J Mol Evol (1990) 30:391–399

Journal of Molecular Evolution

Nonrandomness in Prebiotic Peptide Synthesis

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Summary. We have synthesized and studied the properties of phosphoanhydrides of alanine with guanosine monophosphate, uridine monophosphate, and adenosine monophosphate. This series of compounds allowed us to investigate the specificity of peptide bond formation in a reaction that could have taken place on the prebiotic earth. We asked whether the intrinsic reactivity of the amino acids, the nature of the nucleotide in the anhydride, or the complementary polynucleotide template influences the specificity of the peptide synthesis reaction. We observed that the differential reactivity of the amino acids results in nearest-neighbor preferences during the peptide synthesis, whereas the nature of the nucleotides and the presence of complementary polynucleotides had no influence on the specificity. These results suggest that some peptides would have been more abundant than others on the prebiotic earth and have implications for the study of the origins of the genetic code and protein synthesis.

Key words: Amino acyl nucleotide anhydrides — Specificity in prebiotic peptides — Origin of the genetic code — Origin of protein synthesis — Template effects

Introduction

The origin of protein synthesis is an important aspect of the processes responsible for the origin of life. Spontaneous synthesis of polypeptides from phosphoanhydrides of amino acids and AMP could have played an important role in the early stages of the evolution of protein synthesis by contributing to the pool of polypeptides on the prebiotic earth. These anhydrides appear as active intermediates during the biosynthesis of proteins (Schimmel and Soll 1979). They spontaneously produce peptides in dilute aqueous solutions (Lewinsohn et al. 1967; Paecht-Horowitz and Katchalsky 1967) and could have existed on the prebiotic earth (Mullins and Lacey 1981). Their prebiotic relevance was further appreciated by the observation that certain clays and surfactants dramatically increase the yields and lengths of the peptides produced (Paecht-Horowitz et al. 1970; Armstrong et al. 1978). Anticipating that the amino acyl anhydrides of other nucleotides would also be capable of producing peptides, we have synthesized and studied the properties of alanyl anhydrides of AMP, GMP, and UMP. This series of compounds allows us to address questions concerning the specificity of prebiotic peptide synthesis and the origin of the genetic code. These compounds contain an element from each of the separate worlds of nucleic acids and proteins and may provide the elusive interface that originally brought them together.

It is often assumed in the theories concerning the origin of life that peptides produced in noninstructed syntheses would be random in their sequences (Crick et al. 1976; Eigen and Schuster 1977; Cairns-Smith 1982; Dyson 1985; Weiner and Maizels 1987). However, the differential reactivities of amino acids should influence the order of amino acids in peptides, especially at the nearest-neighbor level. Also, the activating groups attached to the amino acids should influence the outcome of peptide synthesis reactions. Here, we describe experiments that search

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for order in spontaneously assembled peptides brought about by differences in the intrinsic reactivities of amino acids and by the effect of different activating groups (nucleotides).

Research on the origin of the genetic code has led to the hypothesis that the weak affinities that exist between amino acids and their anticodonic nucleotides could have been responsible for the origin of the genetic code (Lacey and Mullins 1983; Khaled et al. 1984; Senaratne 1986; Yarus 1988). An investigation of spontaneous peptide synthesis by amino acyl anhydrides of different nucleotides offers the possibility of determining whether such physical affinities could influence the order of peptide assembly.

Peptide synthesis from amino acyl nucleotide anhydrides occurs in two steps: First, hydrolysis of the anhydride produces a free amino acid and a nucleoside monophosphate. Then, the free amino acid, which is a nucleophile, attacks the carbonyl group of another anhydride molecule, giving rise to a dipeptide. This dipeptide is also a nucleophile, so it too can attack an anhydride, leading to the synthesis of a tripeptide. This reaction continues until the available anhydride is exhausted, resulting in the synthesis of a series of oligopeptides (Katchalsky and Ailam 1967). Utilizing this mechanism, we allowed several different amino acids to compete for the formation of peptide bonds, to explore the degree of ordering in the oligopeptide products, and to study the effect of the nature of the nucleotide within the anhydride on this ordering. Each amino acyl nucleotide anhydride was reacted simultaneously with the set of homocodonic amino acids (glycine, proline, phenylalanine, and lysine). Homocodonic amino acids were chosen so that one of them would be able to find its anticodonic nucleotide in the anhydride, thus improving its chance of forming a peptide bond. We chose a low initial concentration of the anhydride and a high initial concentration of the amino acids to ensure that there would be little polymerization beyond the dimer stage. This reaction is illustrated below:

$$\text{Ala–NMP} + \left\{\begin{matrix}\text{Gly}\\ \text{Pro}\\ \text{Phe}\\ \text{Lys}\end{matrix}\right\} \rightarrow \text{NMP} + \left\{\begin{matrix}\text{Ala–Gly}\\ \text{Ala–Pro}\\ \text{Ala–Phe}\\ \text{Ala–Lys}\end{matrix}\right\}$$

Because an equimolar concentration of each of the four amino acids was used, the relative concentrations of the resultant dipeptides would indicate the capacity of each amino acid to form a peptide bond. A variation in these concentrations would suggest that there is specificity in the formation of peptide bonds.

One of the requirements of a model for prebiotic peptide synthesis is that it should be able to evolve into a system, which, like current protein biosynthesis, takes instructions from polynucleotides. It is possible to envisage a primitive form of such a system: Amino acyl nucleotide anhydrides could interact with polynucleotides through complementary hydrogen bond interactions, and then the orientation effect of these interactions would enhance the yield of peptides. An increase in the rate of peptide synthesis, brought about by polynucleotides, could be considered as a rudimentary form of translation, where the nucleotide part of the anhydride molecule functions as a single nucleotide adapter. The idea of the single nucleotide adapter, first proposed by Lacey et al. (1975), is a simpler version of a number of different models that have been developed for the mechanism of prebiotic translation (Crick et al. 1976; Hopfield 1978; Tyagi 1981; Kuhn and Waser 1982).

As a test of this proposition, each of the three anhydrides was used to carry out peptide synthesis in the presence of its complementary homopolynucleotide under conditions that favor homopolymer–monomer interactions (Howard et al. 1966; Bridson and Orgel 1980; Miles and Frazier 1982; Joyce et al. 1984). If polynucleotides can bring the anhydride molecules close to each other, the degree of polymerization of alanine would increase, as compared to the degree of polymerization when polynucleotides are absent. We compared the pattern of oligopeptide concentrations produced in the presence of the polynucleotide with the pattern in its absence.

Materials and Methods

Materials. *N-t-Boc*-L-alanine, amino acids, peptides, octylsulfonic acid, fluorescamine, the acid forms of the nucleoside 5′ monophosphate, and the homopolynucleotides were obtained from Sigma Chemical Co. The nucleotides were tested for impurities by high-pressure liquid chromatography (HPLC). Because no impurities were found, they were used directly. All reagents for the synthesis of amino acyl nucleotide anhydrides were obtained from Aldrich Chemical Co. The solvents were dried over sodium before use.

Analytical Procedures. HPLC was used to analyze and quantify the substrates and the products in each reaction. A Waters 6000A or a Dupont 830 solvent delivery system and reverse-phase C-18 (Whatman or Supelcosil) columns (25 × 0.46 cm) were used in conjunction with a UV (Dupont) or a fluorescence (American Instrument Co.) detector. The amino acids, peptides, and amino acyl nucleotide anhydrides were detected by postcolumn derivatization with fluorescamine (Benson and Hare 1975). The mobile phase was 10 mM KH_2PO_4 (pH 3.0), unless otherwise identified in the figure legend.

Synthesis, Purification, and Assay of Amino Acyl Nucleotide Anhydrides. Amino acyl nucleotide anhydrides were prepared by the condensation of alanine with AMP, GMP, or UMP, utilizing a method adopted from Armstrong et al. (1979). *Sec*-butyl-chlo-

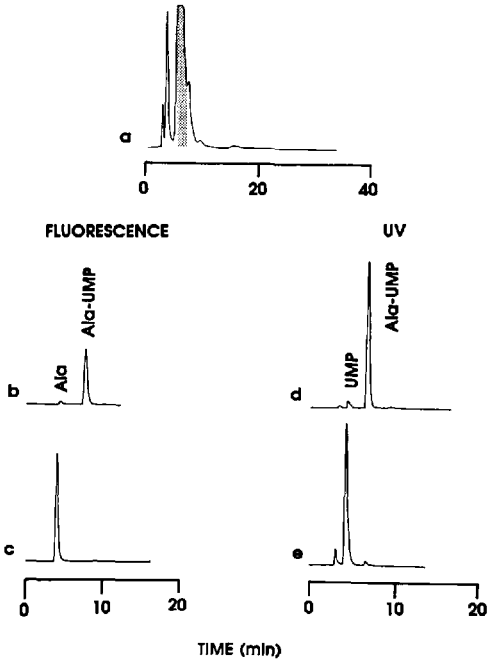


Fig. 1. Purification and assay of Ala–UMP. The effluent representing the shaded areas in the preparative chromatogram (a) was analyzed using a fluorescence and a UV detector. The purified anhydride preparation contained very small amounts of free alanine (b) and UMP (d). After several minutes of boiling (at acidic pH), all of the Ala–UMP was hydrolyzed, generating equimolar amounts of free alanine (c) and UMP (e), the concentrations of which were determined by comparisons with known standards. For the chromatogram (a) the mobile phase was 1 mM KH_2PO_4 (pH 3.0).

roformate, which was used by Armstrong et al. (1979) as a condensing agent, was replaced by *n*-butyl-chloroformate (Tyagi 1987). The alanyl anhydride of CMP could not be made with this procedure. Stringent anhydrous conditions were employed throughout the synthesis. Usually, about 45% of the dry weight of the preparations were amino acyl nucleotide anhydrides, the rest of the material being free amino acid and free nucleotide. Upon storage, additional unidentified impurities appeared in the preparation. The anhydrides were isolated from this mixture on a reverse-phase (Whatman) preparative column (25 × 0.92 cm). In order to minimize hydrolysis during purification, the mobile phase and the column were chilled on ice, and an acidic buffer was used for elution because the amino acyl nucleotide anhydrides are more stable in the acidic pH range (Lewinsohn et al. 1967; Lacey et al. 1984).

A dry anhydride preparation (18–20 mg) was dissolved in 160 μl distilled water and filtered through a Millex-HV4 filter (Millipore Corporation) before loading on the chromatograph. The effluent (7–8 ml) from the shaded peak (Fig. 1a) was collected over ice, acidified with 4 μl of 6 N HCl, and stored in liquid nitrogen. Solutions of all the anhydrides could be stored in this manner for at least a month without degradation. If necessary, the preparations were concentrated by lyophilization. The purity and concentration of each preparation was established by chromatographic analysis prior to use.

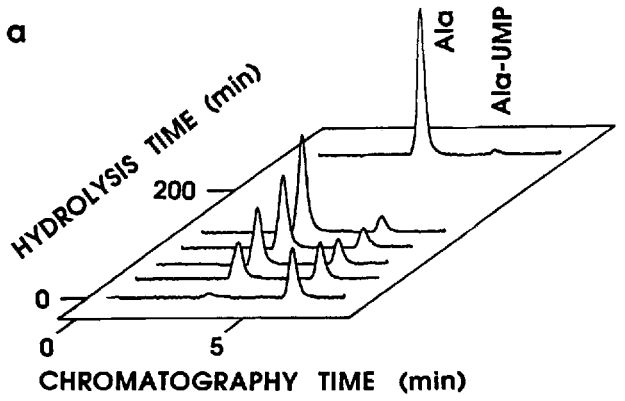
Traditionally, the amino acyl adenylates have been assayed by the hydroxamate assay procedure (Lipmann and Tuttle 1945; Moldave et al. 1959), which detects the presence of anhydride bonds. The hydroxamate assay, however, is relatively insensitive and is not applicable to submillimolar concentrations. Therefore, a chromatographic assay was developed for this purpose. Each anhydride preparation was analyzed by HPLC. The peak corresponding to the anhydride in the chromatogram was identified by its property of releasing equimolar amounts of free alanine and nucleotide upon boiling (at acidic pH); exhibiting a positive hydroxamate reaction; and, under basic conditions, giving rise to oligopeptides. The concentration of the anhydride was determined by the amount of alanine or the amount of nucleotide released upon boiling (Fig. 1b–e). Concentrations obtained from the measurements of the two compounds were in close agreement. The linearity of the estimation range was established by using a number of known alanine and nucleotide standards. Because the fluorescence detection of alanine gave better correlations, it was used for the routine estimation of anhydride concentrations. The sensitivity of the assay was 5 μM.

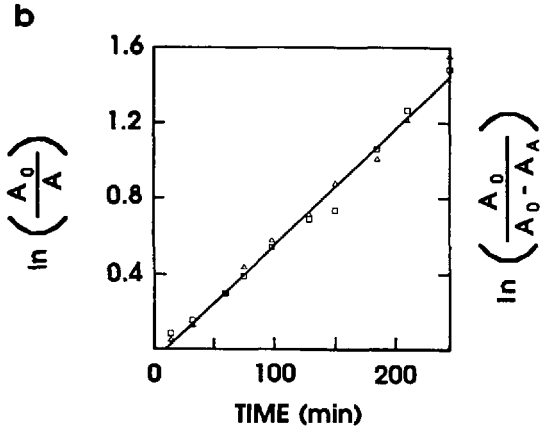
Determination of Rates of Hydrolysis. All the hydrolysis reactions were carried out at 29°C in 2 ml of 0.5 M buffers. The following buffers were used: for the pH range 1–2.1, HCl–KCl; for the pH range 2.5–4.3, KH_2PO_4–H_3PO_4; for pH 5.7, citric acid and sodium citrate; for pH 6.7 and 7.5, Na_2HPO_4–NaH_2PO_4; and for pH 8.0 and 10.5, boric acid and potassium borate. These buffers were prepared according to the methods of Gomori (1955). For hydrolysis reactions in the pH range 1–4.6, the concentrations of the anhydrides were: 0.18 mM Ala–UMP, 0.18 mM Ala–GMP, and 0.07 mM Ala–AMP; and for the pH range, 5.7–10.5, the concentrations were: 8.85 μM Ala–UMP, 17.6 μM Ala–GMP, and 7.28 μM Ala–AMP. The hydrolysis reactions were analyzed in real time in the pH range 1–4.6. At higher pH, however, these reactions were too fast to follow. Therefore, for the pH range 5.7–10.5, the reactions were quenched at various times and analyzed later. Aliquots (400 μl) were withdrawn at various times, mixed with 4 μl 6 N HCl, and immediately frozen in liquid nitrogen. Later, these frozen samples were analyzed by HPLC. The addition of the acid minimized hydrolysis between thawing and chromatography.

Peptide Synthesis in the Absence of Exogenous Amino Acids. Reactions containing 100 μl of 100 mM Ala–GMP in 1.2 M 2,6-lutidine buffer (pH 8.0) were carried out in triplicate for 24 h at 4°C. The reaction mixtures retained their pH at the end of the reactions. In order to remove 2,6-lutidine, the samples were repeatedly freeze dried and redissolved in water until its smell disappeared. Each sample was dissolved in 5 ml water containing 100 μl trifluoroacetic acid and diluted 1–5 with the running buffer used for HPLC. The synthesis of oligopeptides from Ala–UMP and Ala–AMP were performed in the same manner, except that the reactions were initiated with 500 mM anhydride in 1 ml of 20 mM sodium borate buffer (pH 8.0). The pH of the samples were adjusted to 3 by the addition of 1 N HCl before analysis.

Peptide Synthesis in the Presence of Exogenous Amino Acids. In the experiments with a mixture of homocodonic amino acids, three 1-ml reactions, containing 0.5 mM each of glycine, proline, phenylalanine, and lysine, 0.5 mM of one of the alanyl nucleotide anhydrides, and 20 mM sodium borate (pH 8.0), were incubated at 29°C for 24 h. In the experiments where only one amino acid was reacted with an anhydride, each reaction mixture contained 0.5 mM of one of the anhydrides and 0.5 mM of the amino acid. The identity of the dipeptides among the products was established by a comparison with known standards, whereas the identity of the larger peptides was inferred from their relative mobility.

Effect of Complementary Homopolynucleotides on Peptide Synthesis. Reaction mixtures (100 μl), containing 50 mM polycytidine [poly(C)], 50 mM Ala–GMP, 50 mM NaCl, 50 mM $MgCl_2$, and 1.1 M 2,6-lutidine buffer (pH 8.0), were incubated for 1 week at 4°C with constant stirring. The control reactions contained only 50 mM Ala–GMP and the buffer. Poly(C), NaCl, and $MgCl_2$ were lyophilized in the bottom of the tube before





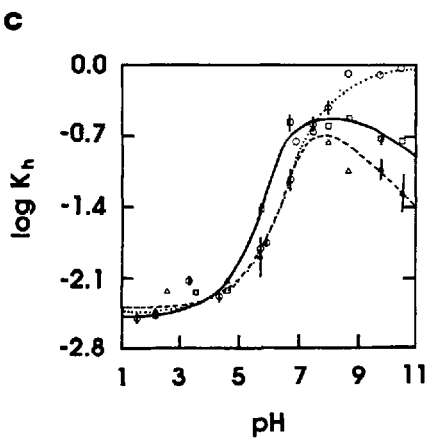


Fig. 2. Determination of the rates of hydrolysis of alanyl nucleotide anhydrides. **a** Hydrolysis of Ala–UMP at pH 2.1 was followed by analyzing the reaction mixture at different times by HPLC. **b** The disappearance of Ala–UMP (△) and the appearance of alanine (□) both follow first order kinetics. A_0 is the initial amount of Ala–UMP; A is the amount of Ala–UMP as a function of time; and A_A is the amount of alanine as a function of time. The slope of the line represents the hydrolysis rate constant K_h. **c** K_h was plotted as function of pH for Ala–UMP (□), Ala–GMP (○), and Ala–AMP (△). The standard deviation of estimation is displayed by the error bars for the values where more than two different measurements of the hydrolysis rate constant were made.

addition of the buffer and the anhydride. There was always some material that remained insoluble. At the end of the reaction, the mixtures maintained their pH. The volatile buffer was removed by repeated lyophilization and dissolution in water. Water (5 ml) and 100 µl of trifluoroacetic acid were added to dissolve the peptides. The precipitated RNA was removed by passing the solution through Millex filters. Amino acid analysis of the precipitate after complete acid hydrolysis showed that it contained no significant amounts of peptides. The supernatant was diluted 1–5 with the running buffer used for HPLC.

Results

Hydrolysis of Amino Acyl Nucleotide Anhydrides

The amino acyl anhydrides of AMP are very unstable molecules as they rapidly hydrolyze in aqueous solutions (Lewinsohn et al. 1967; Lacey et al. 1984). Before the anhydrides of other nucleotides could be used, it was necessary to establish their comparative hydrolytic behavior. The rate of hydrolysis of amino acyl nucleotide anhydrides is highly dependent on pH: their half-life ranges from 200 min at pH 2 to only a few minutes at pH 8. At neutral and basic pH, the hydrolysis is accompanied by peptide synthesis (Lewinsohn et al. 1967). In order to minimize the contribution of peptide synthesis to the disappearance of the anhydride, the hydrolysis reactions were carried out at very low concentrations (micromolar) of the anhydrides. Figure 2a shows the kinetics of hydrolysis of Ala–UMP at pH 2.1. The disappearance of Ala–UMP, as well as the appearance of free alanine, follows pseudo first-order kinetics, as shown in Fig. 2b. The slope of the line indicates that the hydrolysis rate is constant. Using the kinetics of the disappearance of the anhydride, the hydrolysis rate constants of all three anhydrides were determined in the pH range 2.1–10.5 (Fig. 2c). The three anhydrides have very similar hydrolysis profiles, although they tend to diverge in the basic pH range.

Below pH 5, all the Ala–UMP that disappears can be accounted for by the appearance of alanine and UMP. However, above pH 5, more Ala–UMP disappears than appears as alanine and UMP. Additional species appear early during the hydrolysis and then disappear slowly (Fig. 3). The two new species contain both the amino acid and the nucleotide moieties. These two species were isolated. Both of them were found to hydrolyze at a slower rate than that of the anhydride. Their UV spectra had absorbance maxima indistinguishable from UMP and Ala–UMP. Upon boiling, the two species interconverted with each other and broke down into alanine and UMP. The appearance of these species was independent of the initial concentration of the

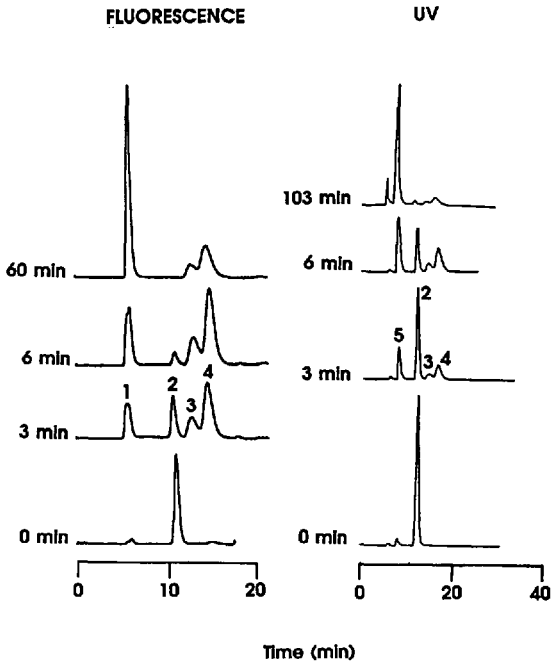


Fig. 3. Detection of intermediates during the hydrolysis of Ala–UMP at pH 6.7. The hydrolysis reactions were stopped at different times and then analyzed by HPLC. The detection in the left panel is based upon the derivatization of primary amino groups by fluorescamine, and the detection in the right panel is based upon UV absorption of the nucleotide moiety. The peaks are numbered as follows: 1, alanine; 2, Ala–UMP; 3 and 4, intermediates; and 5, UMP.

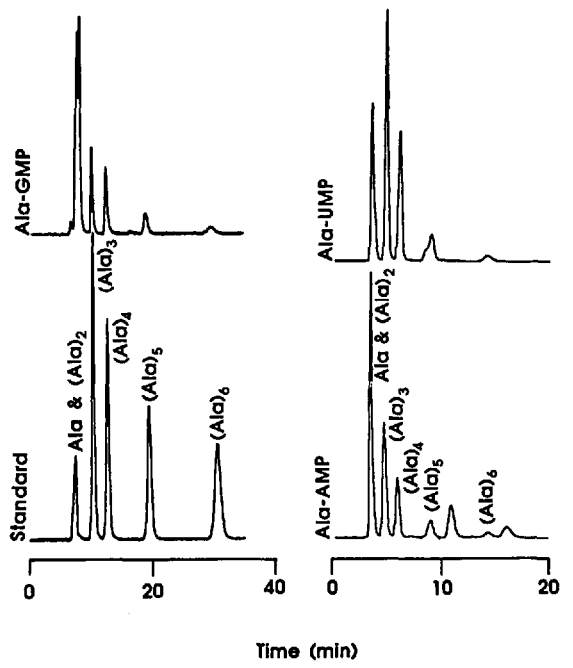


Fig. 4. Chromatographic separation of the oligopeptides produced by alanyl nucleotide anhydrides. A standard containing an equimolar mixture of Ala, $(Ala)_2$, $(Ala)_3$, . . . , and $(Ala)_6$ is included for comparison.

anhydrides. It is likely that the intermediates are 2′(3′)-amino acyl esters, produced by intramolecular migration of alanine from the phosphate to the 2′- or the 3′-hydroxyl group. These intermediates were observed during the hydrolysis of Ala–UMP and Ala–GMP, but not during the hydrolysis of Ala–AMP.

Peptide Synthesis

The enhanced rate of hydrolysis in the basic pH range frees alanine to participate in peptide synthesis, initiating a chain reaction that produces peptides of various lengths. The extent of polymerization depended upon the initial concentration of the anhydrides: the higher the initial concentration of the anhydrides, the higher the extent and degree of polymerization. A 0.1–0.5 M initial concentration of Ala–GMP, Ala–AMP, orAla–UMP gave rise to peptides as large as a hexamer (Fig. 4). When a dry powder of the anhydride was added during the course of the reaction, in order to keep a high steady-state concentration, longer polymers of alanine were seen. The extent and degree of polymerization was independent of the identity of the nucleotide in the anhydride. Peptide synthesis occurred with all three anhydrides over the entire basic pH range. In the acidic pH range, no peptides were synthesized.

Nonrandom Synthesis of Peptides

If other amino acids are present with the amino acyl nucleotide anhydrides, they would be incorporated into the peptides. In order to determine if amino acids differ in their capacity to take part in the peptide bond-forming reaction, an equimolar mixture of glycine, proline, phenylalanine, and lysine was reacted with each of the three anhydrides, in three separate reactions, under conditions that resulted in mainly dipeptide products. The products were separated, identified, and quantified by HPLC. Typical chromatograms are shown in Fig. 5. The yield of each dipeptide was determined by comparison with known standards. The results are shown in Table 1. In each of the three reactions, the relative yield of each dipeptide was different, even though the initial concentration of each amino acid was the same. The yield of Ala–Pro was markedly higher than the other dipeptides in each reaction. However, the pattern of dipeptide formation was very similar, no matter which alanyl nucleotide anhydride was used.

An additional series of experiments was carried out to further explore the differences in the reactivities of amino acids. In these reactions, only one of the four amino acids was present. Under these con-

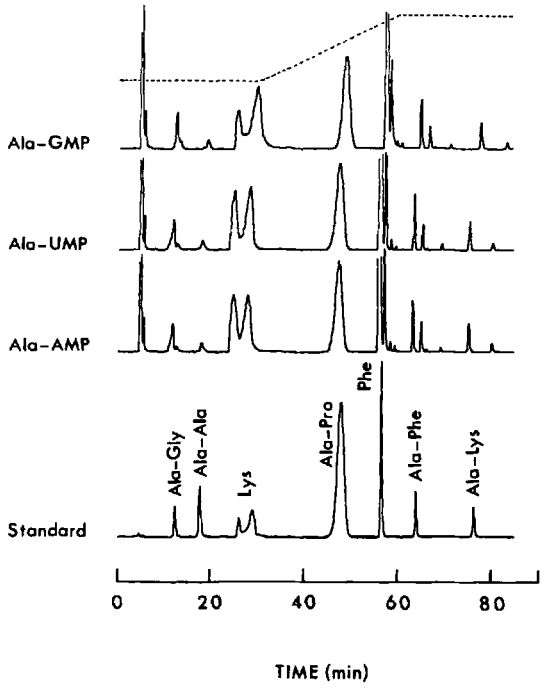


Fig. 5. Separation of dipeptides produced in reactions of alanyl nucleotide anhydrides with the set of homocodonic amino acids. Products of the reactions of an equimolar mixture of Gly, Pro, Phe, and Lys with either Ala–GMP, Ala–UMP, or Ala–AMP were analyzed by HPLC. A standard containing 40 μM Ala–Gly, 50 μM Ala–Ala, 50 μM Lys, 200 μM Ala–Pro, 100 μM Phe, 40 μM Ala–Phe, 40 μM Ala–Lys, and 100 μM Pro is included for comparison. The large peak between Phe and Ala–Phe in the top three chromatograms is Ala–ε–Lys. The detection system exhibited variable sensitivities for different amino acids and peptides, and did not detect proline. The broken line in the top chromatogram indicates the predicted shape of the gradient used for all the chromatographic separations. The initial mobile phase contained 2 mM octyl sulfonic acid in 10 mM potassium phosphate (pH 3.0), whereas the final mobile phase contained 20% acetonitrile in addition to the constituents of the initial mobile phase.

Table 1. Yield (micromolar) of dipeptides in reactions of different alanyl nucleotide anhydrides with an equimolar mixture of four amino acids

Dipeptide	Amino acyl nucleotide anhydride		
	Ala–AMP	Ala–UMP	Ala–GMP
Ala–Pro	126	117	119
Ala–Phe	37	37	35
Ala–Lys	60	71	53
Ala–Gly	47	54	45

The standard deviation of the concentrations of Ala–Pro, Ala–Phe, and Ala–Gly was 4–12% of the mean and for Ala–Lys it was 7–25% of the mean values reported above

Table 2. The ratio of dipeptides to tripeptides

	Ala–AMP	Ala–UMP	Ala–GMP
Ala–Pro : $(Ala)_2$–Pro	4.5	4.7	4.6
Ala–Phe : $(Ala)_2$–Phe	1.2	1.2	1.1
Ala–Lys : $(Ala)_2$–Lys	—	0.9	—
Ala–Gly : $(Ala)_2$–Gly	1.1	1.2	1.1

ditions, peptide synthesis did not stop at the dimer stage, but proceeded further. However, the extent of polymerization depended upon the identity of the amino acid that was present (Fig. 6). In the case of proline, only Ala–Pro, $(Ala)_2$–Pro, and $(Ala)_3$–Pro were observed, with most of the proline appearing in Ala–Pro; whereas, at the other extreme, glycine was most effective in the production of larger oligomers (Fig. 6). The tendency of different amino acids to produce peptides of different lengths was quantified by calculating the ratio of the dimer concentration to the trimer concentration. The smaller this ratio, the farther the polymerization proceeded. These ratios are listed in Table 2. Proline, which produced the largest amount of dimer in competition with other amino acids, resulted in the lowest degree of polymerization when used alone. This parameter, like the pattern of peptide formation in the experiments with the mixture of amino acids, also did not show any significant variation with the nature of the nucleotide. Proline was not only much more efficient than the other amino acids (see Table 1), but it also out-competed its own peptides [Ala–Pro and $(Ala)_2$–Pro] in forming additional peptide bonds (see Table 2). The dimer, Ala–Pro, effectively acted as a sink during the polymerization. These experiments indicate that there is a specificity, at the nearest-neighbor level, that depends upon the nature of the incoming amino acid, but not upon the nature of nucleotide in the anhydride.

Effect of Complementary Polynucleotides on Polymerization

Ala–GMP, Ala–AMP, or Ala–UMP was reacted in the presence or in the absence of its complementary polynucleotide under conditions likely to favor the formation of "monomer–polymer" complexes. HPLC profiles of the products of Ala–GMP in the presence and in the absence of poly(C) are shown in Fig. 7. The amounts of various peptides were determined by comparison with authentic standards of oligoalanine. No significant effect of the polynucleotides was observed on the yields of the polypeptides. Changes in the concentration of poly(C) did not alter this result. A variety of other conditions were explored, in order to see if they would lead to a template effect. A number of buffer systems (borate, cacodylate, phosphate, and 2,6-lutidine) were tested; the entire basic pH range under which pep-

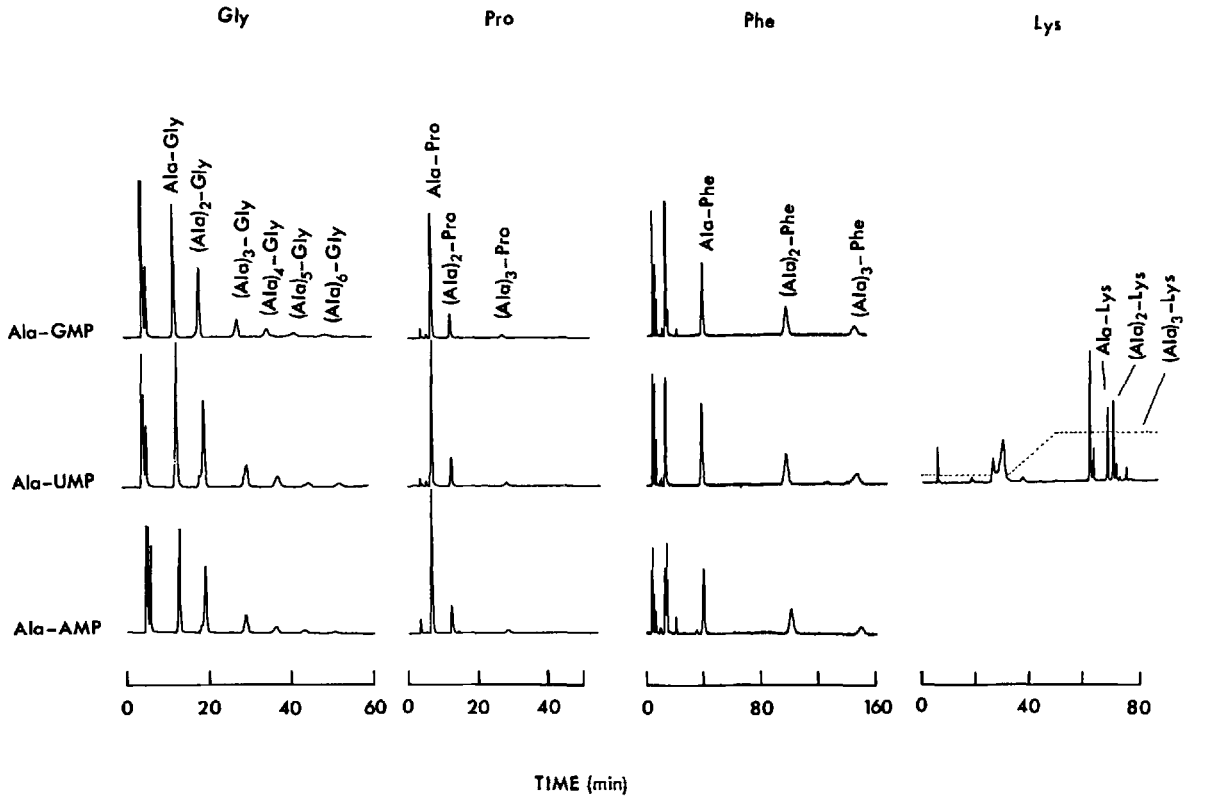


Fig. 6. Analysis of the peptides produced by the reaction of alanyl nucleotide anhydrides with either Gly, Pro, Phe, or Lys. Different mobile phases were used in each separation. Gly: 10 mM KH_2PO_4 (pH 3.0) and 2 mM octyl sulfonic acid; Pro and Phe: 10 mM KH_2PO_4 (pH 3.0); Lys: a gradient of the shape indicated by the broken line was made using 2 mM octyl sulfonic acid in 10 mM potassium phosphate (pH 3.0) as the initial mobile phase, and the same buffer with 20% acetonitrile as the final mobile phase.

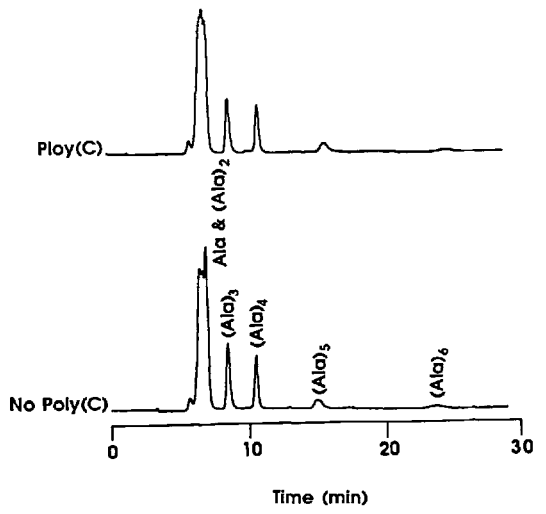


Fig. 7. Chromatographic analysis of alanyl peptides produced by Ala–GMP in the presence and in the absence of poly(C).

tide synthesis takes place was explored; and divalent metal ions were included in the reaction mixtures. No polynucleotide-induced enhancement in the yield of peptides could be seen under any of these conditions. Similar results were obtained for poly(A) with Ala–UMP, and poly(U) with Ala–AMP (Tyagi and Ponnamperuma 1990).

In order to confirm these results by an independent analytical method, the peptide products were subjected to complete acid hydrolysis. The amount of alanine released after hydrolysis was determined by the ninhydrin assay. The ratio of the concentration of amino terminals after hydrolysis to the concentration of amino terminals before hydrolysis is indicative of the average degree of polymerization. This parameter also includes any peptide larger than $(Ala)_6$, that was not detected by HPLC. The ratios were: in the presence of poly(C), 5.58 (and 5.50 in a replicate); and in the absence of poly(C), 5.15. These results confirm that the presence of polynucleotide has no significant effect on the degree of polymerization.

Discussion

The hydrolysis of amino acyl nucleotide anhydrides shows a strong pH dependence. In the acidic pH range, hydrolysis is the only reaction that depletes the anhydrides; in the neutral and basic pH range,

the formation of peptides and 2′(3′)-esters contribute to this process. The sensitive assay system developed here allowed us to conduct the hydrolysis reactions at anhydride concentrations that were low enough to preclude significant amounts of peptide synthesis, thus permitting more accurate determination of the hydrolysis constants. Also, it allowed us to detect the ester intermediates that were not detectable by the techniques used before (Paecht-Horowitz and Katchalsky 1967; Lacey et al. 1984). The formation of 2′(3′)-esters by intramolecular migration of amino acid was suggested by Moldave et al. (1959) and has recently been observed by others (Lacey, personal communication). The mechanism of hydrolysis, ester formation, and peptide synthesis are very similar—it involves a nucleophilic attack of either water, 2′(3′)-hydroxyl groups, or amino acids, respectively, on the carbonyl carbon of the phosphoanhydride link. The pH dependence of the hydrolysis constants for the three anhydrides in the acidic pH range are comparable. The divergence of the constants in the basic pH range probably reflects differences in the degree of ester formation. All three anhydrides produce peptides in the basic pH range, and their capacity to do so is indistinguishable from each other. Also, the degree and extent of polymerization is independent of the nature of the nucleotide in the anhydride. Using these comparative features of the anhydrides, it was possible to carry out the experiments that explored specificity in peptide synthesis.

The relative effectiveness of amino acids in forming dipeptides (Pro $\gg$ Lys > Gly > Phe) does not have any direct correlation with their pKa and hydrophobicity values. The remarkable effectiveness of proline, however, may be due to its highly nucleophilic imino nitrogen. A detailed analysis of the relevant physical attributes of the amino acids would be fruitful only after a more complete survey of the amino acyl nucleotide anhydrides and the free amino acids have been completed.

The differential rate of incorporation of amino acids into dipeptides can be viewed as nearest-neighbor preferences of amino acids during the polymerization. Although these preferences may not influence the sequences of modern proteins, they may have played an important role in determining the nature of primitive peptides, before the advent of nucleic acid-instructed protein synthesis. The existence of these nearest-neighbor preferences would limit the diversity of peptides compared to the diversity if the amino acids were randomly distributed. As a result, some peptides would have been more abundant than others on the prebiotic earth, and possibly these were the ones that carried out the first tasks of catalysis.

Evidence for preferential production of certain organic molecules is scattered throughout the literature on chemical evolution (Fox 1980). It has been observed before that the composition of peptides is different from the composition of the amino acids they are derived from. For example, Nakashima et al. (1977) polymerized a dry mixture of glutamic acid, glycine, and tyrosine by heating. The largest single fraction was a tripeptide. Statistically, based on random assignments (including the bonds with γ-carboxyl group of glutamic acid), 36 different tripeptides should have contained tyrosine, but actually only two did.

The experiments described here were designed to explore the degree of ordering brought about by the intrinsic activities of amino acids, as well as by the influence of the specific interactions that might occur between the nucleotide in the anhydride and the incoming amino acids. These interactions, which have been implicated in the origin of the genetic code, did not have any detectable influence on the specificity of peptide synthesis. This observation, however, should not be interpreted as evidence against the relevance of amino acid–nucleotide interactions in the origin of the genetic code.

The results of the experiments designed to study the transition from noninstructed peptide synthesis to nucleic acid-directed peptide synthesis disagree with the proposition that monomer–polymer interactions between amino acyl nucleotide anhydrides and complementary polynucleotides could lead to an enhancement in the degree of polymerization of amino acids. The conclusion is true for all three pairs of alanyl nucleotide anhydrides and their complementary homopolynucleotides. Several factors could render the proposed template effects impossible. In the system of amino acyl nucleotide anhydrides and polynucleotides, the organized monomer–polymer complexes may not form at all. If they do form, the stereochemical disposition of the amino acids may not be favorable for the step of peptide formation. In the light of these results, it becomes necessary to postulate the existence of prebiotic adapters that are more complex then single nucleotides.

Acknowledgments. We appreciate the discussions we had with Professor Fred Eirich during the development of this work. This work was supported by National Aeronautics and Space Administration grant NGR-21-002-317-21.

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Received June 14, 1989/Revised and accepted November 10, 1989