POLYMERIZATION ON THE ROCKS: THEORETICAL INTRODUCTION

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Abstract. It is difficult if not impossible to synthesize long polymers of amino acids, nucleotides, etc., in homogeneous aqueous solution. We suggest that long polymers were synthesized on the surface of minerals in a prebiotic process analogous to solid-phase synthesis. Provided that the affinity of a mineral for an oligomer increases with the length of the oligomer, adsorption must become essentially irreversible for sufficiently long oligomers. Irreversibly adsorbed oligomers may be elongated indefinitely by repeated cycles in which the mineral with its adsorbed oligomers is first incubated with activated monomers and then washed free of deactivated monomer and side-products. We discuss in some detail the formation of oligomers of negatively-charged amino acids such as glutamic acid on anion-exchange minerals such as hydroxylapatite or illite. We show that the average length of adsorbed oligomers at steady state, $\overline{n}$, depends on the balance between the rate of chain elongation and the rate of hydrolysis, and we derive a very approximate formula for $\overline{n}$.

1. Introduction

Most theories of the origin of biological organization assume that organic polymers were formed abiotically on the primitive earth and somehow evolved into a genetic system. There seems to be a consensus that polymer lengths in the range 30–100 would be needed to get a self-replicating system started (Szostak and Ellington, 1993). We are unaware of any reports of synthesis of peptides or oligonucleotides in homogeneous aqueous solution that yield products in this size range. Clearly this obstacle must be overcome before realistic models of early evolution can be proposed.

The major groups of biological polymers are formed from their monomers by dehydration. It is well recognized that water competes to a greater or lesser degree with all such polymerization reactions by attacking the activated monomer. In batch polymerizations, therefore, it is rarely possible to convert a significant proportion of the input of activated monomers to oligomers say ten monomer units long or longer. This is the most important obstacle that must be overcome.

Bernal was the first to propose that molecules adsorbed on minerals, particularly on clays, were essential for the origins of life (Bernal, 1951). Many authors have elaborated on this important idea. They have suggested, for example, that clay minerals acted as catalysts for polymerization reactions. Recently, Ferris and his coworkers have shown that montmorillonite, a clay mineral, does indeed catalyze the formation of oligonucleotides from activated mononucleotides (Ferris, 1993; Ferris and Ertem, 1993). However, the products do not extend much beyond the 10 mer.

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Some years ago we suggested that adsorption of oligonucleotides to anion-exchange minerals such as hydroxylapatite might make possible the prebiotic equivalent of solid phase synthesis (Gibbs *et al*., 1980). Sufficiently long oligomers might adsorb irreversibly to a mineral surface, and these might be elongated indefinitely by incubation in a dilute solution of an activated monomer or by occasional immersion in a more concentrated solution. Here we discuss this idea in detail and in later papers (Hill, Jr. *et al*., 1998; Liu and Orgel, 1998) we present relevant experiments on the polymerization of amino acids. We will show that 'polymerization on the rocks' does allow one to synthesize long oligomers from dilute solutions of activated monomers. A preliminary discussion of some of our results has appeared (Ferris *et al*., 1996).

2. Choices of an Experimental System

The scheme we propose is a general one applicable to any polymer–mineral system in which the affinity of the polymer for the mineral surface increases with the length of the polymer. We have chosen to work mainly with hydroxylapatite because a great deal of information concerning its properties as an adsorbent can be deduced from published work on hydroxylapatite chromatography (Bernardi, 1971, 1973). Hydroxylapatite binds negatively-charged monomers or polymers with increasing affinity as the magnitude of their charge increases. Furthermore, oligomers are readily eluted from the mineral by solutions of inorganic phosphates or pyrophosphates. We have also carried out experiments with illite, a naturally-occurring aluminosilicate, to demonstrate that our results are not restricted to man-made materials.

Among the many candidates for a monomer-activating agent pair, we chose to concentrate on glutamic acid (glu) activated by carbonyl diimidazole (CDI). It is a reaction that we have already shown to proceed efficiently in concentrated aqueous solutions (Böhler *et al*., 1996). The mechanism of the reaction guarantees that no diketopiperazine can be formed (Ehler and Orgel, 1976). Oligomers of glutamic acids adsorb to hydroxylapatite and are eluted efficiently by low concentrations (10–20 mM) of potassium pyrophosphate. Finally, glutamic acid is a very simple molecule, and so can be considered as a model for the kind of negatively-charged monomer that could have accumulated on the primitive earth. We have also carried out less complete series of experiments with aspartic acid and O-phospho-L-serine.

3. Adsorption and Elongation on a Mineral Surface

The free energy of adsorption of a polymer on the surface of a solid will usually depend linearly on the number of residues in the polymer, once a critical length is exceeded. We define $\Delta(\Delta F_n)$ as the additional free energy of adsorption obtained

when a sufficiently long *n* mer is extended to an (*n* + 1) mer. This can be determined from the ratio of distribution coefficients between water and the mineral surface for the *n* mer and (*n* + 1) mer.

$$\Delta(\Delta F_n) = \frac{\text{conc. of } n \text{ mer in solution}}{\text{amount of } n \text{ mer adsorbed}} \Big/ \frac{\text{conc. of } (n\ +\ 1) \text{ mer in solution}}{\text{amount of } (n\ +\ 1) \text{ mer adsorbed}}$$

Direct measurement for oligoglutamic acid on hydroxylapatite suggests that the distribution coefficient increases by a factor of about 4.2 for each added glutamate residue, that is $\Delta(\Delta F_n) \approx$ –850 cals (Hill, Jr. *et al.*, 1998). $\Delta(\Delta F_n)$ is larger for polymers of aspartic acid and serine phosphate, but we could not obtain quantitative estimates because adsorption from aqueous solution was almost complete even for short oligomers.

Another important parameter is the residence time of a polymer adsorbed to a mineral surface. The way in which this quantity varies with length can be estimated roughly from a knowledge of $\Delta(\Delta F_n)$. The rate of adsorption of molecules from the solution onto unoccupied sites on the mineral surface will be determined by the collision rate. This does not vary rapidly with the length of the polymer for sufficiently long polymers. Consequently, since the distribution coefficient is given by $K = K_{\text{on}}/K_{\text{off}}$, the residence time on the surface will increase by a factor of RT $\ln\{\Delta(\Delta F_n)\}$ for each increment of length. In the case of glutamic acid the half-time for desorption should, therefore, increase by about a factor of 4 with each additional residue. Experiments showed that the half-time is long for $(\text{glu})_{10}$ and higher oligomers adsorbed on hydroxylapatite. Consequently oligomers in the size range 20–30 repeating units will not elute in pure water even on a geological time scale.

Consider next the behavior of an adsorbed oligo (glutamic acid) molecule bathed in a dilute solution of activated monomer that is maintained at a constant concentration. Let the half-time for chain extension be $t_{e1/2}$. Oligomers with residence times much less than $t_{e1/2}$, although they may concentrate on the surface, will equilibrate freely between the surface and the solution. Once an oligomer reaches a length such that its residence time is comparable to $t_{e1/2}$, it has a substantial chance of elongating to give a longer oligomer with lifetime about $4t_{e1/2}$. Such a molecule will with even higher probability elongate to give an oligomer with residence time about $16t_{e1/2}$ before it escapes from the surface. At this point the molecule will usually elongate indefinitely, since it has little further chance of escaping.

4. The Consequences of Hydrolysis and Chain Termination

There are two well-recognized processes that may prevent the indefinite elongation of molecules stably adsorbed on a surface, namely hydrolysis and chain termination. They have very different consequences.

For simplicity consider a family of homopolymers, say in the size range 20–30 monomer units adsorbed on a mineral surface. The rate of elongation of the primers should be almost independent of length, but proportional to the concentration of activated intermediate, say $\lambda = k_e c_o$, where k_e is the elongation rate constant and c_o is the concentration of the activated monomer. The rate of hydrolysis of an oligomer of length n would be $(n-1)k_h$, where k_h is the rate constant for the hydrolysis of a bond. We assume that k_h is independent of the length of the oligomer and the position of a bond within the oligomer. In a system in steady state the average molecular weight will be constant, but the number of molecules will increase with time. We may write

$$\overline{n}(\overline{n}-1) \approx \overline{n}^2 \approx \frac{\lambda}{k_h} \tag{1}$$

and

$$\frac{\mathrm{d}N}{\mathrm{d}t} = (\overline{n}-1)k_h \tag{2}$$

where $\overline{n}$ is the mean chain length and N is the number of adsorbed oligomers (see Appendix).

It follows that the mean length of a molecule depends, at first sight counter-intuitively, on $\sqrt{k_e/k_h}$. This places severe restrictions on the length that hydrolyzable molecules can attain. Furthermore, if no molecules are lost from the surface, the number of molecules on the surface grows exponentially with a doubling time of approximately $\log_e 2/(\overline{n}-1)k_h$.

Consider, for example, the difficulty of accumulating long polyesters on a mineral surface. Suppose the half-time for hydrolysis of an ester bond is one week, and that the required mean chain length is 30. Then the half-time for extension must be 1/900 weeks, or about 5 h. It is hard to envisage an elongation process as rapid as this that could occur in aqueous solution on the primitive earth. The half-life for hydrolysis of an internal glycine-glycine peptide bond is about 500 yr at 25 °C (Radzicka and Wolfenden, 1996), so it should be possible to accumulate 30 mers of glycine if the half-time for extension is of the order of 8 months. Hydrolysis would be slower for most amino acids that are substituted on the α-carbon atom, so extension could be slower for them. Formula (1) provides a semi-quantitative justification for the well-recognized conclusion that only polymers that are very stable against hydrolysis can grow to substantial length in an aqueous environment under prebiotic conditions. Oligomers linked by amide or phosphodiester bonds are the most plausible candidates; polyethers, polythioethers, polysulfides, polyureas are among the many other possibilities.

The consequences of (2) are also important, and to some extent counter-intuitive. In the absence of hydrolysis, an increase in the number of oligomers on a surface depends entirely on accumulation of primers from an external source or on the synthesis of primers on the surface. This increase will normally be linear in time.

Hydrolysis, although it restricts the length of polymers on the surface, makes exponential synthesis possible. Chain extension followed by hydrolysis is an autocatalytic mechanism. I have called this the 'Sorcerer's Apprentice' effect (Orgel, 1986). There are two reservations about this conclusion. First the products of hydrolysis must be of the same chemical type as the substrate, so that each product can elongate. Secondly, the average length of the molecules on the surface must be sufficiently long that loss of shorter hydrolysis products from the surface does not reduce the average number of irreversibly adsorbed product molecules produced in each fission to one or less.

Chain termination, like hydrolysis, results in the reduction of the attainable mean chain length, but, unlike hydrolysis, also reduces the number of extendable oligomers. If the probability of chain termination is P_t and the probability of chain extension is $(1-P_t)$, the maximal achievable proportion of oligomers longer than n falls off as $(1-P_t)^{n-1}$. This will not usually be a serious problem; if P_t = 0.05, for example, the proportion of molecules 30 or more residues long is 21%. Combination of chain termination with hydrolysis leads to the regeneration of a growing chain, but one that is shorter than the parental chain-terminated oligomer.

The model described above has interesting consequences when the concentration of the activated intermediate is low. Very few molecules long enough to adsorb irreversibly will be formed. Consequently, each mineral particle is likely to be 'nucleated' by one or a very few primers. Subsequently, rounds of growth and hydrolysis are likely to lead to the coverage of the surface exclusively by the descendants of one or of a very small number of original primers.

5. Elution by Inorganic Salts

The free-energy of adsorption of negatively charged polymers on positively charged mineral surfaces is strongly influenced by dissolved salts, for example sodium chloride or sodium phosphate – this is the basis of hydroxylapatite chromatography (Bernardi, 1971, 1973). The effects of salt concentration must be taken into account in any consideration of prebiotic polymerization reactions on mineral surfaces. Increasing the salt concentration will increase the minimum oligomer length needed for irreversible adsorption. Consequently a high constant concentration of salt as in sea-water may lead to adsorbed products different from those obtained with fresh water.

Variations in salt concentration provide a mechanism for dispersing polymers – as in anion exchange chromatography, oligomers accumulated on a surface at low salt concentration will be desorbed at higher salt concentrations. They may then be re-adsorbed at new sites when the salt concentration falls again. On the one hand, dispersion may favor replication when a mineral surface becomes completely covered with polymer. On the other, dispersion is a disruptive process, and

may be disadvantageous in heteropolymer systems if replication depends on the simultaneous presence of two or more macromolecules.

6. Copolymers

The presence of more than one activated amino acid in the homogeneous phase greatly complicates the pattern of oligomers formed on the mineral surface. Presumably, negatively-charged amino acids will be represented disproportionately in the short oligomeric primers which initiate oligomerization on the surface, since the strength of adsorption increases rapidly with negative charge. However, once a firm 'anchor' is formed, there is not necessarily any advantage to accumulating further negative charge. The exploration of multicomponent systems, particularly the search for template-effects, is a part of our future program.

7. Conclusions

Minerals with anion-exchange properties may have functioned in the prebiotic synthesis of negatively-charged polymers in much the same way that solid supports function in the laboratory synthesis of peptides and oligonucleotides. Once the size of an oligomer exceeds a certain minimum, adsorption is essentially irreversible. Then a molecule on the surface can be extended indefinitely, without interference from unactivated monomers or short oligomers in solution. The latter can be removed by 'washing', as in conventional solid-phase procedures. In principle, mineral surfaces in contact with very dilute solutions of activated monomers could in time become covered with high molecular-weight material.

The production by this method of long polymers from dilute solutions of polymers is, of course, a very inefficient process. Unless the mineral surface catalyzes the polymerization process very effectively, the great majority of activated monomers hydrolyze in solution or condense to short oligomers that are washed away. The production of high local concentrations of long oligomers adsorbed to mineral surfaces is achieved only at a very great cost in the efficiency of utilization of activated molecules. However, if a genetic system can only be formed from long oligomers, it is better to produce them inefficiently than not to produce them at all.

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Appendix

We assume that the rate of extension of a chain of length *n* by addition of monomers having concentration c_o is $k_e c_o = \lambda$, independent of *n*. We also assume that the rate of hydrolysis of a chain of length *n* is $k_h(n-1)$. We define

$$\overline{n}(t) = \sum p_n n(t) \,, \tag{1}$$

where p_n is the proportion of adsorbed molecules with length *n*.

First consider the consequence of chain extension: after time Δt the probability of extension of any chain is $\lambda \Delta t$. Consequently, after summing over *n*,

$$\overline{n}(t + \Delta t) = \overline{n}(t) + \lambda \Delta t \,.$$

Next consider the consequences of hydrolysis for a molecule of length *n*. After time $\Delta(t)$ the probability of hydrolysis is $k_h(n-1)\Delta t$. Then the average number of molecules present is $1 + k_h(n-1)\Delta t$ and the average length declines to

$$\frac{n}{1 + k_h(n-1)\Delta t} \approx n\{1 - k_h(n-1)\Delta t\} \,.$$

Substituting in Equation (1)

$$\begin{aligned}
\overline{n}(t + \Delta t) &= \sum p_n n\{1 - k_h(n-1)\Delta t\} \\
&= \overline{n}(t) - \sum p_n k_h n(n-1)\Delta t \\
&\approx \overline{n}(t) - \sum p_n k_h n^2 \Delta t \\
&= \overline{n}(t) - k_h(\overline{n^2})\Delta t \,.
\end{aligned}$$

At steady state, the rates of extension by addition of monomers and shortening by hydrolysis must be equal. Thus:

$$\lambda \approx k_h(\overline{n^2})$$

Provided the distribution of lengths is reasonably sharp (Hill, Jr. *et al.*, 1997)

$$\lambda \approx k_h \overline{n}^2$$

$$\overline{n} \approx \sqrt{\frac{\lambda}{k_h}} \,.$$

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