Efficient Oligomerization of Negatively-Charged *β*-Amino Acids at −20 °C

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It is often possible to concentrate an aqueous solution by cooling. Provided the solutes are insoluble in ice, they are concentrated in the liquid phase when the solution is partially frozen. This procedure may be referred to loosely as eutectic concentration. Eutectic concentration has been used on a number of occasions to facilitate second-order reactions in initially dilute solutions.[1−4] Here, we describe a remarkable acceleration of the rate of polymerization of *β*-amino acids by a water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), when the solution is cooled to −20 °C.

In our initial experiments, we made up solutions 1 mM in *β*-glutamic acid and 1 mM in EDAC and allowed them to stand at 25, 0, or −20 °C for 2 days. Only at the lowest temperature was a significant yield of peptides (>50%) obtained (Figure 1). The maximum in the elution profile corresponds to a peptide with length in the range of 15−20 units. Next, we studied the concentration dependence of the reaction. We found that the efficiency of polymerization and the length distribution of the products were unchanged over the concentration range of 0.1−100 mM at −20 °C, but were strongly concentration dependent at 0 and 25 °C (Figure 2). The efficiency of the reaction at −20 °C is greater than any that we could achieve at 0 °C or 25 °C, even when we used saturated solutions of the amino acid in the latter cases.

To confirm that the high yield of products at −20 °C was due to concentration and not a simple effect of temperature, we explored the effect of 5.6 M sodium chloride on the products of the reaction (Figure 3). We found that the presence of salt

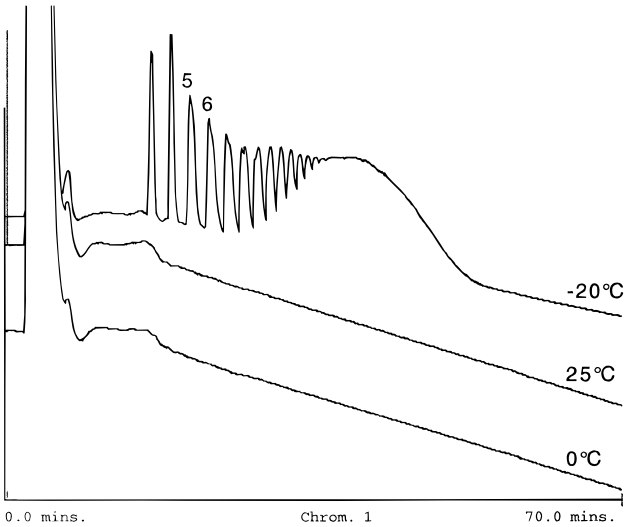

Figure 1. HPLC elution profiles of products from the EDAC-induced oligomerization of 1 mM *β*-glutamic acid at different temperatures. The pentamer and hexamer were identified by electrospray mass spectroscopy. The corresponding peaks are labeled in the profile. The reaction was carried out at indicated temperatures for 24 h using 1 mM EDAC as condensing agent. The products were eluted from the RPC-5 column with a linear gradient of $NaClO_4$ (0−0.048 M in 64 min) at pH 8.0 at a flow rate of 1 mL/min.

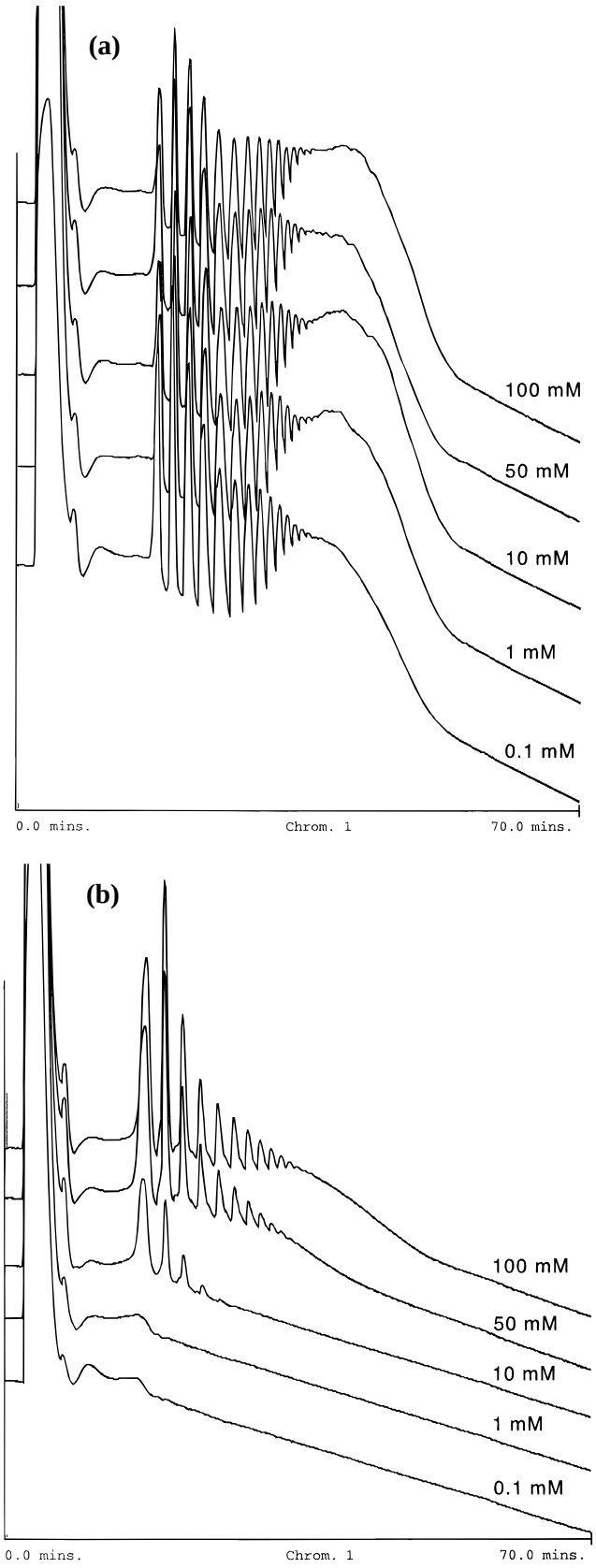

Figure 2. HPLC elution profiles of products from the EDAC-induced oligomerization of various concentrations of *β*-glutamic acid at −20 °C (a) or room temperature (b) after 24 h. The reaction mixture contained appropriate concentration of *β*-glutamic acid (pH 6.0) and the same concentration of EDAC. The products were eluted from the RPC-5 column with a linear gradient of $NaClO_4$ (0−0.048 M in 64 min) at pH 8.0 at a flow rate of 1 mL/min.

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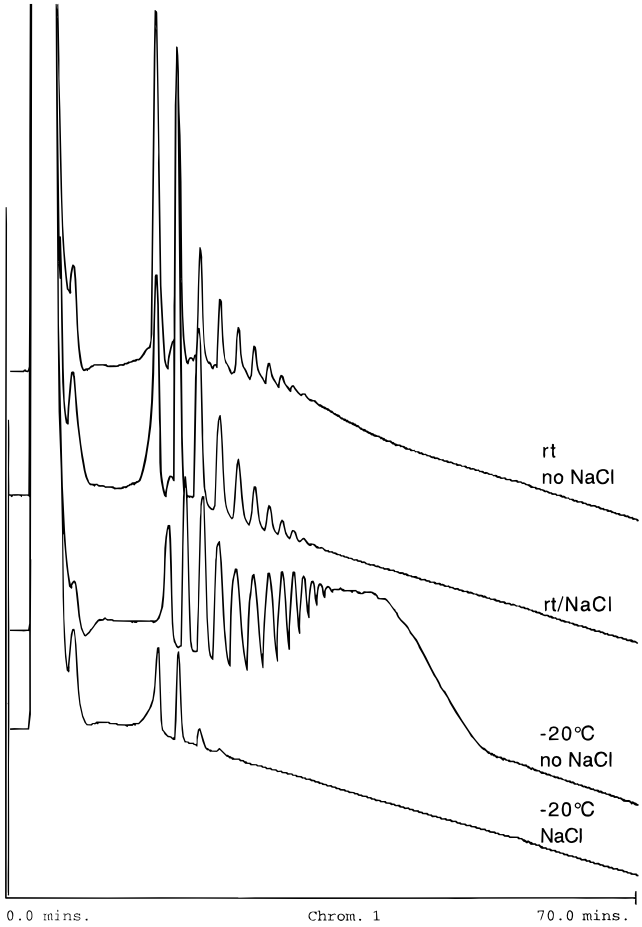

Figure 3. The effect of salt on the EDAC-induced oligomerization of β-glutamic acid. The reaction mixture contained 50 mM β-glutamic acid (pH 6.0) and 50 mM EDAC. NaCl (5.65 M) was added to some of the reaction mixtures. The reaction was carried out at −20 °C or at room temperature for 24 h. The products were eluted from the RPC-5 column with a linear gradient of $NaClO_4$ (0−0.048 M in 64 min) at pH 8.0 at a flow rate of 1 mL/min.

somewhat increased the yield of peptides formed at room temperature. At −20 °C, the presence of salt prevented the separation of ice and suppressed the polymerization reaction. We also eliminated the effect of freezing on the pH of the solution as a cause of the enhanced polymerization by showing that no comparably efficient polymerization occurred in the pH range of 4.5−8.0 at room temperature.

The effect of freezing is not restricted to the oligomerization of β-glutamic acid. We obtained very similar results with aspartic acid. We also find that oligomers of α-glutamic acid and β-aspartic acid, α-glu_3 and β-asp_6, in dilute solution undergo efficient ligation at −20 °C, even though no products can be detected in the corresponding reactions carried out at room temperature. The reaction is not applicable to monomeric α-amino acids which yield diketo piperazines when treated with EDAC in concentrated solution. The efficient oligomerization of aspartic acid is presumably dependent on the formation of peptide bonds involving the β-carboxyl group.

The finding that EDAC at −20 °C is an effective activating agent for carboxyl groups suggests that many condensations presently carried out at room temperature could be carried out more efficiently and perhaps more specifically at −20 °C. It is also possible that prebiotic reagents analogous to EDAC, for example cyanamide, would be effective condensing reagents at −20 °C after sufficient long times of incubation.

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Supporting Information Available: Experimental procedures for the EDAC-induced oligomerization of β-amino acids at −20 °C (3 pages). See any current masthead page for ordering and internet access instructions.

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