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Thermal Syntheses of Polypeptides from N-Boc-Amino Acid(Aspartic Acid,β-Aminoglutaric Acid) Anhydrides

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N-t-Butyloxycarbonyl-amino acid(aspartic acid:Asp, glutamic acid:Glu, β-aminoglutaric acid: β-Agl)anhydrides were deprotected upon heating at temperatures slightly higher than the melting points of these compounds and polypeptides were synthesized easily in high yield.

The N-t-butyloxycarbonyl(N-Boc) group, which has been used as a convenient protecting group in peptide synthesis, is easily removable by acid treatment$^{1)}$ and also slightly thermolabile in aqueous solution.$^{2)}$ In this investigation, it was found that the Boc group was also easily removed from N-Boc-amino acids without any solvents upon heating. Employing this new property of N-Boc-group, thermal syntheses of polypeptides were carried out. Some results of the thermal elimination reaction of Boc group from N-Boc amino acids are shown in Table 1.

Table 1. Deprotection of N-Boc-amino acids upon heating

Boc-Amino acid	Reaction conditions		Free amino acid recovery$^{a)}$/%
	Temp/°C	Time/h	
Boc-L-Ala	140	2	80
Boc-L-Val	140	2	70
Boc-L-Asp	130	1	81

a) Free amino acid recovery was determined by amino acid analysis of the reaction mixture.

After appropriate heating of N-Boc-amino acids, the reaction products were dissolved in 10 mM sodium hydrogen carbonate and analyzed by means of an amino acid analyzer. The amino acid analyses showed good recovery. These results indicate that N-Boc-amino acids were deprotected by heating to give corresponding free amino acids.

We tried to apply this new property of N-Boc-amino acids for the syntheses of polypeptides. It is well known that carboxylic anhydrides can easily be attacked by amino group to form amide bonds. We chose anhydrides[3)] of N-Boc-DL-aspartic acid(Asp), N-Boc-L-Asp, N-Boc-DL-glutamic acid(N-Boc-DL-Glu), and N-Boc-β-aminoglutaric acid (N-Boc-β-Agl) for the thermal syntheses of polypeptides. Scheme 1 shows the reaction pathway for the syntheses of polypeptides using N-Boc-amino acid anhydrides 3a-d by heating.

$$\underset{\text{1a-d}}{\text{AA}} \xrightarrow[\text{NaOH}/H_2O]{(\text{Boc})_2O} \underset{\text{2a-d}}{\text{N-Boc-AA}} \xrightarrow[\text{AcOEt}]{\text{DCC}} \underset{\text{3a-d}}{\text{N-Boc-AA anhydride}}$$

$$\xrightarrow[\Delta]{-CO_2,\ -CH_2{=}C(CH_3)_2} \underset{\text{4a-b, 4d}}{\text{Polypeptides}} \quad \text{or} \quad \underset{\text{4c}}{\text{Pyroglutamic acid}}$$

Scheme 1. Preparation of N-Boc-AA(amino acid) anhydrides and their heating reactions. 1a: DL-aspartic acid; 1b: L-aspartic acid; 1c: DL-glutamic acid; 1d: β-aminoglutaric acid. Boc: t-butyloxycarbonyl, DCC: dicyclohexylcarbodiimide; AcOEt: ethyl acetate.

These anhydrides melted first upon heating and then generated gases to form white products. Such thermal reactions were carried out at temperatures slightly higher than the melting points of each starting material. The resulting white solids were purified by means of gel filtration to give a white amorphous powder after lyophilization. The IR spectra of the white powder showed typical absorption bands for acidic polypeptides (1710 cm^{-1}: -COOH; 1650 cm^{-1}: amide I; 1540 cm^{-1}: amideII). The molecular weights of the polypeptides were estimated to be between 3000 to 5000 by means of gel filtration(Sephadex G-25). The amino acid recovery of the purified products after acid hydrolysis(in 6M-HCl at 110 $^{\circ}$C) showed that the polypeptides were composed of more than 95% amino acid. The yields of the purified polymeric products after thermal reactions under various conditions are listed in Table 2.

Elemental analyses of the purified polypeptides agreed with those of the calculated values based on the amino acid residue. One hour of heating was sufficient to complete the polymerization of anhydrides of Boc-DL-Asp and Boc-β-Agl. No increase in the yields was observed on longer heating as shown in Table 2. To the best of our knowledge, this is the first synthesis of poly-β-Agl. It could be considered that in these thermal polycondensations, Boc-group could be removed first and the resulting amino acid anhydrides would polymerize to afford

Table 2. Formation of polypeptides from N-Boc-amino acid anhydrides by heating

Substrate	Reaction conditions		Yield[a]		Elemental analysis(%)		
(melting point)	Temp /°C	Time/h	%		C	H	N
3a (122-124 °C)	130	1	91	Calcd[b] Found	39.28 39.22	4.78 5.04	11.45 11.00
		4	89				
		12	93				
	140	1	89				
	150	1	90				
3b (135-137 °C)	130	1	90	Calcd[c] Found	39.87 40.08	4.68 5.03	11.62 11.08
	140	1	89				
3c (104-106 °C)	110	1	(93)[d]				
3d (160-162 °C)	165	1	90	Calcd[e] Found	44.64 44.64	5.69 5.85	10.41 10.48

a) Yield of polypeptide is expressed by the ratio of amino acid residue in the product to the substrate.

b) $C_4H_5NO_3\cdot0.4H_2O$

c) $C_4H_5NO_3\cdot0.3H_2O$

d) Yield of pyroglutamic acid.

e) $C_5H_7NO_3\cdot0.3H_2O$

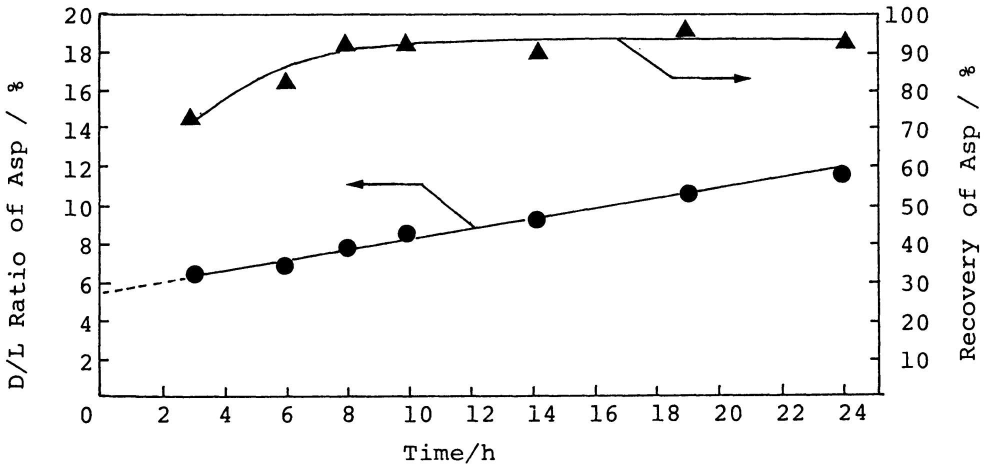

Fig. 1. Recovery and D/L ratio of Asp in the acid hydrolysate of poly-L-Asp obtained from Boc-L-Asp anhydride upon heating at 130 °C.

polypeptides. However, Boc-DL-Glu anhydride gave only pyroglutamic acid in good yield. This means that the intramolecular reaction was predominant in the thermal reaction of Boc-DL-Glu anhydride.

The ratio of D-Asp to L-Asp was 7.5% in the 8 hour's acid hydrolysis of the polymers obtained by heating of Boc-L-Asp anhydride(3b) at 130 °C for 1 h. It was found that eight hours' heating was sufficient to complete the hydrolysis. The D/L ratio was determined by the optical resolution of enantiomeric N-trifluoroacetyl-Asp diisopropylesters by gas liquid chromatography using a chiral glass capillary column (Chirasil-Val III).[4] The actual D/L ratio of Asp in poly-L-Asp before hydrolysis was estimated as 5.5% by extrapolating the value of D/L ratio at 0 h(Fig. 1). The extent of racemization in the thermal synthesis of poly-L-Asp was found to be low, while complete racemization of L-Asp took place in the thermal polycondensation of free L-Asp at 200 °C to form anhydropolyaspartic acid.[5]

In this study, it was found that the thermal polymerizations of anhydrides of Boc-Asp and Boc-β-Agl proceeded at much lower temperatures than that for the polymerization of free Asp. And the racemization of L-Asp was rather low in the thermal polymerization reactions. We believe this method could be applicable for the syntheses of various polyamino acids using Boc-amino acids and Boc-peptides. The determination of the ratio of β- to α-linkage in the thermally synthesized polyaspartic acid is now under investigation.

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