CATALYSIS OF THE OLIGOMERIZATION OF O-PHOSPHO-SERINE, ASPARTIC ACID, OR GLUTAMIC ACID BY CATIONIC MICELLES

CHRISTOF BÖHLER, AUBREY R. HILL, JR. and LESLIE E. ORGEL
The Salk Institute for Biological Studies, Post Office Box 85800, San Diego, CA 92186–5800, U.S.A.

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Abstract. Treatment of relatively concentrated aqueous solutions of O-phospho-serine (50 mM), aspartic acid (100 mM) or glutamic acid (100 mM) with carbonyldiimidazole leads to the formation of an activated intermediate that oligomerizes efficiently. When the concentration of amino acid is reduced tenfold, few long oligomers can be detected. Positively-charged cetyltrimethyl ammonium bromide micelles concentrate the negatively-charged activated intermediates of the amino acids at their surfaces and catalyze efficient oligomerization even from dilute solutions.

Introduction

Catalysis by micelles in aqueous solution has been studied for more than two decades. This work has focussed on reactions involving hydrolysis, aminolysis and nucleophilic substitution reactions (Broxton and Lucas, 1994; Fendler and Fendler, 1975; Menger, 1991; Menger and Fei, 1994) and, more recently, on oxidation (Engbersen *et al.*, 1990; Panigrahi and Mishra, 1993) and decarboxylation (Patel *et al.*, 1994) reactions. We are aware of only one report of micellar catalysis of a polymerization reaction (Armstrong *et al.*, 1978). In this paper we report the catalysis by cetyltrimethyl ammonium bromide (CTAB) micelles of the polymerization of activated, negatively-charged amino acids.

Carbonyldiimidazole (CDI), in aqueous solution, brings about the oligomerization of amino acids and N-monoalkylated amino acids including glycine, sarcosine, alanine, phenylalanine and histidine (Ehler and Orgel, 1976; Ehler *et al.*, 1977). This method has been used to generate oligopeptides attached to oligonucleotides by a linker containing an amino group. The latter reaction is readily monitored by gel-electrophoresis when the amino acids are neutral or cationic, but this method of analysis fails for negatively-charged amino acids (Zieboll and Orgel, 1994).

The formation of oligopeptides in these reactions proceeds by the initial formation of an intermediate (I) which is in equilibrium with the N-carboxyanhydride (II) (Figure 1). A free primary or secondary amino group reacts with (II) to form an amide bond and leaves a carbamic acid terminus. The latter decarboxylates rapidly to generate a new amino group (Ehler, 1976). Repetition of this sequence of reactions generates successively longer peptides attached to the initiating amino group.

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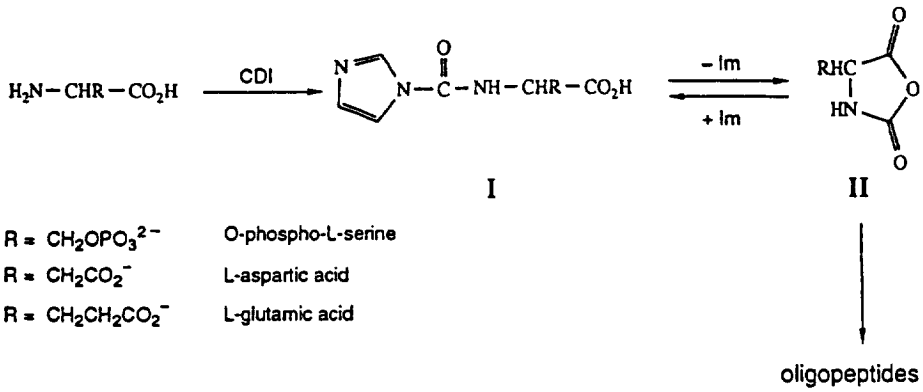

Fig. 1.

Materials

Reagent grade chemicals were used throughout. O-phospho-L-serine, L-aspartic acid, L-glutamic acid and cetyltrimethyl ammonium bromide were purchased from Sigma Chemical Company. Carbonyldiimidazole was from Aldrich Chemical Company.

Methods

Preparation of reaction mixtures

Reaction mixtures were prepared by adding an amino acid stock solution at pH 8 to solid CDI in an Eppendorf-tube. To study micelle-catalyzed reactions, CTAB was added to the amino acid stock solution. The tubes were incubated at 16 °C in a waterbath for appropriate times. Uncatalyzed reaction mixtures were analyzed by withdrawing aliquots and diluting them with water. CTAB-catalyzed reactions were treated the following way: Equal volumes of the reaction solution and 0.1 M $NaClO_4$-solution were mixed in an Eppendorf-tube. Precipitation was observed immediately. The tubes were vortexed and centrifuged for 5 min at 0 °C and 10,000 rpm. Aliquots of the supernatant were diluted with H_2O and analyzed by HPLC. In every case 0.25 μmole of amino acid was applied to the column.

Analysis of reaction mixtures

HPLC was carried out on a stainless steel column (0.39 × 30 cm) packed with RPC-5 material (Joyce *et al.*, 1984). A Waters model 680 automated gradient controller was used in combination with a Waters model 510 solvent delivery system. A linear gradient of $NaClO_4$ (0 to 0.03 M over 40 min) at a flow rate of

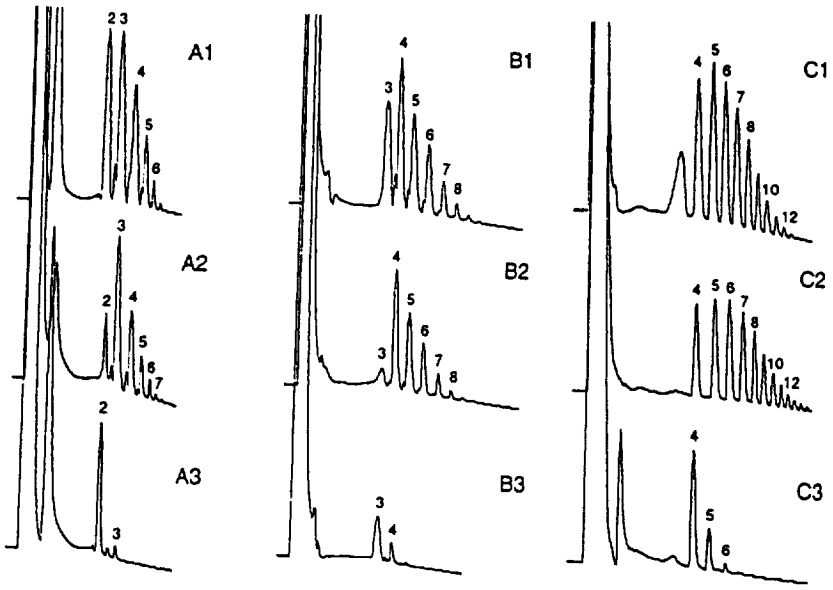

Fig. 2. Polymerization of O-phospho-L-serine (A1-A3), L-aspartic acid (B1-B3) and L-glutamic acid (C1-C3) activated by carbonyldiimidazole (CDI). Samples were analyzed by HPLC on RPC-5 after 1 day (A and B) or 6 h (C). A1: 50 mM O-phospho-L-serine, 100 mM CDI; A2: 5 mM O-phospho-L-serine, 20 mM CDI, 20 mM CTAB; A3: 5 mM O-phospho-L-serine, 20 mM CDI. B1: 100 mM L-aspartic acid, 200 mM CDI; B2: 10 mM L-aspartic acid, 20 mM CDI, 20 mM CTAB; B3: 10 mM L-aspartic acid, 20 mM CDI. C1: 100 mM L-glutamic acid, 200 mM CDI; C2: 10 mM L-glutamic acid, 20 mM CDI, 20 mM CTAB; C3: 10 mM L-glutamic acid, 20 mM CDI. The lengths of the oligomers corresponding to selected peaks in each chromatogram are indicated in the figure. The lengths were determined by co-injection with authentic sample in the case of L-aspartic and L-glutamic acid and by co-injection with material isolated by paper chromatography in the case of O-phospho-L-serine.

1 ml/min was used to elute the oligomers formed in the reaction. Chromatography was performed at pH 8 in the presence of 0.002 M Tris-$HClO_4$. The eluate was monitored at 200 nm using an ABI Analytical Kratos Division model Spectroflow 757 UV detector. Paper chromatography was carried out on Whatman 3MM paper using a mixture of n-propanol (55%), a concentrated NH_4OH solution (10%), and H_2O (35%). Oligopeptides were visualized on the paper with ninhydrin spray (1% ninhydrin, 4% acetic acid in n-butanol. The paper was heated to 100 °C to visualize the spots).

Results

We have studied the oligomerization of O-phospho-L-serine by carbonyldiimidazole (CDI) in some detail, using high performance liquid chromatography (HPLC) on RPC-5 to analyze the products (Joyce, Inoue *et al.*, 1984). When a 0.05 M solution of O-phospho-L-serine is treated with a twofold excess of CDI at pH 8 and 16

°C a series of oligomers is revealed by HPLC (Figure 2, A1). Paper chromatography of the reaction products reveals a corresponding series of peptides (data not shown). Analysis by the ninhydrin method shows that the yield of oligomers substantially exceeds 50%. Co-injection of material eluted from paper chromatograms or of a commercial sample with the complete reaction mixture allows us to assign lengths to the products that are separated by HPLC as indicated in Figure 2.

When the concentration of O-phospho-L-serine is reduced to 0.005 M, the pattern of products detected is very different. The longest detectable oligomer is now the trimer (Figure 2, A3). However, 0.005 M O-phospho-L-serine in the presence of 0.02 M CTAB, a concentration above its critical micelle concentration (Fendler and Fendler, 1975), yields a series of oligomers up to the 7-mer (Figure 2, A2). Catalysis of the formation of (I) is not involved, since the same results are obtained if (I) is generated first and CTAB added later.

The striking effect of CTAB micelles described above is not restricted to the polymerization of O-phospho-L-serine. In less extensive studies we obtained similar results with L-aspartic acid and L-glutamic acid (Figure 2, B and C).

Discussion

The micelles formed by CTAB carry positive charges on their surfaces. The use of a quaternary amine makes it unlikely that the reactants are specifically oriented on the surface of the micelle and also makes it unlikely that acid-base catalysis is important. We believe that negatively-charged monomers are concentrated close to the cationic surface, and that the polyanionic oligomers, once formed, attach to the surface with an affinity that increases with their length. Thus the N-terminus of an oligomer is in an environment where the concentration of the activated amino acid exceeds that in free solution. Consequently the probability that an oligomer will be extended increases with its length, at least until it is long enough to attach stably to a micelle.

Micellar catalysis of polymerization reactions has been proposed as an important aspect of prebiotic chemistry (Oparin, 1924). We believe this to be the first description of the catalysis of the polymerization of a negatively charged monomer on a cationic micelle. We anticipate that the effect will prove a general one for monomers carrying either a positive or a negative charge, adsorbed on micelles carrying the opposite charge.

A full description of the CDI-mediated oligomerization of negatively-charged amino acids, including a discussion of the temperature and concentration dependence of the reaction and of the catalysis of the reaction by metal ions, will be published in due course.

Acknowledgements

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