the existence of the RNA world includes the discovery of over 17 RNA catalysts (ribozymes) that catalyze a diverse array of organic reactions (Joyce, 1998). Second, the RNA of the ribosome catalyzes peptide bond formation (Ban et al., 2000), a finding that suggests that the first ribosome contained only RNA. Proteins were added later to enhance its efficiency. Third, the biosynthesis of DNA has RNA monomers as biosynthetic intermediates. This suggests the biosynthesis of RNA evolved first and the biosynthesis of DNA evolved after the biosynthesis of RNA.

Many scientists feel that RNA was not formed directly by prebiotic processes because of the complex structure of RNA monomers (Orgel, 1998; Schwartz, 1998). They note that a plausible prebiotic synthesis of RNA monomers has not been accomplished. Instead they suggest a structurally simpler genetic polymer formed first that eventually evolved to RNA. The absence of strong evidence for the prebiotic synthesis of the monomers for such a polymer has convinced us to continue work on the direct prebiotic synthesis of RNA.

The synthesis of RNA from a phosphorylated nucleoside monomer is thermodynamically impossible in the presence of water. Hydrolysis of the phosphate ester bond of 5'-AMP (Fig. 1) releases 2.2 kcal/mol so that energy input was necessary to form the phosphate ester

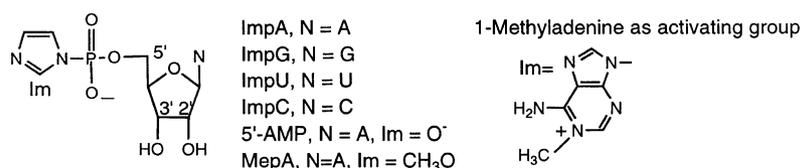


Fig. 1. RNA monomers used in this study.

bond between two nucleotides. One way to provide the energy is to attach an activating group to the phosphate, which provides the energy to form the phosphate ester bond. In the studies to be described, this activating group has been the imidazole (Sulston et al., 1968) or 1-methyladenine (Prabahar and Ferris, 1997) groups (Fig. 1). The use of the 1-methyladenine group is attractive because it is assumed that adenine was present on the primitive Earth since it is likely that 1-methyladenine was present on the primitive Earth as well (Levy and Miller, 1996).

## 2. Montmorillonite-clay catalysis of RNA oligomer formation

Dissolution of an activated monomer in a aqueous solution containing  $Mg^{2+}$ , montmorillonite clay which is buffered to pH 8 generated RNA oligomers in 2–3 days that contain from 2 to 14 monomer units (Ferris and Ertem, 1993; Ertem and Ferris, 1996; Ding et al., 1996; Kawamura and Ferris, 1999). The montmorillonite clay also catalyzes the hydrolysis of the activated monomer but the rate of oligomer formation is ten times greater than the hydrolysis rate (Kawamura and Ferris, 1994). Hydrolysis is the principal reaction pathway and low yields (~1%) of 2- and 3-mers are formed when the reaction is performed under the same conditions in the absence of montmorillonite. Montmorillonite is a much less efficient catalyst than the enzymes that catalyze RNA synthesis from activated monomers in contemporary biological systems since the RNAs oligomers formed are linked by 2',5'- and 3',5'-phosphodiester bonds. Other bonding includes the formation of cyclic dimers and monomers linked by pyrophosphate groups. The formation of cyclic dimers and pyrophosphate groups is blocked if there is a methyl group on the 5'-phosphate group of one of the monomers (Wang, unpublished). This was observed in the reaction of adenosine-5'-methylphosphate (Fig. 1) with ImpU (Fig. 1).

### 2.1. Site of catalysis

Montmorillonite clays are composed of two-dimensional sheets (platelets) some of which are stacked on top of each other like a deck of cards. The sheets are held together by cations bound to the negatively charged surfaces of the sheets. Each sheet is composed of two outer layers composed of tetrahedral silicate groups and an inner layer composed of octahedral aluminate groups. Oxygen atoms link these layers covalently. Isomorphous substitution of  $Al^{3+}$  for  $Si^{4+}$  in the tetrahedral layer and  $Mg^{2+}$  for  $Al^{3+}$  in the octahedral layer occurs during the formation of clay sheets. These substitutions are the source of the negative charges on the clay lattice (Grim, 1968).

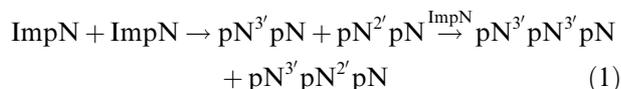
There are two possible sites for the reaction of activated monomers on montmorillonite, between the layers or at the edges of the clay platelets. Binding studies suggest that the activated monomers bind in the interlayer (Ertem and Ferris, 1998) by van der Waals interaction between the purine or pyrimidine rings of the nucleotides to the silicate layers on the montmorillonite sheets. The larger purine nucleotides bind more strongly than pyrimidine nucleotides. Edge sites, with silanol and  $Al(HOH)^+$  groups, may also catalyze reactions. The selective placement of large groups in the interlayer and at the edge sites established that the reaction was proceeding in the interlayer between the clay sheets.

### 2.2. Sequence selectivity

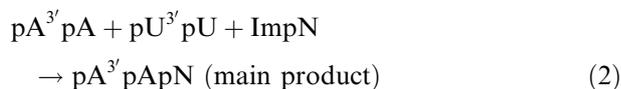
The RNA world scenario requires RNAs that are sufficiently long to store genetic information and to catalyze reactions. Theoretical studies suggest that RNAs that contain at least 40–60 monomer units are required to accomplish these tasks (Joyce and Orgel, 1999; Szostak and Ellington, 1993). Previously it was noted that long oligomers are not formed in the absence of catalysis. One of the reasons for the failure to make longer oligomers is that all possible isomers form in the absence of a catalyst that controls the reaction pathway. The enzymes of contemporary biological systems exert exquisite control over the RNA sequences formed. As noted previously, we do not expect a clay mineral to be as specific a catalyst as a biological polymerase but we do expect it to show some selectivity in its reactions.

In studies completed to date, we note the following control by montmorillonite over the course of the RNA synthesis:

1. The initial step in the formation of dimers from monomers gives both 2',5'- and 3',5'-phosphodiester bonds (Ferris and Ertem, 1993). In the subsequent step of the elongation the 3',5'-linked dimer reacts ~5 times faster than the corresponding 2',5'-linked isomer to give trimers in which the phosphodiester bond at the 5'-end of the chain is 3',5'-linked Eq. (1) (Wang, unpublished)



2. Elongation of a dimer with a purine nucleotide at the 3'-position elongates about five times more rapidly than if there is a pyrimidine nucleotide at that position Eq. (2) (Wang, unpublished)



3. The montmorillonite-catalyzed reaction of an equimolar mixture of four activated monomers yields different amounts of the 16 possible dimers (Ertem and Ferris, 2000). Of these 16 dimers half account for 84% of the reaction products. All of the eight major products have a purine nucleotide at the 5'-position and four of these also have a purine nucleotide at the 3'-position. Studies on the elongation of selected dimers to trimers are in progress (Miyakawa, unpublished).

Theoretical studies mentioned above indicate that an oligomer containing at least 48 monomer units in length is required for it to generate a complementary strand by template-directed synthesis with low levels of error and to catalyze reactions. If two copies of a 48 mer contained all possible isomers weighs  $10^{28}$  g, a value close to the mass of the Earth. Obviously, there was not  $10^{28}$  g of activated monomer available on the primitive Earth to generate (Joyce and Orgel, 1999) two copies of each 48 mer so there must have been a limit on the number of sequences formed to have generated the requisite RNA and catalysis is a likely way for selective synthesis to have been accomplished.

### 2.3. Template-directed synthesis

Template-directed synthesis of the complementary RNA strand, which in turn is used to form the original template by template-directed synthesis, is the route by which RNA is replicated in biological systems. It is generally agreed that a similar replication pathway occurred on the primitive Earth. The sequences of RNA, and hence its information content, would have been preserved in this way.

The first step in the replication of RNA, the non-enzymatic synthesis of the complementary strand has been investigated thoroughly (Orgel, 1992). He and his coworkers found that the reaction of an activated monomer of G (ImpG, Fig. 1) would react on an oligo(C) template to form the complementary oligo(G)s.

In the studies described above the RNA template was linked by 3',5'-phosphodiester bonds. Since the oligomers formed by montmorillonite catalysis contain both 2',5'- and 3',5'-phosphodiester bonds and also contain pyrophosphate linkages and cyclic oligomers it was not certain if this heterogeneous mixture would serve as a template for template-directed synthesis. It was observed that the longer oligo(C)s formed by the reaction of ImpC in the presence of montmorillonite do initiate the reaction of the activated monomers of G to form oligo(G)s (Ertem and Ferris, 1997). In addition, when those oligomers that contained 3',5'-links were removed from the oligo(C) template mixture the remaining 2',5'-linked oligomers served as templates for the formation of the complimentary oligo(G)s.

### 2.4. Reactions of D, L-mixtures

Activated monomers present on the primitive earth would be expected to contain both D- and L-enantiomers. It is known that template-directed synthesis is strongly inhibited when a monomer unit of the opposite handedness to the template is incorporated into the complementary chain (Joyce et al., 1984). This finding raised the question of whether the montmorillonite-catalyzed synthesis of RNA oligomers is also strongly inhibited when both enantiomers are incorporated into the RNA. The initial investigation of the reaction of D, L-ImpA showed partial inhibition by the observation that the longest oligomer formed was an 8 mer while the longest oligomer formed from one enantiomer was a 10 mer (Ertem et al., 1999; Joshi et al., 2000; Urata et al., 2001). Analysis of the linear dimers formed from racemic mixtures of ImpA and ImpU revealed that a 60:40 ratio of the D, D- & L, L-dimers to D, L- & L, D-dimers were formed (Joshi et al., 2000).

### 2.5. Generation of longer RNA oligomers

RNA oligomers with chain lengths much longer than 10 mers are required to have initiated the first life on Earth. As noted previously, theoretical studies show that oligomers greater than 40 mers are required to have RNAs that undergo high fidelity template-directed synthesis and that catalyze reactions. Initial studies demonstrated that it was possible to generate long oligomers in "feeding reactions". In these studies a 10 mer primer was bound to the montmorillonite and the activated monomers (ImpA) was added to the primer daily for a total of 14 days to generate an oligomer which contained more than 40 mononucleotides (Fig. 2) (Ferris et al., 1996). More recent studies showed that reaction worked for both ImpA and ImpU oligomerization reactions but only 30 mers were obtained from ImpU (Ferris, 2002b).

Research in progress demonstrated the more efficient synthesis of longer oligomers using monomers activated with 1-methyladenine (Prabakar and Ferris, 1997) (Huang, unpublished). The use of an adenine derivative

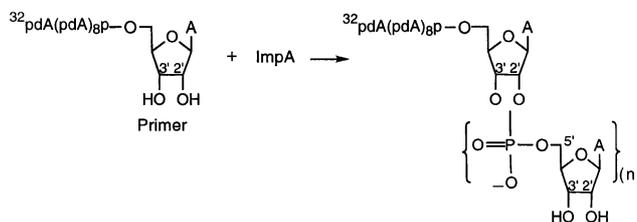


Fig. 2. Elongation of a 10-mer primer by adding activated monomer (ImpA) daily. The maximum chain length of  $n$ , the number of monomer units added, is about 40. The monomers bind to the primer by 2',5'- or 3',5'-links.

as the activating group is a more prebiotic type reagent than the imidazole group because adenine is formed from HCN and is present in the Murchison meteorite.

### 3. Conclusions

Catalysis was essential for the formation of biopolymers on the primitive Earth. The RNA oligomers that initiated the RNA world may have formed by the mineral-catalyzed polymerization of RNA monomers. The oligomers formed contain 2',5'- and 3',5'-links, pyrophosphate groups and some cyclic nucleotides. These oligomers can serve as templates for the formation of the complementary RNA. The montmorillonite catalyst shows sequence selectivity in the oligomers formed and tends to generate an excess of homochiral products (all D and L) from a racemic mixture of starting material. Adding activated monomers to a 10-mer primer daily for 10–14 days period forms RNA oligomers containing chains of more than 40 nucleotides. Relatively short time periods for forming these longer oligomers were observed using 1-methyladenine as the nucleotide activating group.

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