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The Synthesis of Poly-L-arginine*1

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N^{ω}-Tosyl-L-arginine-N^{α}-carboxylic acid anhydride hydrochloride was obtained as a crystal by the reaction of N^{α}-carbobenzyloxy-N^{ω}-tosyl-L-arginine and thionyl chloride. After treatment with silver oxide to remove the hydrochloride, N^{ω}-tosyl-L-arginine-N^{ω}-carboxylic acid anhydride (NCA) was prepared; it was then polymerized to poly-N^{α}-tosyl-L-arginine ($M \fallingdotseq 16200$, $n \fallingdotseq 52$), which was subsequently converted into poly-L-arginine hydrochloride by treating it with hydrogen fluoride and by passing it through a column of the hydrochloride form of the IRA-400 resin.

Poly-L-arginine is an interesting model of the natural basic proteins, such as curpeine or sarmine, containing large amount of arginine.

The preparation of the *N*-carboxylic acid anhydride (NCA) of the L-arginine derivative has been studied in order to synthesize poly-L-arginine,

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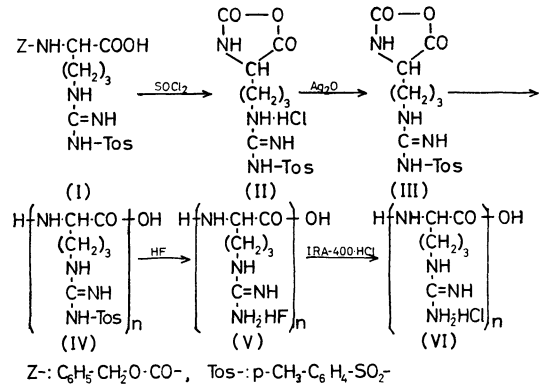
but the results were almost entirely unsuccessful with regard to the chemical specificity of the guanido group, although all of native amino acids except arginine have been converted into their NCA's. However, arginine NCA was recently prepared in the case of the arginine derivative whose N^{δ}-imino group was protected as the carbobenzyloxy group, because when the arginine derivative is treated by the Leuchs method[1] or the Fuchs-Farthing method,[2] the α-carboxyl group tends to form a closed ring with the N^{δ}-imino group of arginine. Poly-L-arginine has been prepared by the following three methods: **a**) poly-ornithine, which had itself been prepared from δ-protected ornithine NCA, was guanidized with 1-guanyl-3,5-dimethyl pyrazole[3] or with methyl isothiourea[4] into polyarginine; **b**) poly-L-nitroarginine, which had been prepared by the *N*-carbothiophenyl method,[5] was reduced in formic acid with stannous chloride into poly-L-arginine,[6] and **c**) $N^{\omega},N^{\omega'}$-dicarbobenzyloxy-L-arginine NCA hydrochloride,[7] which had been prepared from $N^{\alpha},N^{\omega},N^{\omega'}$-tricarbobenzyloxy-L-arginine and thionyl chloride, was polymerized, after treatment with silver oxide in order to remove its hydrochloride, into poly-$N^{\omega},N^{\omega'}$-dicarbobenzyloxy-L-arginine, which was then decarbobenzyloxylated with hydrogen bromide in acetic acid in order to convert it into poly-L-arginine.[7]

However, the quantitative guanidation of all the ornithine residues of poly-ornithine is not easy with the method a), poly-L-arginine is hard to get in a high polymer with the method b), and $N^{\alpha},N^{\omega},N^{\omega'}$-tricarbobenzyloxy-L-arginine can not easily be prepared in a good yield in the method c) and, for the complete decarbobenzyloxylation of poly-$N^{\omega},N^{\omega'}$-dicarbobenzyloxy-L-arginine, it is necessary to employ the drastic step of heating it at 50°C for 90 min in 6N HBr - glacial acetic scid. The chemical properties of the N^{ω}-carbobenzyloxy group are so different from those of an ordinary *N*-carbobenzyloxy group that such a drastic step to removing the protecting group is not generally applicable to the synthesis of an arginine copolymer without a cleavage of the peptide bond.

In this investigation, the synthesis of N^{ω}-tosyl-L-arginine NCA was studied, because the nature of N^{α}-carbobenzyloxy-N^{ω}-tosyl-L-arginine is such that it is hard to form a N^{δ}-closed ring with the α-carboxyl group in peptide syntheses and the tosyl of guanido group can easily be removed by hydrogen fluoride. N^{ω}-Tosyl-L-arginine NCA hydrochloride was obtained for the first time as a crystal without protecting an N^{δ}- or N^{ω}-imino group of L-arginine. After the removal of hydrogen chloride from the NCA hydrochloride with silver oxide, the resulting syrupy NCA was ploymerized into poly-N^{ω}-tosyl-L-arginine which was then converted to poly-L-arginine with hydrogen fluoride.[8]

Results and Discussion

The synthetic route of poly-L-arginine is summarized in Scheme 1.



Scheme 1. The synthetic route of poly-L-arginine hydrochloride.

A syrupy N^{ω}-tosyl-L-arginine NCA (III) was obtained from II by treating the dry acetone solution with silver oxide in order to remove the hydrogen chloride and by evaporating the solvent after the acetone solution had been passed through a column of dry active charcoal. This NCA was immediately dissolved in dimethylacetamide (DMA), dioxane, hexamethyl phosphoramide (HMPA), or dimethyl sulfoxide (DMSO), and polymerized at room temperature in a sealed flask with a mercury manometer. The highest molecular weight was obtained in DMA ($M\fallingdotseq16200$, $n\fallingdotseq52$, $[\alpha]_D^{30}$ +9.9°). The polymerization in dioxane and DMSO gave a lower polymer ($M\fallingdotseq8400$, $n\fallingdotseq27$, $[\alpha]_D^{30}$ +7°). The difference in $[\alpha]_D$ between the two polymers may be due to the difference in molecular weight, for their hydrolysates, when treated with 6N hydrochloric acid at 110°C for 22 hr, gave only L-arginine without any racemization. Poly-N^{ω}-tosyl-L-arginine (IV) was treated with hydrogen

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2) A. C. Farthing and R. J. W. Reynolds, *Nature*, **165**, 647 (1950).
3) S. Ariely, M. Wilcheck and A. Patchornik, *Biopolymers*, **4**, 91 (1966).
4) E. Katchalski and P. Spitnik, *J. Amer. Chem. Soc.*, **73**, 3992 (1951).
5) T. Hayakawa, T. Mizuno and J. Noguchi, *Nippon Kagaku Zasshi*, **81**, 618 (1960).
6) T. Hayakawa, Y. Fujiwara and J. Noguchi, This Bulletin, **40**, 1205 (1967).
7) T. Hayakawa, Y. Kondo, H. Yamamoto and Y. Murakami, *ibid.*, **42**, 479 (1969).
8) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, This Bulletin, **40**, 2164 (1967); S. Sakakibara, Y. Kishida, R. Nishizawa and Y. Shimonishi, *ibid.*, **41**, 438 (1968); S. Sakakibara, N. Nakamizo, Y. Kishida and S. Yoshimura, *ibid.*, **41**, 1477 (1968).

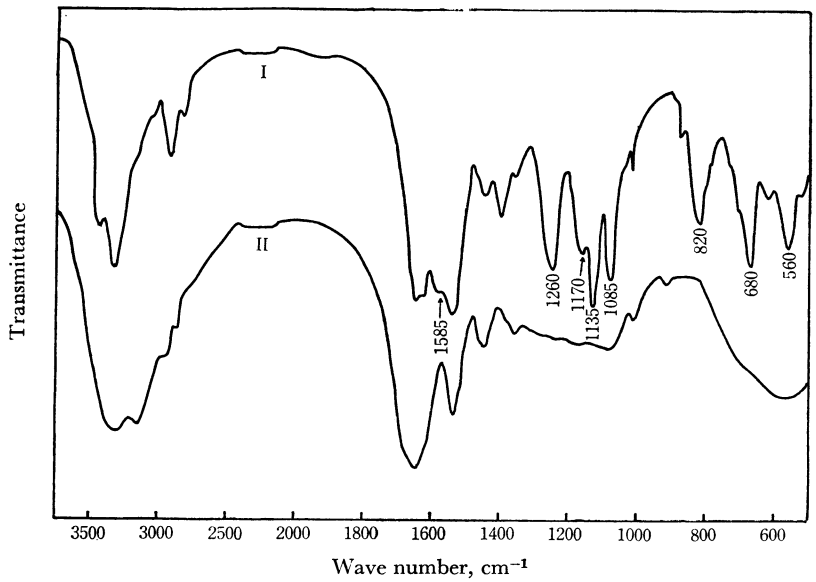

Fig. 1. Infrared absorption spectra of poly-N^{ω}-tosyl-L-arginine (I) and poly-L-arginine hydrochloride (II).

fluoride[8] at 0°C for 1 hr to remove the tosyl groups and then converted into poly-L-arginine hydrochloride by passing it through a column of the HCl type of resin (IRA-400). The infrared spectrum of poly-L-arginine (Fig. 1) shows the absence of the absorptions at 1585, 1265, 1085, 820, 680, 560, 1170, and 1135 cm^{-1} which are due to the benzene and SO_2 groups of the tosyl residue. The hydrolysate of poly-L-arginine gave only L-arginine (R_f 0.16)*2 and did not show any trace of ornithine (R_f 0.12)*2 or citruline (R_f 0.20)*2 on paper chromatography. Therefore, the complete detosylation proceeded by means of the HF method.[8]

Experimental

N^{α}-Carbobenzyloxy-N^{ω}-tosyl-L-arginine (I). N^{α}-Carbobenzyloxy-L-arginine,[9] which had been prepared from L-arginine hydrochloride and carbobenzyloxy chloride, was tosylated with tosyl chloride according to the procedure of Ramachandran and Li.[10]

N^{ω}-Tosyl-L-arginine NCA Hydrochloride (II). Five grams (10.8 mmol) of I were dissolved in 10 m*l* of thionyl chloride at room temperature for 5 hr, and then the excess thionyl chloride was evaporated *in vacuo* at room temperature. The residual syrup was crystallized with dry ether, and the crystal was centrifuged and washed with ether. It was then recrystallized from acetone and ether. Yield, 3.8 g (90%); mp 75°C (dec.); $[\alpha]_D^{23}$ −9.0° (*c* 2.6, acetone). The equivalent molecular weight, as determined by CH_3ONa titration, was 390 calcd 390).

Found: C, 43.4; H, 5.5; N, 13.8; Cl, 8.8%. Calcd for $C_{14}H_{19}O_5N_4SCl$: C, 43.1; H, 4.9; N, 14.3; Cl, 9.1%.

N^{α}-Tosyl-L-arginine NCA (III). Two grams (5.1 mmol) of II were dissolved in 20 m*l* of dry acetone, and then 2 g (8.0 mmol) of silver oxide were added. After the mixture had been stirred at room temperature until the chloride ion in the solution disappeared, it was filtered and the filtrate was passed through a column of dry active charcoal.[11] The colorless solution was evaporated *in vacuo*, and N^{ω}-tosyl-L-arginine NCA was obtained as a syrup. Yield, 1.6 g (89%). The NCA test with barium hydroxide was positive. The fresh syrup was used in the polymerization.

Poly-N^{ω}-tosyl-L-arginine (IV). a) N^{ω}-Tosyl-L-arginine NCA (III), 1.6 g (4.5 mmol), was dissolved in 4.2 m*l* of DMA and polymerized by the addition of triethylenediamine (TEDA) (0.045 mmol) as an initiator at room temperature over a 3-day period. After the mixture had been refluxed for 2 hr on a boiling water bath, it was cooled and poured into water to precipitate the polymer. The precipitate was then centrifuged and washed water. Yield, 0.7 g (50%). $[\alpha]_D^{30}$ +9.9° (*c* 2.0, DMF). The equivalent molecular weight was 16200 ($n \fallingdotseq 52$); it was measured by carboxyl-end titration with 0.02N CH_3ONa in methanol. b) 2 g (5.6 mmol) of IV were dissolved in 8 m*l* of dioxane and then polymerized with TEDA (0.028 mmol). The polymer which was separated out of the solution after it was stood for 16 hr was redissolved with the addition of DMSO (4 m*l*), and then the solution left to stand for a further 3 days. The reaction mixture was treated by the same procedure as that of a). Yield, 1.6 g (94%); $[\alpha]_D^{23}$ +7° (*c* 2.0, DMF). The equivalent molecular weight as determined by 0.02N CH_3ONa titration, was 8400 ($n \fallingdotseq 27$).

*2 Developed with an *n*-butanol-glacial acetic acid-water mixture (4 : 1 : 5).

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11) J. Noguchi, N. Nishi, M. Itaya and S. Tokura, *Kogyo Kagaku Zasshi*, **69**, 745 (1966).

Found: C, 47.8; H, 6.15; N, 16.8%. Calcd for $(C_{13}H_{18}O_3S \cdot H_2O)_n$: C, 47.8: H, 6.10; N, 17.2%.

Hydrolysis of Poly-N^{ω}-tosyl-L-arginine. IV was hydrolyzed with 6N hydrochloric acid at 110°C for 22 hr; when the hydrolysate was then analyzed by paper chromatography, it showed only one spot of arginine with ninhydrin. The quantity was determined by means of a colorimetric densitometer and compared with that of standard ariginine, it corresponded to the arginine content of the polymer. The optical rotation of hydrolysate was $[\alpha]_D^{19}$ +22.3° (*c* 0.7, 6N HCl); this is in good agreement with that of a standard arginine hydrochloride and shows no racemization.

Poly-L-arginine Hydrochloride (VI). Poly-N^{ω}-tosyl-L-arginine (IV) (1.4 g), containing 1.4 m*l* of anisole was treated with liquid hydrogen fluoride at 0°C for 1 hr by the procedure of Sakakibara *et al.*[8] After evaporating the hydrogen fluoride, the poly-L-arginine hydrofluoride (V) was taken out with ether and alcohol. It was dissolved in 10 m*l* of water and dialyzed against water. The solution was then treated with a HCl form of the IRA-400 resin in order to remove the fluoride ion and then evaporated *in vacuo*. The residue was washed with alcohol and dried. Yield, 0.6 g (62%); $[\alpha]_D^{17}$ −71.8° (*c* 1.9, water).

Found: C, 39.1; H, 7.52; N, 25.9; Cl, 16.7%. Calcd for $(C_6H_{13}ON_4Cl \cdot \frac{1}{2} \cdot C_2H_5OH)_n$: C, 39.3; H, 7.49; N, 26.0; Cl, 16.5%.

Hydrolysis of Poly-L-arginine Hydrochloride. A part of the VI was hydrolyzed with 6N hydrochloric acid at 110°C for 22 hr; when the hydrolysate was then analyzed by paper chromatography, it showed only one spot of arginine. The optical rotation of the hydrolysate was $[\alpha]_D^{22}$ +22.5° (*c* 1.0, 6N HCl); this is in good agreement with standard L-arginine hydrochloride and shows no racemization of the polymer.

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