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OLIGOMERIZATION OF NEGATIVELY-CHARGED AMINO ACIDS BY CARBONYLDIIMIDAZOLE

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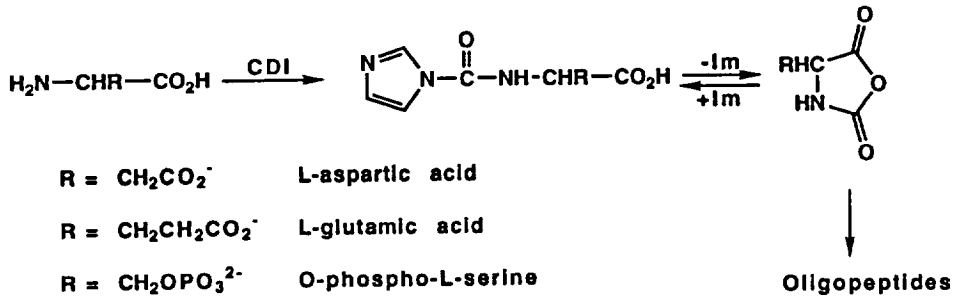
Abstract. The carbonyldiimidazole-induced oligomerizations of aspartic acid, glutamic acid and O-phospho-serine are amongst the most efficient reported syntheses of biopolymers in aqueous solution. The dependence of the yields of products on the concentrations of reagents, the temperature and the enantiomeric composition of the substrate amino acids are reported. Catalysis by metal ions, particularly by Mg^{2+}, is described. These reactions do not generate significant amounts of material in the size-range of several tens of residues that are thought to be needed for a polymer to function as a genetic material.

Introduction

It is very difficult to carry out polymerization reactions that involve dehydration in a homogenous aqueous environment. Several methods of circumventing this problem have been proposed in the literature of prebiotic chemistry (Miller and Orgel, 1974). The most straightforward – heating to dryness in an atmosphere of low humidity – rarely produces a homogeneous and useful product. The most successful oligomerizations reported up to the present use mineral surfaces to concentrate reactants and in some cases to catalyze the ensuing reaction (Ferris and Ertem, 1993; Ferris *et al.*, 1996; Kawamura and Ferris, 1994). We have begun a detailed investigation of the oligomerization of the negatively charged amino acids on ion-exchange minerals such as hydroxylapatite and illite.

In this paper we describe some background experiments on the oligomerization of the amino acids aspartic acid, glutamic acid and O-phospho-serine in aqueous solution. We have studied in some detail the effect of temperature, reagent concentrations, metal ion concentrations and the enantiomeric composition of the substrate on the reaction rate and on the composition of the final product mixture. This information was essential for the success of our subsequent experiments on the influence of mineral surfaces on the progress of the reactions.

We have used carbonyldiimidazole (CDI) in all of the experiments reported here, on account of its convenience and because it is amongst the most efficient of the known condensing reagents for amino acids (Ehler and Orgel, 1976). In later papers we will also describe the use either of water-soluble carbodiimides as condensing agents or of pre-activated amino acids such as thio acids as substrates. The mechanism of the CDI-induced polymerization of amino acids has been described in detail elsewhere (Brack, 1987; Ehler, 1976; Ehler and Orgel,



Scheme 1.

1976). Here it is only necessary to note that the amino acid-CDI system generates N-carboxyanhydrides, a group of compounds known to polymerize efficiently in aqueous solution (Scheme I).

Materials and Methods

L-Aspartic acid, L-glutamic acid, O-phospho-L-serine and short all-L-oligomers of aspartic and glutamic acids were obtained from Sigma Chemical Company. 1,1' carbonyldiimidazole was from Aldrich Chemical Company.

Reaction mixtures were prepared by adding stock solutions of amino acids at pH 8 to solid CDI in 1.7 ml microfuge tubes. Tubes were incubated at various temperatures. The pH was checked at zero time and at the end of the reactions and remained in the range 8 ± 0.3. Aliquots were withdrawn at the specified times, diluted with HPLC initial buffer and stored at –85 °C until analyzed.

HPLC analysis was performed on an RPC-5 analytical column using a Waters model 680 automated gradient controller in conjunction with a Waters model 510 solvent delivery system and a model 712 WISP. The reaction products were eluted with a linear gradient of $NaClO_4$ (0–0.03 M in 40 minutes) at pH 8.0 in the presence of 2 mM Tris-$HClO_4$ at a flow rate of 1 ml/min. The eluate was monitored at 200 nm using an ABI Analytical Kratos Division Spectraflow 757 Absorbance Detector. In our routine analyses, 0.25 μM or 0.5 μM of an amino acid was applied to the column.

The peaks corresponding to the short oligomers of aspartic and glutamic acids were identified by co-injection of the reaction mixture with a commercial sample of the appropriate dimer, trimer or tetramer. The short oligomers of O-phospho-serine were identified by co-injection of the reaction mixtures with samples of short oligomers eluted from paper chromatograms. Very approximate estimates of the yields of products were made by visual inspection of paper chromatograms sprayed with ninhydrin and, in the case of reactions involving O-phospho-serine, also with the Hanes-Isherwood reagent (Zweig and Sherma, 1972). These estimates were confirmed for a number of reaction mixtures containing glutamic acid by

calibrating peak areas in the elution profile against the peak area obtained from a known amount of authentic $(Glu)_3$.

Paper chromatography was performed on Whatman 3MM paper using the descending method. The solvent system was n-propanol:water:NH_4OH, 55:35:10. Samples were applied in duplicate or triplicate along with the corresponding amino acid monomer as marker. In the case of aspartic and glutamic acids authentic samples of commercially available short oligomers were also used as markers. After approximately 24 h of development, the papers were dried and one reaction lane and a marker lane were cut and sprayed with ninhydrin. In the case of O-phospho-serine, one lane was also sprayed with the Hanes-Isherwood reagent.

Results

We carried out a large number of experiments on the polymerization of aspartic acid, glutamic acid and O-phospho-serine using different concentrations of the amino acids and CDI at temperatures between 0 °C and 25 °C. Elution profiles of the products of some typical reactions at 16 °C are illustrated in Figure 1. Similar patterns of products are obtained at 0 °C and 25 °C, but the final yields of longer oligomers are somewhat larger at 0 °C and somewhat smaller at 25 °C (Figure 2). The reactions are essentially complete after one day at 16 °C and higher temperatures, and after 14 days at 0 °C. We confirmed that the reaction can also be carried out in much shorter times at temperatures up to 70 °C.

The elution profiles, in the case of glutamic acid, consist of a simple series of peaks corresponding to successive oligomers. The dimer of glutamic acid is not retained on the column. In typical experiments carried out at 16 °C using 0.1 M amino acid, the oligomers up to about the 18-mer can be detected (Figure 1d). The total yield of oligomers of length 3 or higher is estimated from the HPLC traces to be about 90%. Paper chromatography suggests that at least 90% of the input glutamic acid is converted to dimers and higher oligomers. When the concentration of glutamic acid is reduced to 0.02 M the length of the longest detectable oligomer is reduced to 10 (Figure 1c) and the total yield of oligomers estimated from paper chromatograms is reduced to about 50%.

The elution profiles are more complicated when aspartic acid is used as the substrate (Figure 1 a,b), but a series of major peaks is easily recognized. These correspond to the α-linked peptides of length 3 and up. We believe that the subsidiary peaks correspond to peptides containing one or more β-linkage. The longest oligomers detected are the 13-mer and the 8-mer, when the concentrations of aspartic acid are 0.1 M and 0.02 M, respectively. The total yield of oligomers estimated from paper chromatograms is about 75% when the substrate concentration is 0.1 M and about 30% when it is 0.02 M.

Elution profiles of the products from reactions of O-phospho-serine at 16 °C are also complicated by a number of minor peaks (Figure 1 e,f), perhaps due to

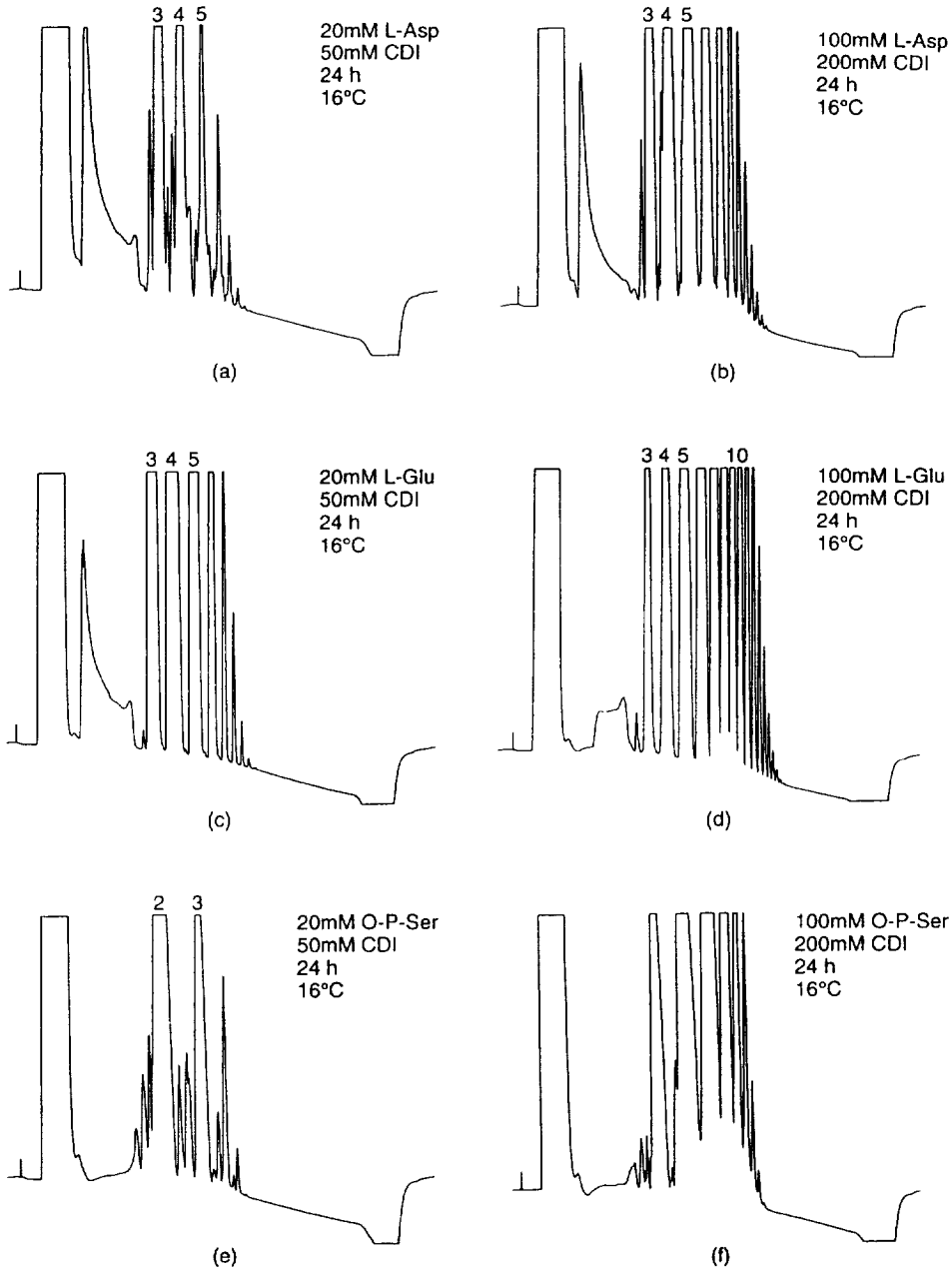


Figure 1. Elution profiles of products from the CDI-induced oligomerization of aspartic acid, glutamic acid and O-phopho-serine.

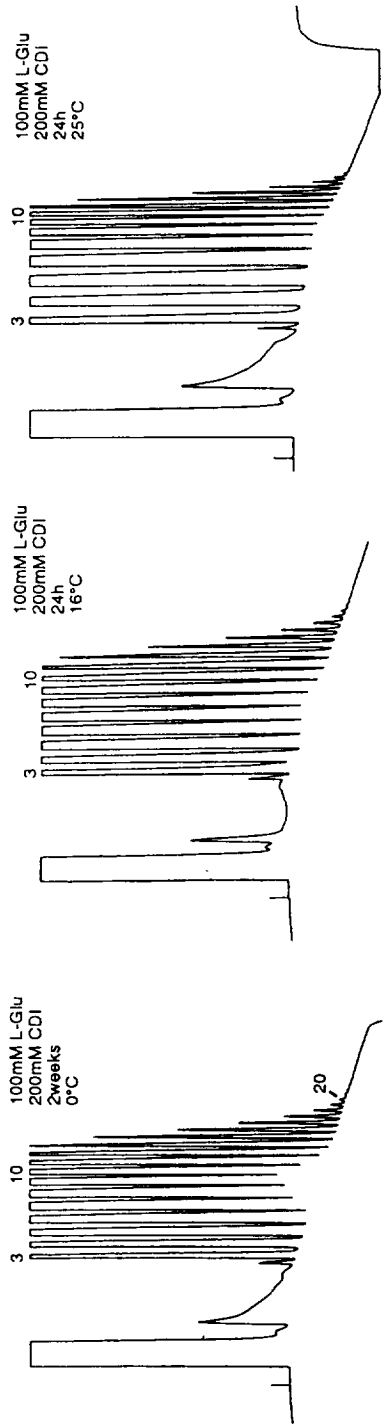


Figure 2. Elution profiles of products from the CDI-induced oligomerization of glutamic acid at 0 °C, 16 °C and 25 °C.

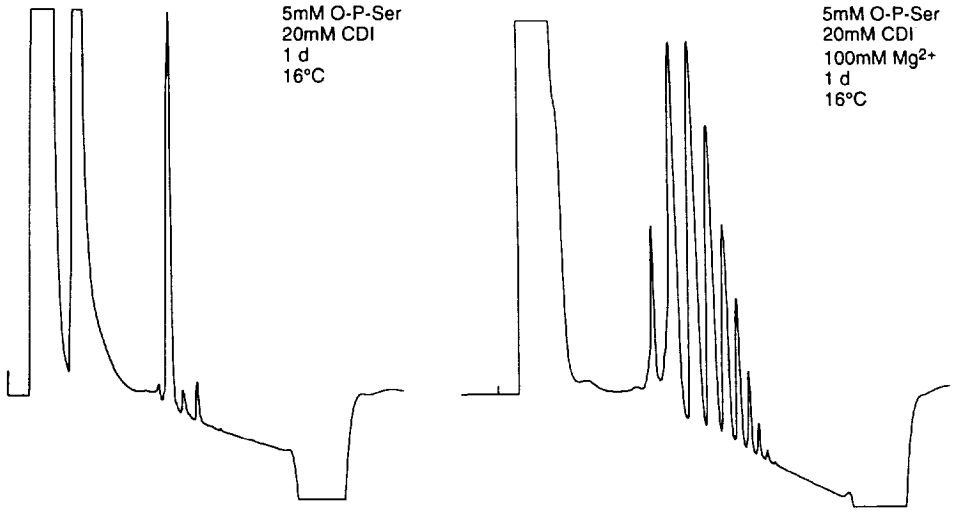


Figure 3. Elution profiles of products from the CDI-induced oligomerization of O-phospho-serine in the presence or absence of 0.1 M Mg^{2+}.

side-reactions that occur on the phosphate group. Nonetheless a series of major peaks are present extending to the 9-mer when the amino acid concentration is 0.1 M and to the 5-mer when it is 0.02 M. The paper chromatograms indicate that at least 90% of the input is converted to oligomers at the higher concentrations of substrate and more than 50% at the lower.

The oligomerization reactions of each of the amino acids are catalyzed by divalent metal ions, particularly by the Mg^{2+} ion. The effect is greater for O-phospho-serine, perhaps because it carries the largest charge (Figure 3). Catalysis is most easily demonstrated when the concentration of the amino acid is low, but is detectable at higher concentrations of the amino acid (data are not shown). Catalysis by Ca^{2+} and Mn^{2+} is generally comparable to but somewhat less extensive than with Mg^{2+}. Zn^{2+}, Co^{2+} and Pb^{2+} do not catalyze the oligomerization reaction.

The substitution of DL-glutamic for L-glutamic acid in general led to a reduction in the yield of oligomers obtained and to a decrease in the length of the longest detectable oligomer. In no case was the effect large.

Discussion

The CDI-induced oligomerizations of aspartic and glutamic acids that are described above are remarkably efficient compared to most other polymerizations in aqueous solution that have been reported. The oligomerization of O-phospho-serine is somewhat less efficient, but still is unusually efficient for a dehydration reaction in aqueous solution. Nonetheless, the reactions do not produce oligomers longer than about the 10-mer in appreciable yield even when the concentration of amino

acid is as high as 0.1 M. The longest oligomer of O-phospho-serine that we have detected is the 9-mer. Simular results for the micelle-catalyzed reaction have been reported earlier (Böhler *et al.*, 1996).

Current opinion suggests that a polymer must have a length of at least several tens of residues to function as a genetic/catalytic unit (Szostak and Ellington, 1993). Furthermore the concentrations of activated amino acids, nucleotides etc., in prebiotic aqueous environments is likely to have been very much lower than 0.1 M. Clearly it is unlikely that the first 'genetic' polymers were formed in homogeneous aqueous solution as the result of polymerization reactions resembling those that have been reported so far. In subsequent papers we will first describe in some detail a specific model in which short negatively-charged primers are adsorbed on the surface of an anion-exchange mineral and extended by repeated 'feedings' to oligomers whose length is limited only by their stability to hydrolysis. We will then demonstrate that long oligomers of negatively-charged amino acids can be synthesized in a simulation of this 'prebiotic' model. (A preliminary account of that work is included in (Ferris, Hill Jr *et al.*, 1996).

Acknowledgements

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