

The Role of Metal Ions in Chemical Evolution: Polymerization of Alanine and Glycine in a Cation-Exchanged Clay Environment

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Summary. The effect of the exchangeable cation on the condensation of glycine and alanine was investigated using a series of homoionic bentonites. A cycling procedure of drying, warming and wetting was employed. Peptide bond formation was observed, and the effectiveness of metal ions to catalyze the condensation was $\text{Cu}^{2+} > \text{Ni}^{2+} \approx \text{Zn}^{2+} > \text{Na}^+$. Glycine showed 6% of the monomer incorporated into oligomers with the largest detected being the pentamer. Alanine showed less peptide bond formation (a maximum of 2%) and only the dimer was observed.

Introduction

Various models have been proposed for the composition of the primitive atmosphere on Earth, and, when such atmospheres are subjected to energy inputs (e.g., electrical discharge, heat, UV irradiation), organic molecules of varying degrees of complexity are formed (Miller and Orgel, 1974). Support for the theory of chemical evolution has also been found by the identification of complex organic compounds in extraterrestrial materials such as meteorites (Kvenvolden et al., 1971; Lawless et al., 1971; Lawless et al., 1974). But, in addition to the biological synthesis of organic molecules in primitive environments, a number of other steps in the origin of life process must have occurred. Among these are: 1) a selection for the biological subset of monomers from the random population of organic compounds available, 2) a mechanism for the concentration of this subset so that subsequent interactions and chemical reactions could occur, and 3) a mechanism for the formation of polymers such as polypeptides and polynucleotides which is a necessary preamble to the formation of proteins and nucleic acids. To elucidate the latter, several investigators have attempted to carry out prebiotic synthesis of polypeptides. A large number of polymeric substances has been made using metaphosphate esters as the condensing agent (Schramm et al., 1962; Schramm, 1965). These polymers were obtained by heating the metaphosphate esters of the monomer to above 100°C. Others have made polyamino acids from free amino acids by heating them on pieces of lava at 170°C. By this method spheres of poly-

peptides have been produced which have some catalytic activity (Fox and Harada, 1960; Rohlfing and Fox, 1963).

Using amino acids in the form of adenylates (Katchalsky, 1973), it has been shown that polymerization of these high-energy molecules occurs in the presence of montmorillonite at a pH close to 7.0. After a few hours, polypeptides containing up to 60 amino acids were produced with the distribution showing a discrete spectrum of the chain lengths (Paecht-Horowitz et al., 1970). Diglycine has been synthesized by heating glycine in the presence of hydroxyapatite or orthophosphate. The yield of diglycine was increased by the addition of inorganic cyanate (Flores and Leckie, 1973). Amino acids have also been condensed in aqueous solution using cyanamide as a condensing agent (Ponnamperuma and Peterson, 1965; Flores and Ponnamperuma, 1972), and using carbodiimide (Cavadore and Previero, 1969).

These approaches to peptide synthesis use different ways to overcome the thermodynamic barrier for the polymerization of amino acids. Heating to high temperatures, using condensing agents, or activating the amino acids before polymerization places severe restrictions on the general concept of abiogenic amino acid condensations under primitive earth conditions.

Recently (Lahav et al., 1978), glycine was polymerized on both bentonite and kaolinite by applying wetting, drying, and heating cycles to the clay-glycine system. The clay-glycine system was heated to 94°C and polypeptides containing up to five glycine units were detected. Fluctuating systems like these might correspond to cyclic wetting and evaporation from lagoons and small lakes. This method for polymerizing amino acids has the obvious advantage of not using activated amino acids, high temperatures, or condensing agents, and thus appears to be more reflective of possible primordial conditions.

Clays have been considered by some investigators (Katchalsky, 1973; Paecht-Horowitz et al., 1970; Lahav et al., 1978; Lahav and Chang, 1976; Cairns-Smith, 1971) since Bernal (1951) suggested them as providing the most likely surface onto which small molecules could be adsorbed and concentrated as a first step leading to condensation. However, in the pH range (7.5 ± 1) believed to have prevailed in the primitive ocean, amino acids are not readily adsorbed.

In previous studies we have found that at a pH of about 7.0 cation-exchanged clays can selectively concentrate by adsorption (Lawless et al., 1977) and selectively decarboxylate (Levi and Lawless, 1977) different classes of amino acids, sparing those protein amino acids which are used by our present-day biology. These results implied that the interactions of amino acids with various cation-exchanged clays were important in prebiotic chemistry. This importance is reflected in the necessary presence of metal ions in terrestrial life. It has been speculated (Lahav and Chang, 1976), however, that amino acids strongly adsorbed on clays would not be as effective in subsequent polymerization reactions as their less strongly adsorbed counterparts. This paper reports a study of the effect of metal ions in the clay matrix on the polymerization products and chain length of oligopeptides formed when amino acid clay mixtures are subjected to a fluctuating environment of wetting, drying, and heating.

Experimental

The clay used was bentonite B-235 (Fisher Scientific) cation-exchanged with one metal ion Na^+ , Cu^{+2} , Zn^{+2} , or Ni^{+2} . The method of preparing the cation-exchanged clay has been described elsewhere (Theng, 1974).

Two ml of 2×10^{-2} molar solution (4×10^{-5} moles) of glycine (or alanine) in triply distilled water at neutral pH was added to 20 mg bentonite in a round bottom tube. The suspension was sonicated for 1 min three times, mechanically suspended after each sonication, and stored at 4°C for one day. The fluctuating conditions used were as follows: 1) drying the clay-amino acid in a vacuum oven at 45°C for two days, 2) heating at 94°C for five days, 3) addition of 1/2 ml of water, followed by sonication and mechanical suspension as above. These steps were repeated eight times. Each of the different homoionic clays used in this study was subjected to exactly the same procedures. Thus, differences in oligopeptide yield and chain length can be attributed to the effect of the metal ion in the cation-exchanged clay. In the absence of clay, no oligomer formation was observed when alanine and glycine were cycled as above.

After the eight cycles, the products were extracted from the clay with 2 ml of 3N NH_4OH by sonicating three times for 20 min with mechanical shaking between followed by 12 h at 4°C . The clays were centrifuged and the NH_4OH supernatant removed. This procedure was repeated and the solutions combined. Studies (Lahav et al., 1978) have shown that diglycine can be removed in this manner from Na^+ -clays with no detectable hydrolysis. Identification of glycine and alanine oligopeptides was based on their elution times in an automatic amino acid analyzer (Beckman 120C) as compared with known oligopeptide standards. Analysis by both amino acid analyzer and gas chromatography showed only compounds attributable to the experiments and showed no contaminants.

Results and Discussion

Table 1 shows the percent oligomerization for glycine as a function of the metal cation. As can be seen, substitution of the exchangeable cation affects both the polymer yield and the chain length appreciably. Copper was the most effective metal for production of high-yield, long-chain oligoglycine while sodium was the least effective. The gradual decrease in concentration of the higher homologs on the copper-clay indicates with

Table 1. Oligomerization of glycine on homoionic-bentonite^a

	Di	Tri	Tetra	Penta	Σ	Percent polymerization ^b
Na	17.2 ^a	---	---	---	17.2	0.9
Ni	29.5	6.3	---	---	35.8	1.8
Cu	69.9	41.4	10.4	2.8	123.6	6.2
Zn	25.6	3.3	---	---	28.9	1.4

^aAll results are expressed as nmoles of glycine incorporated in the product/mg clay; initial concentration $2 \mu\text{moles glycine/mg clay}$

^bGiven as percent of the monomer incorporated in oligomers

Table 2. Oligomerization of D, L alanine on homoionic-bentonite^a

	Di	Percent polymerization ^b
Na	14.6	0.7
Ni	21.2	1.1
Cu	40.6	2.0
Zn	24.6	1.2

^aAll results are expressed as nmoles of alanine incorporated in the product/mg clay; initial concentration 2 μ moles alanine/mg clay

^bGiven as percent of the monomer incorporated in oligomers

some certainty that small amounts of successive higher homologs exist; since other investigators found that peptide yield on the sodium-clay reaches a maximum for glycine after 11 to 27 cycles (Lahav et al., 1978), it is felt that more than eight cycles should produce higher yields and longer chains of oligoglycine on the copper and other cation-exchanged clays. This is the subject of further studies. Oligomerization of alanine also occurs on the same clays (Table 2) but at a lower yield.

This difference is attributed to a higher activation energy for alanine peptide bond formation than for the corresponding glycine peptide bond formation. The preferential oligomerization of glycine over alanine is most pronounced in the Cu-bentonite matrix, with the yield of oligoglycine being 3.1 times the yield of oligoalanine. The difference in the relative yield becomes very small when the condensation is taking place in the Zn-bentonite matrix. This observation suggests that the amino acids incorporated into the oligopeptides could possibly be selected from a mixture, as a function of the relative concentration of the cation present on the clay.

The mechanism believed to be operative for polymerization is the formation of metal-glycine and of metal-alanine complexes both in aqueous solution and in the interlayer space of the clay. This complex formation is favored at neutral pH values (Theng, 1974). As the amino acid-clay system is dried, the acidity of the clay surface increases (Solomon et al., 1971; Lahav, 1972) and, under such conditions, the carboxyl group of the amino acid is 'free' (Jang and Condrate, 1972).

While the chemical synthesis of a peptide bond involves the activation of either the carboxyl or the amino group (Bodansky and Ondetti, 1966), generally it is the former group which is activated to make its carbon atom more electrophilic and hence more susceptible to nucleophilic attack.

Thus, the relatively high yield of polypeptides obtained in our experiments suggests that the transition metals are responsible, through direct or indirect interactions, for the activation of the carboxyl carbon, making it susceptible to a nucleophilic addition by the amino group on a noncomplexed amino acid. Since the stability constants of the transition metals with peptides are lower than with the amino acids (Greenstein and Winitz, 1961), it is proposed that with every wetting cycle oligopeptides are replaced by amino acids and that this redistribution of oligomers frees them for subsequent

reactions. Such a mechanism would account for the decreasing concentration of the oligomers with increasing chain length (Table 1).

It is concluded that, by using various cation-exchanged clays, the yield of oligopeptides is significantly increased and, rather than decreasing reactivity (Lahav and Chang, 1976), the strongly adsorbed amino acids have increased reactivity.

The results reported here present the opportunity to consider the broader questions regarding the origin of life. It has been suggested that metal-ions were important in the processes which led to the origin of life (Egami, 1974; McClendon, 1976; Orgel, 1974) and that the metal ion content of living systems is a reflection of this importance (Banin and Navrot, 1975).

It has also been suggested that clays were important in the initiation of chemical evolution, and that the primitive genetic material was probably a crystal of some sort and possibly a layer-lattice silicate (Cairns-Smith, 1971). The data reported here, plus the selective adsorption and selection decarboxylation of amino acids (a detailed discussion of these latter results will be presented elsewhere) suggest that, rather than considering each inorganic component (metal-ions and clays) separately, the simultaneous interaction of both clays and metal ions with organic molecules should be considered. It thus appears that the metal ions in the clay matrix could have played a key role in the processes believed to have been important for the origin of life on earth.

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