An Attempt to Convert Glycine Residues to Threonine Residues in Polyglycine

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Some attempts have already been made to introduce side chains into the glycine residues of synthetic or naturally-occurring polypeptides[1,2].

Regarding the "fore-protein" formation, for example, Akabori postulated that polyglycine was produced according to the polymerization of aminoacetonitril and that then various side chains were introduced into the glycine residues of polyglycine, thus leading to the fore-protein[3]. In order to prove his hypothesis, Akabori et al. tried to introduce side chains into polyglycine which had been dispersed on the kaolinite surface by means of aldehydes in the presence of some basic catalysts. It was thus shown that small amounts of serine and threonine were formed from the glycine residues in the polymer by reactions with aqueous formaldehyde and acetaldehyde respectively[2].

The present authors have attempted the conversion of the glycine residues in polyglycine to threonine residues by means of acetaldehyde in a non-aqueous medium. Although they were unsuccessful in obtaining polythreonine from polyglycine in the present study, appreciable amounts of threonine could be isolated from the hydrolyzates of the reaction product of polyglycine with acetaldehyde in liquid ammonia. The spatial configuration of the threonine thus formed was also examined by the aid of infrared absorption spectroscopy.

Experimental

Polyglycine. — Polyglycine was prepared by Leuchs' *N*-carboxylic anhydride method[4]. The

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[1] T. Wieland and K. Dose, *Angew. Chem.*, **66**, 781 (1954).

[2] S. Akabori, K. Okawa and M. Sato, This Bulletin, **29**, 608 (1956).

[3] S. Akabori, *Science (Kagaku)*, **25**, 54 (1955).

[4] Y. Go and H. Tani, This Bulletin, **14**, 510 (1939).

average degree of polymerization of the polymer was estimated to be 93～97 by titrating the terminal carboxyl groups[5] with sodium methoxide in dimethylformamide.

The Condensation of Acetaldehyde with Polyglycine and Acid Hydrolysis.—Five hundred milligrams of polyglycine, together with a piece of ferric nitrate crystal, were dispersed in 100 ml. of liquid ammonia in a dry ice-acetone bath. Then, 400 mg. of metallic sodium were added, the mixture was stirred for 80 min., and 50 ml. of freshly distilled acetaldehyde were added dropwise into the above reaction mixture. This was stirred for two hours under cooling and then left overnight at room temperature. During this period, the liquid ammonia evaporated. The residual brown oily substance was dried over concentrated sulfuric acid under reduced pressure. The residue was then hydrolyzed with 6 N hydrochloric acid for 15 hr. at 110°C. The hydrolyzate of the modified polyglycine was evaporated to dryness on a steam bath.

The Isolation of Threonine from the Acid Hydrolyzate of the Modified Polyglycine.—The isolation of threonine was carried out utilizing the O → N acetyl shift reaction[6]. The above-dried hydrolyzate was dissolved in 40 ml. of glacial acetic acid, and dry hydrogen chloride gas was saturated to convert threonine into its *O*-acetyl derivative. The O→N acetyl shift reaction of *O*-acetyl threonine was performed at pH 8 in a pH-stat (Radiometer Co., Demmark) for 4 hr. at 31°C. The reaction mixture was treated with ion exchange resin as in the procedures of Fujiwara et al.[6] One hundred and nine milligrams of crude *N*-acetyl threonine were thus obtained. It was hydrolyzed with 6 N hydrochloric acid. The dried hydrolyzate was dissolved in 5 ml. of methanol, and 0.1 ml. of triethylamine was added; 5.3 mg. of the crystals (m. p., 208～209°C with decomp.) were obtained. The results of paper electrophoresis (500 V., 0.2 mamp./cm., in 1 N acetic acid, pH 2.5) and infrared absorption spectra showed that the material was threonine.

Glycine was recovered from the adsorbed fraction on the resin column, which was used after the O→N acetyl shift reaction for the separation of *N*-acetyl threonine from glycine, by eluting it with 2 N ammonium hydroxide. It was recrystallized from water-ethanol, 410 mg. (m. p., 237°C with decomp. Found: C, 32.08; H, 6.54; N, 18.42. Calcd. for $C_2H_5NO_2$: C, 32.00; H, 6.71; N, 18.66%).

Results and Discussion

As for the formation of the threonine residue from glycine residue in polyglycine, an aldol-type condensation is expected to occur under certain conditions which activate the methylene group in the glycine residue. Akabori et al.[7] found that the formation of a glycine copper chelate complex brought on the activation of the methylene group, and they succeeded in synthesizing threonine by an aldol-type condensation of acetaldehyde. Moreover, they attempted to introduce some side-chain groups into polyglycine which had been dispersed on a clay, kaolinite, and suspended in aqueous alkaline media with formaldehyde or acetaldehyde[2]. In these experiments 0.4～3.1%[*1] of glycine residues were shown to be converted to serine and 1.4～1.5%, to threonine residues, but polyglycine which had not been dispersed on kaolinite was not affected under similar conditions.

In the present investigation, it was shown that 7～9%[*2] of the glycine residues converted to threonine residues. The analyses were made by means of paper chromatography of their 2, 4-dinitrophenyl derivatives after hydrolysis of the modified polymers. Therefore, it seems that the use of liquid ammonia in place of water as a reaction medium is more favorable for this aldol-type condensation reaction[8].

The condensation reaction seemed to occur not only at the *N*- and *C*-terminal glycyl residues in polyglycine but also at the non-terminal residues, since one terminal residue was calculated as being approximately 1% on the basis of the measurement of the average degree of polymerization.

The chromatogram of the 2, 4-dinitrophenyl derivatives of the hydrolyzates of the modified polyglycine showed four spots, 2, 4-dinitrophenyl (DNP) glycine, DNP-threonine, 2, 4-dinitrophenol and one unknown spot which located at the position between threonine and dinitrophenol on a two-dimensional paper chromatogram (*n*-butanol saturated with aqueous ammonia, a 1.5 M phosphate buffer (pH 6)). However, the only amino acids obtained after the isolation procedure are glycine and threonine. Therefore, the amount of an unknown spot on the chromatogram may be insignificant.

It is of interest to examine the spatial configuration of threonine thus obtained from the modified polyglycine. As has already been described, Akabori et al. identified serine and threonine chromatographically from the hydrolyzates of the formaldehyde- and acetaldehyde-treated polyglycine, but they did not isolate the hydroxy amino acids thus formed. In the present studies, liquid ammonia was used as a

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[5] M. Sela and A. Berger, *J. Am. Chem. Soc.*, **77**, 1893 (1955).

[6] S. Fujiwara, S. Morinaga and K. Narita, This Bulletin, **35**, 438 (1962).

[7] M. Sato, K. Okawa and S. Akabori, ibid., **30**, 937 (1957).

[*1] Molar per cent.

[*2] The isolated threonine amounted approximately to only one per cent. This small value probably results from the fairly complex recovering procedure.

[8] K. Shimo and R. Asami, *Hisui-Kenkyusho Hokoku* (*Tohoku University*), **4**, 67 (1954); ibid., **4**, 75 (1954). In these papers, Shimo et al. attempted the introduction of side chains into the glycine residues in 2, 5-diketopiperazine (glycine anhydride) in liquid ammonia with alkyl halides and they obtained the *N*-substituted compounds.

reaction medium and the threonine formed was isolated by the use of the O→N acetyl shift reaction⁶⁾.

The threonine isolated from the modified polyglycine was examined by infrared absorption spectroscopy. The absorption spectra showed the threonine sample to contain appreciable amounts of allothreonine⁹⁾. Estimation of the amount of contaminated allothreonine was made by the use of characteristic bands at 702 and 619 cm^{-1} ¹⁰⁾. The values of 64.4% for threonine and 35.6% for allothreonine were obtained.

Summary

When polyglycine was reacted with acetaldehyde in liquid ammonia in the presence of metallic sodium, it was observed that 7∼9% of the threonine residues were formed from glycine residues. From the hydrolyzate of the modified polyglycine, threonine could be isolated by the aid of the O→N acetyl shift reaction. It was shown that the threonine thus isolated was 35.6% allothreonine.

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9) K. Kodera, Y. Sato and T. Takahashi, *Yakugaku-Kenkyu*, **30**, 38 (1958).

10) T. Furuyama and S. Senoh, This Bulletin, **36**, 126 (1963).