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The Synthesis of Poly-L-arginine Hydrobromide and Copolymers of L-Arginine and Other Amino Acids*1

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Pure crystalline $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine N-carboxyanhydride hydrochloride was obtained from $N^{\alpha,\omega,\omega'}$-tricarbobenzyloxy-L-arginine and thionyl chloride. It was polymerized to poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine. This polymer was converted to poly-N^{ω}-carbobenzyloxy-L-arginine by treating it with alcoholic potassium hydroxide; also, both carbobenzyloxy groups of the protected polymer were completely removed into poly-L-arginine by treating them with hydrogen bromide in glacial acetic acid. Several copolymers containing L-arginine residue were also prepared by the copolymerization of the above NCA and the NCAs of other amino acids, such as γ-benzyl-L-glutamate, L-alanine, and N^{ε}-carbobenzyloxy-L-lysine.

The natural α-amino acids found in protein give polyamino acids upon the polymerization of all the corresponding α-amino acid N-carboxyanhydride (α-amino acid NCA) except arginine. Because of the high basicity of the guanido group, the preparation of the pure NCA of L-arginine derivative is difficult and has not been reported in detail.[1,2]

These synthetic methods of producing poly-L-arginine have been reported: (a) poly-L-ornithine prepared by the NCA method was guanidized into poly-L-arginine with 1-guanyl-3,5-dimethylpyrazole or isomethylthiourea,[3,4] and (b) poly-L-nitroarginine prepared by the N-carbothiophenyl method was reduced to poly-L-arginine hydrochloride with stannous chloride in formic acid.[5] In the former case, however, it is very difficult to guanidize quantitatively the ornithyl residues of poly-L-ornithine into poly-L-arginine, and in the latter case, no polymer with a high molecular weight has been prepared. In addition, it is difficult to synthesize copolymers with other amino acids by the use of the above methods.

In this report, the synthesis of poly-L-arginine hydrobromide and copolymers of L-arginine and other amino acids, such as L-glutamic acid, L-alanine, and L-lysine, will be described. The synthetic route of poly-L-arginine (V) is summarized in Eq. (1).

Dicarbobenzyloxy-L-arginine NCA hydrochloride (II) was obtained from tricarbobenzyloxy-L-arginine (I)[6] by the use of thionyl chloride in dioxane. The infrared absorption spectrum of Compound II showed absorptions at 1850 cm^{-1} and 1785 cm^{-1}. These might correspond to the C=O groups in the five-membered ring of NCA. Poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine was prepared as follows: NCA hydrochloride (II) was treated with silver oxide in dry acetone to remove any hydrogen chloride and was then polymerized in dioxane by adding triethylamine as an initiator. Polymerization was allowed to proceed at room temperature, the solution became a clear gel after several days. The molecular weight was found to be about 30000 by titration with N/50 CH_3ONa in methanol. Poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine (III) was treated with alcoholic potassium hydroxide to convert it into poly-N^{ω}-carbobenzyloxy-L-arginine (IV) by the elimination of one carbobenzyloxy group of the protected guanido group of III. Poly-L-arginine hydrobromide was obtained by treating III or IV with HBr in glacial acetic acid at 50°C. The acid hydrolyzate of poly-L-arginine hydrobromide gave only one spot of arginine upon paper chromatography and showed no racemization of arginine. The copolymerization of $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine NCA and other amino acid NCAs (γ-benzyl-L-glutamate, L-alanine and N^{ε}-carbobenzyloxy-L-lysine) was performed. The yield, molecular weight, and amino acid composition of each copolymer were determined. The molar ratio was determined quantitatively by paper chromatography

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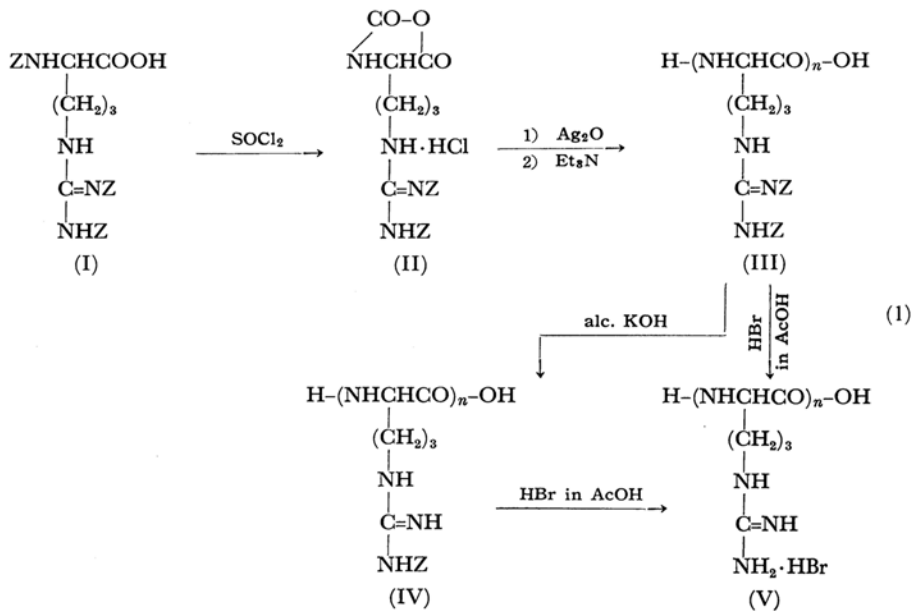

Z: $C_6H_5CH_2OCO-$

after acid hydrolysis. The results of the elemental analyses of these copolymers are shown in Table 1. In a way similar to that of poly-L-arginine synthesis, one carbobenzyloxy group of the arginine residue and the benzyl group of glutamate in these copolymers were removed (see Table 2) by treating them with alcoholic potassium hydroxide. Also, all the protecting groups of the copolymers were removed by treating them with hydrogen bromide in glacial acetic acid. The liberated homopolymer and copolymers were soluble in water and showed positive Biuret and Sakaguchi reactions.

Poly-L-arginine and the copolymers containing a L-arginyl residue provide a valuable model of natural basic proteins, such as salmine and clupeine, which have a high arginine content. The physicochemical properties of these polymers, such as the infrared spectra, X-ray analyses, and optical rotatory dispersion, will be reported in the near future.

Experimental

$N^{\alpha,\omega,\omega'}$-Tricarbobenzyloxy-L-arginine(I). $N^{\alpha,\omega,\omega'}$-Tricarbobenzyloxy-L-arginine (I) was prepared from L-arginine hydrochloride and carbobenzyloxy chloride as has been described by Zervas *et al.*[6]

$N^{\omega,\omega'}$-Dicarbobenzyloxy-L-arginine NCA Hydrochloride (II). To a solution of tricarbobenzyloxy-L-arginine (I) (2.0 g) in 5 m*l* of dioxane, thionyl chloride (1.0 m*l*) was added; the mixture was then allowed to stand for 5 hr at room temperature. When to this mixture dry ether was added, a white precipitate was obtained. This precipitate was filtered, washed with dry ether, and recrystallized from dry acetone and ether. Yield, 1.45 g (83.1%); mp 62°C (decomp.).

Found: C, 54.71; H, 5.37; N, 11.04; Cl, 6.94%. Calcd for $C_{23}H_{25}O_7N_4Cl$: C, 54.71; H, 4.99; N, 11.10; Cl, 7.02%.

$N^{\omega,\omega'}$-Dicarbobenzyloxy-L-arginine NCA. To a solution of $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine NCA hydrochloride (II) (1.45 g) in 20 m*l* of dry acetone, dry silver oxide (1.5 g) was added. The mixture was stirred until it showed negative in a chloride test and was then passed through a dry charcoal column to purify it.[7] The acetone in the clear filtrate was removed to dryness *in vacuo*; an oily residue was thus obtained. Yield 1.0 g (83.3 %). The residue was used for immediate polymerization.

Poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine (III). To a solution of $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine NCA (1.0 g) in 5 m*l* of dioxane, 0.0028 m*l* of triethylamine was added as an initiator; the mixture was polymerized in a sealed tube at room temperature. The polymerization proceeded until a clear gel was obtained. After one week, the reaction mixture was heated in a boiling water bath for 5 hr. After it had been cooled, the mixture was stirred into 100 m*l* of water. The polymer was collected by centrifugation, washed with water, and dried. Yield, 0.8 g (88.0%); $[\alpha]_D^{20}$ +13.1° (*c* 0.6, dioxane) and $[\alpha]_D^{20}$ −20.3° (*c* 0.5, trifluoroacetic acid). Equiv. mol wt≒30000 (by titration of the carboxyl end group with N/50 CH_3ONa in methanol).

Found: C, 62.25; H, 5.79; N, 13.11%. Calcd for $(C_{22}H_{24}O_5N_4)_n$: C, 62.25; H, 5.70; N, 13.20%.

Poly-$N^{\omega,\omega'}$-carbobenzyloxy-L-arginine (IV). To a solution of poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine (III) (200 mg) in 1.2 m*l* of dioxane, 1.2 m*l* of 0.6 N potassium hydroxide in 95% ethanol was added and shaken for 2 hr at room temperature. The solution was acidified with acetic acid. After the removal of the organic solvent, the polymer was precipitated by the addition of water. The polymer was collected by centrifugation

7) J. Noguchi, N. Nishi, M. Itaya and S. Tokura, *Kogyo Kagaku Zasshi* (*J. Chem. Soc. Japan, Ind. Chem. Sect.*), **69**, 745 (1966).

and dried. Yield, 102 mg (74.4%); $[\alpha]_D^{20}$ +20.4° (*c* 0.2, 2-chloroethanol) and $[\alpha]_D^{20}$ −28.6° (*c* 0.3. trifluoroacetic acid).

Found: C, 57.89; H, 6.16; N, 19.02%. Calcd for $(C_{14}H_{18}O_3N_4)_n$: C, 57.92; H, 6.25; N, 19.30%.

Poly-L-arginine Hydrobromide (V). (a) Poly-$N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine (100 mg) was dissolved in 10 m*l* of 6 N hydrogen bromide in glacial acetic acid and stirred for 90 min at 50°C. The reaction mixture was then evaporated to dryness *in vacuo*, and the residue was treated with dry ether. The polymer was collected by centrifugation and washed with ether. This polymer was dissolved in a minimum amount of water and reprecipitated with ethanol. It was then collected by centrifugation, washed with ether, and dried. Yield, 32 mg (57.1%) (b) Poly-N^{ω}-carbobenzyloxy-L-arginine (115 mg) was treated in the way described above. Yield, 57.5 mg (61.2%); $[\alpha]_D^{20}$ −49.2° (*c* 1.0, water).

Found: C, 28.64; H, 5.60; N, 21.56%. Calcd for $(C_6H_{13}ON_4Br\cdot H_2O)_n$: C, 28.25; H, 5.93; N, 21.96%.

Hydrolysis of Poly-L-arginine Hydrobromide. A solution of poly-L-arginine hydrobromide (62.5 mg) in 10 m*l* of 6 N hydrochloric acid was hydrolyzed at 105°C for 24 hr. The hydrolyzate gave only arginine upon paper chromatography. The hydrolyzate was $[\alpha]_D^{20}$ +26.3°, which agrees with the value of authentic L-arginine.

Copoly(γ-benzyl-L-glutamate, $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine)(2 : 1). To a solution of γ-benzyl-L-glutamate NCA (0.7 g) and $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine NCA (0.62 g) in 13 m*l* of dioxane, 0.0055 m*l* of triethylamine was added as an initiator, and the mixture was polymerized in a sealed tube at room temperature for 5 days. The reaction mixture was heated for 2 hr in a boiling water bath, and then poured into water. The polymer precipitated was collected by centrifugation and dried. Yield, 1.0 g (87.2%). The analytical data and the molecular weight are shown in Table 1.

Other Copolymers. Copoly(γ-methyl-L-glutamate, $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine) (2 : 1); copoly(L-alanine, $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine) (1 : 1) and (2 : 1); copoly(N^{ε}-carbobenzyloxy-L-lysine, $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine) (3 : 2) and (2 : 1) were prepared by the copolymerization of $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine NCA and the corresponding amino acid NCAs in the molar ratios presented above. The yields, the molecular weights, and the analytical data are shown in Table 1.

Hydrolysis of Copolymers. A portion of these polymers were hydrolyzed with 6 N hydrochloric acid

Table 1. Copolymers of $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine and other amino acids

Copolymer	Amino acid Mole ratio	Yield %	Amino acid composition Mole ratio	Molecular weight	Molecular formula	Found % C	Found % H	Found % N	Calcd % C	Calcd % H	Calcd % N
$N^{\omega,\omega'}$-Di-Z-L-Arg N^{ε}-Z-L-Lys	2 3	84.4	2 3.2 [a]	12000	$(2C_{22}H_{24}O_5N_4\cdot 3C_{14}H_{18}O_3N_2)_n$	62.85	6.24	11.92	63.14	6.29	11.99
$N^{\omega,\omega'}$-Di-Z-L-Arg N^{ε}-Z-L-Lys	1 2	96.8	1 2.1 [a]	12000	$(C_{22}H_{24}O_5N_4\cdot 2C_{14}H_{18}O_3N_2)_n$	63.18	6.40	11.63	63.28	6.37	11.81
$N^{\omega,\omega'}$-Di-Z-L-Arg L-Ala	1 1	69.7	1 1.2	6100	$(C_{22}H_{24}O_5N_4\cdot C_3H_5ON)_n$	60.21	6.03	14.27	60.60	5.90	14.13
$N^{\omega,\omega'}$-Di-Z-L-Arg L-Ala	2 1	70.5	1.8 1	17000	$(2C_{22}H_{24}O_5N_4\cdot C_3H_5ON)_n$	61.40	5.89	13.92	61.36	5.81	13.70
$N^{\omega,\omega'}$-Di-Z-L-Arg γ-Benzyl-L-Glu	1 2	68.2	1 1.9	6400	$(C_{22}H_{24}O_5N_4\cdot 2C_{14}H_{13}O_3N)_n$	64.69	6.07	9.64	64.02	5.84	9.74
$N^{\omega,\omega'}$-Di-Z-L-Arg γ-Methyl-L-Glu	1 2	75.3	1 2.0	5000	$(C_{22}H_{24}O_5N_4\cdot 2C_6H_9O_3N)_n$	57.82	6.05	11.58	57.46	5.96	11.83

a) Determined after dinitrophenylation.

Table 2. Copolymers of N^{ω}-carbobenzyloxy-L-arginine and other amino acids

Copolymer	Amino acid Mole ratio	Yield %	Molecular formula	Found % C	Found % H	Found % N	Calcd % C	Calcd % H	Calcd % N
N^{ω}-Z-L-Arg N^{ε}-Z-L-Lys	2 3	86.4	$(2C_{14}H_{18}O_3N_4\cdot 3C_{14}H_{13}O_3N_2)_n$	61.90	6.84	14.24	61.48	6.63	14.34
N^{ω}-Z-L-Arg L-Ala	1 1	77.2	$(C_{14}H_{18}O_3N_4\cdot C_3H_5ON)_n$	54.07	6.70	18.67	53.82	6.64	18.46
N^{ω}-Z-L-Arg L-Glu	1 2	73.8	$(C_{14}H_{18}O_3N_4\cdot 2C_5H_7O_2N\ H_2O)_n$	53.29	6.28	15.83	53.92	6.41	15.72

TABLE 3. COPOLYMERS OF L-ARGININE HYDROBROMIDE AND OTHER AMINO ACIDS

Copolymer	Amino acid Mole ratio	Yield %	Molecular formula	Found % C	Found % H	Found % N	Calcd % C	Calcd % H	Calcd % N
L-Arg·HBr L-Lys·HBr	2 3	73.0	$(2C_6H_{12}ON_4 \cdot 3C_6H_{12}ON_2 \cdot 5HBr \cdot 2H_2O)_n$	32.24	6.14	16.35	32.07	6.19	16.21
L-Arg·HBr L-Ala	1 1	78.6	$(C_6H_{12}ON_4 \cdot C_3H_5ON \cdot HBr \cdot H_2O)_n$	33.52	5.87	21.63	33.32	6.16	21.41
L-Arg·HBr L-Glu	1 2	83.3	$(C_6H_{12}ON_4 \cdot 2C_5H_7O_2N \cdot HBr \cdot 2H_2O)_n$	38.66	6.04	16.65	38.48	6.26	16.83

at 105°C for 24 hr. The amino acid compositions were determined quantitatively by paper chromatography. The data are shown in Table 1.

Copoly(L-alanine, N^{ω}-carbobenzyloxy-L-arginine) (1 : 1). To a solution of copoly(L-alanine, $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine) (1 : 1) (200 mg) in 0.9 m*l* of dioxane, 0.9 m*l* of 0.6 N potassium hydroxide in 95% ethanol was added. The mixture was shaken at room temperature for 2 hr. The mixture was then acidified with acetic acid, and the polymer precipitated by the addition of water. The polymer was collected by centrifugation, washed with water, ethanol, and ether, and dried. The yield and the analytical data are shown in Table 2. Copoly(L-glutamic acid, N^{ω}-carbobenzyloxy-L-arginine) (2 : 1) and copoly(N^{ε}-carbobenzyloxy-L-lysine N^{ω}-carbobenzyloxy-L-arginine) (3 : 2) were prepared in a similar way by treatment with alcoholic potassium hydroxide. The yield and the analytical data are shown in Table 2.

Copoly(L-lysine hydrobromide, L-arginine hydrobromide) (3 : 2). Copoly(N^{ε}-carbobenzyloxy-L-lysine, $N^{\omega,\omega'}$-dicarbobenzyloxy-L-arginine)(3 : 2)(200 mg) was dissolved in 10 m*l* of 6 N hydrogen bromide in glacial acetic acid, stirred for 90 min at 50°C, and evaporated to dryness *in vacuo*. The residue was treated with dry ether. The polymer was collected by centrifugation, washed with ether, and dried. This polymer was redissolved in a minimum amount of water and reprecipitated with ethanol and ether. The yield and the analytical data are shown in Table 3. From the corresponding protected copolymers, copoly(L-glutamic acid, L-arginine hydrobromide) (2 : 1) and copoly(L-alanine, L-arginine hydrobromide) (1 : 1) were prepared in the same way. The yield and the analytical data are shown in Table 3.

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