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The Aqueous Thermal Polycondensation of Asparagine and Isoasparagine and the Structure of Polyaspartic Acid

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Introduction

It has been known that the formation of peptide bonds from free amino acids in an aqueous solution is difficult. However, it was demonstrated by Kovács and Nagy (KOVÁCS and NAGY, 1961) that polyaspartic acid could be formed by refluxing an aqueous solution of asparagine. The detailed structure of the resulting polyaspartic acid was not clarified yet, however, the structure of the polyaspartic acid was assumed to be poly-β-aspartic acid because of the intermolecular transamidation of β-amide of asparagine.

In this communication, the structure of polyaspartic acid prepared from asparagine and also from isoasparagine by refluxing the aqueous solution was studied. The structure of the polyaspartic acid was confirmed as polypeptide by IR absorption sprectra and N-terminal analyses.

Experimental

A typical preparation of polyaspartic acid was as follows: the pH of the solution of asparagine (20 g) in 100 ml of 1 M NaCl aqueous solution was adjusted to 7.0 with sodium hydroxide and the solution was refluxed for 3 days. The reaction mixture was acidified to about pH 2.0, and the solution was dialyzed against distilled water for 3 days. The dialyzed polymer was lyophilized and 2.8 g of polymer was obtained. In a similar way, an isoasparagine solution (pH 7.0) was also refluxed for 3 days and polyaspartic acid was obtained.

In order to obtain information on the linkage of the aspartyl residue in the polymer, the dissociation behavior of the carboxyl groups in the polypeptides was investigated by the potentiometric titration. The polymer (30 mg) was dissolved in 30 ml of 0.5N KCl solution and was titrated with 0.1N KOH. The molar concentrations of α- and β-carboxyl groups were determined by the titrational data.

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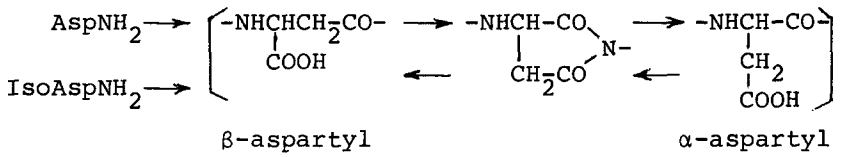
Results and Discussion

The analysis of the titrational data was performed by a modified Speakman's method (SPEAKMAN, 1940) which was used for the determination of the concentration of α- and γ-carboxyl groups in the copoly(Glu, Ala) prepared by thermal polycondensation (KOKUFUTA et al, in press). The intrinsic dissociation constants used for the analysis were 3.15 ± 0.1 (α-COOH) and 4.35 ± 0.1 (β-COOH). These values were adequately used in the previous study as the intrinsic dissociation constants of α- and β-carboxyl groups in the thermaly prepared polyaspartic acid (KOKUFUTA et al, 1977). The result indicates clearly that both polyaspartic acids prepared from asparagine and isoasparagine have α- and β-aspartyl residues and the ratio of α- and β-aspartyl residues is approximately 75 : 25∼85 : 15. The result indicates that the mechanism of the polyaspartic acid formation from asparagine and isoasparagine is not a simple intermolecular transamidation, but the reaction could involve another mechanism to interconvert the α- and β-aspartyl residues. The five membered imide structure is a probable intermediate in the interconversion of the α- and the β-aspartyl residues during the aqueous thermal condensation. The five membered imide was hydrolyzed easily at neutral pH to form α- and β-aspartyl



polyaspartic acid

residues. If this is the case, the ratio of α- and β-aspartyl residues of the polymer might be determined thermodynamically. When the aqueous thermal polycondensation was carried out in a lower pH (pH 4.2), the ratio of α- and β-aspartyl residue was 40 : 60. The proposed interconversion mechanism of α- and β-aspartyl residues could also suggest the formation of asparaginyl (or isoasparaginyl) residues in the polymer by aminolysis of the intermediate five membered imide with ammonia which was liberated by the transamination or by the hydrolysis of the starting material. The aqueous thermal polycondensation of asparagine and isoasparagine is not only interesting as a possible abiotic origine of polypeptide on the primitive earth, but also interesting as an origine of α-aspartyl residue in the protoprotein.

TABLE

Aqueous Thermal Polycondensation of Asparagine and Isoasparagine

	Reaction condition		IR (cm^{-1})				$\overline{DP}$[b]	COOH (m mol/l)		α-linkage (%)
	Solvent	pH	imide	COOH	amide I	amide II		α	β	
$AspNH_2$	1N NaCl	7.0	-	1720	1650	1550	135	1.45±0.38	4.48±0.30	75±6
$IsoAspNH_2$	1N NaCl	7.0	(1780)[a]	1710	1640	1540	165	1.02±0.55	5.58±0.55	85±8
$AspNH_2$	H_2O	4.2	-	1730	1620	1560	90	3.95±0.31	2.78±0.27	40±5

[a] The polyaspartic acid shows weak IR absorption band of five membered imide at 1780 cm^{-1}. One of the absorption bands of imide (1720 cm^{-1}) overlaped with that of carboxyl group (1720 cm^{-1}).

[b] $\overline{DP}$: degree of polymerization which was determined by the N-terminal amino group analysis.

Summary

Both asparagine and isoasparagine were converted into polyaspartic acids by refluxing the corresponding aqueous solutions at neutral pH in the presence of a salt. The properties of the resulting polyaspartic acids were studied. The dissociation behavior of the carboxyl groups in the polypeptides were studied by the potentiometric titration. The analysis of the titrational data was performed by a modified Speakman's method. The analysis show that both polyaspartic acids prepared from asparagine and isoasparagine have α- and β-aspartyl residues and the ratio of α- and β-aspartyl residues is 75 : 25∼85 : 15. A possible mechanism for the formation of α- and β-aspartyl residues in the polymer was discussed.

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