Note

Stereospecificity in Papain-catalyzed Oligopeptide Synthesis

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In enzyme-mediated peptide synthesis, some proteinases synthesize water-insoluble polymers of α-amino acids from their esters.1-4 To prepare oligopeptides having some functional properties, we have studied an enzymatic oligomerization of dialkyl esters of L-aspartic and L-glutamic acids in detail,5,6 where their insoluble products could be converted to fully water-soluble forms and analyzed by conventional techniques.

In this study, we indicate that DL-glutamic acid diethyl ester (Glu-di-OEt) is a good candidate for the substrate of papain-catalyzed synthesis of long-chain L-glutamic acid oligomers.

Table I shows overall reaction yields (calculated from the amount of Glu consumed) and water-insoluble product yields (calculated from the Glu contents of acid-hydrolyzed precipitates) obtained by incubation of Glu-di-OEt containing different ratios of D- and L-isomers with papain (EC 3.4.22.2; recrystallized, Sigma Chemical Co.). The reaction did not yield any oligomerized products from the D-isomer alone, but both yields increased with increasing the ratio of the L-isomer. The D- and L-isomers introduced into peptide bonds were measured by separating both

Table I. Effects of the DL-Ratio of the Substrate on Oligopeptide Synthesis Catalyzed by Papain

A reaction mixture containing 100 mM of each substrate shown in the table was incubated at 25°C for 24 hr with 10 μM papain in 0.5 M phosphate buffer (pH 7.5) in the presence of 1 mM dithiothreitol and 1 mM EDTA.

<table>
<thead>
<tr>
<th>Glu-di-OEt (mm)</th>
<th>Overall reaction yield (%)</th>
<th>Water-insoluble product yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D : L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 : 0</td>
<td>&gt;1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>75 : 25</td>
<td>29.3</td>
<td>12.8</td>
</tr>
<tr>
<td>50 : 50</td>
<td>55.6</td>
<td>20.0</td>
</tr>
<tr>
<td>25 : 75</td>
<td>62.8</td>
<td>29.5</td>
</tr>
<tr>
<td>0 : 100</td>
<td>82.5</td>
<td>42.2</td>
</tr>
<tr>
<td>100 : 100</td>
<td>56.2</td>
<td>20.9</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of D- and L-Isomers in the Reaction Products Obtained from DL-Glu-di-OEt.

Fig. 2. Anion Exchange Chromatograms of the Whole Reaction Mixtures Obtained from L- and DL-Glu-di-OEt.

L- or DL-Glu-di-OEt (100 mM each) was incubated with papain under the conditions shown in Table I. Saponified whole reaction mixtures were put on a column of Protein Pak G-QA (8.2 × 75 mm, Waters), and eluted with a NaCl concentration gradient (0.1 to 0.5 M over 30 min) in 50 mM Tris-HCl buffer (pH 9.0) at a flow rate of 1 ml/min. The figure on the top of each peak refers to its polymerization degree determined by FAB-MS.
isomers that were present in the reaction mixture and the acid-hydrolysate of the precipitate on reversed phase chromatography using a mobile phase composed of \(N,N\text{-dimethyl }L\text{-phenylalanine and copper acetate as a chiral eluent by the method of Wernicke.}\) As shown in Fig. 1, over 80% of the \(L\)-isomer and nearly 30% of the \(D\)-isomer in the racemic substrate were converted to peptides after 24 hr of the reaction. It is noteworthy that only 4.6% of the \(D\)-isomer was incorporated in the water-insoluble product that consisted of more than five residues. Figure 2 shows anion exchange chromatograms of the saponified whole reaction mixtures prepared from \(L\)- and \(DL\)-Glu-di-OEt. There were no significant differences in the more highly polymerized product distribution, but a larger amount of di- and tripeptides was detected in the product derived from the racemic substrate, which indicated that 93% of the \(D\)-isomer used in the reaction was contained in the short-chain peptide fraction, mostly as dipeptides.

Since the peptide bond formation catalyzed by papain can be explained by the acyl-enzyme mechanism, we also deduced that the oligomerization of the amino acid ester with a free \(\alpha\)-amino group is initiated by the enzyme in a two-step process: the first is the acylation of Cys-25 of papain by one molecule of Glu-di-OEt and the second is the deacylation (aminolysis) by another Glu-di-OEt. Berger and Schechter have demonstrated that the substrate binding site of papain comprises seven subsites, \(S_1\) - \(S_6\) and \(S_7\) (the numbering being away from the catalytic point), and that the \(S_2\) subsite is strictly stereospecific for \(L\)-amino acid residues and the \(S_7\) subsites are also highly specific for \(L\)-residues. These properties of the active site are fairly compatible with our observation. In the early stage of the oligomerization the \((L\text{-})\text{-dipeptide ester will be formed preferentially and then elongated to long-chain }L\text{-oligomers through the reaction with }L\text{-Glu-di-OEt and/or }L\text{-peptide esters. After a significant amount of }L\text{-Glu-di-OEt is consumed (long-chain }L\text{-oligomers are accumulated as a water-insoluble product), the }L\text{-dipeptide ester can be formed through the aminolysis by }D\text{-Glu-di-OEt the amount of which in the reaction system greatly exceeds that of the }L\text{-isomer at this time. A large portion of the }L\text{-dipeptide ester may remain unreacted in the late stage of the reaction, because it cannot form the acyl-enzyme and the concentrations of }L\text{-peptides and }L\text{-Glu-di-OEt remaining in the solution will be insufficient to cause further reaction. The time-lag observed in the use of the }L\text{- and }D\text{-isomers supports the above view, although the reaction mechanism of the oligomerization needs to be investigated further.}

References

6) K. Aso, T. Miyamoto and T. Uemura, Abstracts of Papers, the Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry, Nagoya, April, 1988, p. 117.