Synthetic Amphoteric Polypeptide. I. Syntheses and Some Properties of Linear Amphoteric Polypeptides

By Heimei YUKI, Shumpei SAKAKIBARA and Hisaya TANI

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Introduction

The behavior of synthetic amphoteric polypeptides is of great interest, because of their relationship to those of proteins. With this in mind, Akabori, Tani and Noguchi[1] have synthesized copoly-1 : 1-(L-glutamic acid, DL-lysine), the first synthetic amphoteric polypeptide. This substance, unfortunately, had some unfavorable properties as a "model protein". Its solubility in water was too great, due to its relatively low molecular weight; also it differed from proteins in that all of the component amino acid residues had the ionisable side chain groups.

It would be desirable to obtain a high molecular weight polypeptide having acidic, basic and neutral amino acid residues in proportions nearly equal to those in protein. Therefore, copoly-1 : 1 : 3-(DL-glutamic acid, DL-lysine, DL-phenylalanine) (A) and copoly-1 : 1 : 1.9-(L-glutamic acid, L-lysine, L-leucine) (B) have been synthesized and some properties of their solutions investigated.

Preparations

The copolymerizations were carried out with mixtures of N-carboxy anhydrides of γ-benzyl DL-glutamate, ε-N-carbobenzoxy-DL-lysine and DL-phenylalanine (I, II, III respectively) and of γ-benzyl L-glutamate, ε-N-carbobenzoxy-L-lysine and L-leucine (IV, V, VI respectively). To obtain a homogeneous high molecular polypeptide, a suitable solvent must be used for the polymerization. The results obtained with several solvents tried in these experiments are shown in Table I. It was found that chlorobenzene was the most suitable solvent, giving the polymers having the highest specific viscosities. In nitrobenzene and in ethyl phenylacetate, the reaction mixtures formed transparent gels or highly viscous solutions. The polymer produced in benzene and its reduction product were found to be heterogeneous in their solubility behavior. In dioxane and tetrahydrofuran the polymerization could not proceed; after evaporating the solvents in vacuo the crystalline masses, supposedly mixtures of the N-carboxy anhydrides, were recovered. These could now be polymerized in any of the suitable solvents mentioned above, the polymerization proceeding apparently in the same manner as when fresh monomer mixtures were used. It has been observed previously[2] that N-carboxy-α-amino acid anhydride was very stable in purified acetone. The dioxane and tetrahydrofuran used in these experiments contained traces of an aldehydic substance, which might have some stabilizing effect on the monomers.

The polymers were reduced with phosphonium iodide and converted into the hydriodides of corresponding amphoteric polypeptides. The hydriodides gave the free polypeptides quantitatively on treatment with triethylamine. Dialyses of these hydriodides were also carried out to remove

1) S. Akabori, H. Tani and J. Noguchi, *Nature*, **167**, 159 (1951); H. Tani and J. Noguchi, *Chem. High Polymers, Japan*, **8**, 57 (1951).

2) H. Tani and H. Yuki, *Chem. High Polymers, Japan*, **8**, 62 (1951).

TABLE I

Monomers	Solvents	Results	Yield (%)	η_{sp} *3)
(A) I, II, III*1)	Nitrobenzene	Transparent gel	100	
(1 : 1 : 3)	Ethyl Phenylacetate	Opalescent gel	100	
	Benzene	Gel particles in viscous soln.	94	
	Dioxane	No change		
(B) IV, V, VI*2)	Chlorobenzene	Clear viscous soln.	94	0.48
(1 : 1 : 2)	Nitrobenzene	Viscous soln., partially gelling	70	0.15
	Ethyl Phenylacetate	Transparent gel	95	0.31
	Chloroform	Soln. of low viscosity		
	Tetrahydrofuran	No change		

*1) The polymerizations were carried out by heating at 70°C for 60 hr.
*2) The polymerizations were carried out with about 8% solution at 60°C for 8 hr. and then at room temperature for ten days.
*3) The specific viscosities of 0.1% solution of the polymers in chloroform at 30°C.

hydriodic acid, but the polypeptides obtained here still contained a small amount of hydrogen iodide and about half of the polypeptides were lost. Such fractionation caused during dialysis may produce changes in the ratios of the amino acid residues composing the polypeptides, if, in each case, the compositions of the individual molecules are not the same. However, the elementary analyses and the amounts of lysine residues estimated from van Slyke's amino-nitrogen determinations indicated that these polypeptides contained the amino acid residues in the same molar ratios as those of the starting monomer mixtures.

Titration curves (see Fig. 1) of these polypeptides in aqueous solutions showed typical amphoteric properties and the carboxyl group contents calculated from the hydrogen ion combining capacities corresponded to the values obtained from analytical data. These polypeptides were soluble in dilute mineral acids and in dilute alkalis, but were insoluble in water over a rather wide range of pH, i.e., 4.0–10.8 for polypeptide A and 4.5–10.2 for polypeptide B, respectively.

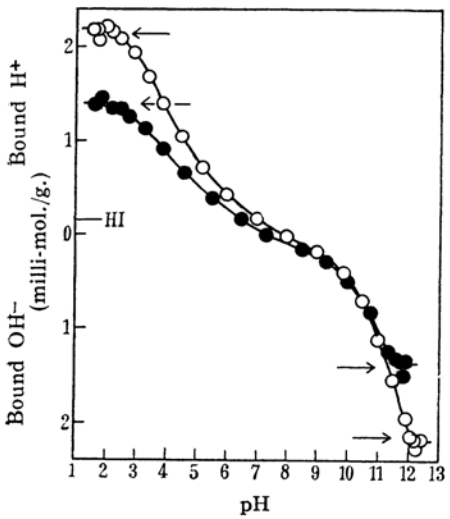

Fig. 1. Titration curves of free amphoteric polypeptides.
●: Copoly-1 : 1 : 3-(DL-glutamic acid, DL-lysine, DL-phenylalanine).
○: Copoly-1 : 1 : 1.9-(L-glutamic acid, L-lysine, L-leucine).
Arrows represent the theoretical end points respectively.

Properties of the Solution

The properties of the aqueous solution of polypeptide B were studied, using the dialysed solution of the hydriodide. The dialysed solution had

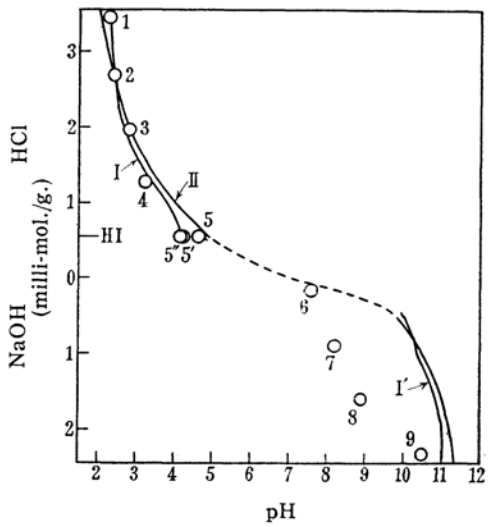

Fig. 2. Titration curves of copoly-1 : 1 : 1.9-(L-glutamic acid, L-lysine, L-leucine).
Curves I and I': Titration of the dialysed solution by 0.1 N HCl and 0.1 N NaOH respectively. Curve II: Titration of a solution of the dry sample in hydrochloric acid with 0.1 N NaOH. The dotted line indicates the insoluble region. The numbered circles represent the pH of the solutions for viscosity measurements, 400 hr. after the preparation of solutions.

a pH value of 4.3. It contained 0.27 mol. of hydriodic acid per lysine residue of the polypeptide. The resulting polypeptide had an isoelectric point at about pH 7.3 as shown in the titration curve in Fig. 2.

The relation of the reduced viscosity to the concentration of the polypeptide was linear in the concentration below about 1%. The intrinsic viscosity was 0.70 (see Fig. 3).

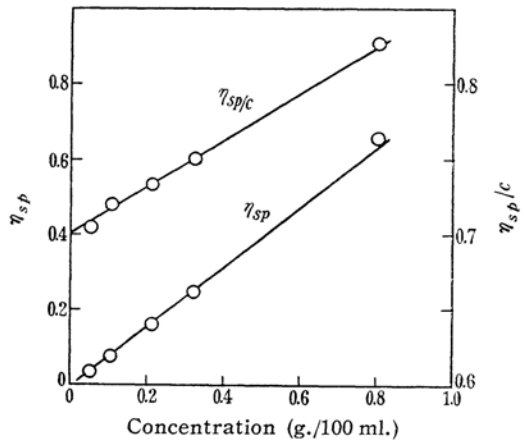

Fig. 3. Relationship between viscosity and concentration.

Because of the insolubility of this polypeptide in the pH range near its isoelectric point, the pH-dependence of viscosity could be measured only outside of this insoluble region. The results (see Fig. 4) showed that the viscosity increased gradually towards its isoelectric point from both sides and that it was considerably higher in the acidic than in the alkaline region.

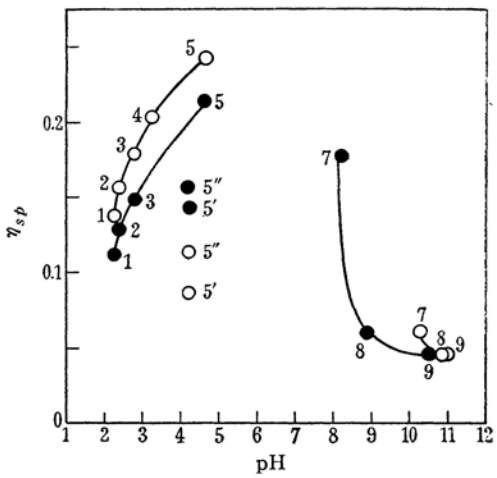

Fig. 4. Relationship between specific viscosity and pH: ○, at 17–52 hr.; ●, at 305–355 hr., after the preparation of the solutions.

The viscosities of solutions of this polypeptide changed gradually with time. In acidic solution the growth of mould was found occasionally, but it is not certain whether or not such bacterial decomposition of the polypeptide caused this decrease in viscosity (see Fig. 5). In alkaline solution the viscosity remained constant for a rather long period and then suddenly began to increase linearly with time. The final solution exhibited thixotropy (see Fig. 6). It was observed also that at the beginning of this increase in viscosity the solution began to show opalescence, which became gradually strong with time, and that the

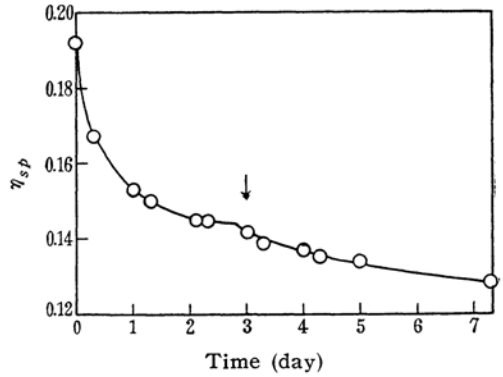

Fig. 5. Change of specific viscosity of solution No. 1. The arrow represents the appearance of mould.

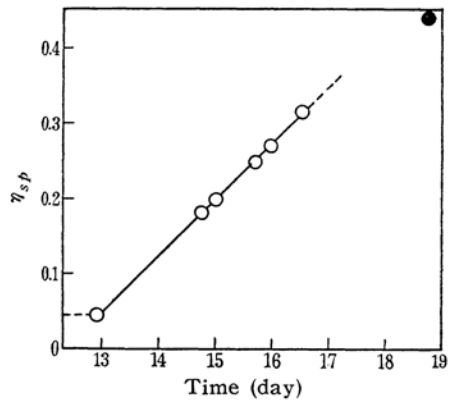

Fig. 6. Change of specific viscosity of solution No. 7.
●: Thixotropy.

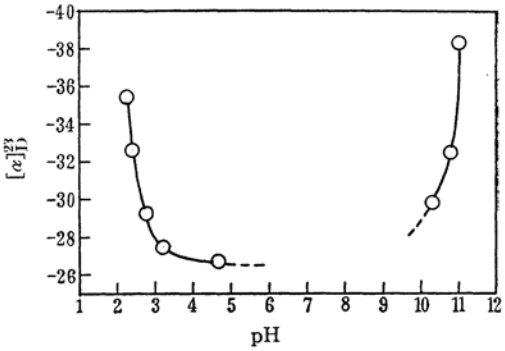

Fig. 7. Relationship between optical rotation and pH.

pH of the solution shifted toward neutrality, as a result of absorption of atmospheric carbon dioxide.

The pH dependence of optical rotatory power of this polypeptide was observed in the soluble region. All of the observed values were laevo-rotatory, the absolute magnitudes decreasing toward the isoelectric region (see Fig. 7).

Discussion

From the results of studies[3] on synthetic vinyl polymers, it has been stated that poly-ampholytes are insoluble in water in any pH range, if the dissociation constant of either the acidic or the basic group is very small, or if the number of one of these groups is much less than that of the other. In the polypeptides obtained in this research, the acidic and basic groups have pK′ values of 4.3 and 10.5 respectively[4], and hence have sufficient dissociation constants to form dipolar ions in their isoelectric regions; moreover, the numbers of these groups are equal. Although the insolubility of these polypeptides in the isoelectric regions may be presumed to be due to the large contribution of the hydrophobic side chain groups of leucine or phenylalanine residues, which are contained in relatively high proportions, high molecular weight copoly-1 : 1-(L-glutamic acid, L-lysine)[5], which contains no such hydrophobic side chain group, also had a wide insoluble region around its isoelectric point.

For solutions of synthetic amphoteric vinyl polymers[3,6,7] and of some of natural proteins[8], it has been reported that viscosity exhibits a minimum at the isoelectric point and a maximum on each side. This viscometric behavior has been interpreted as due to the coiling and stretching of long chain molecules caused by the electrostatic attraction and repulsion between positive and/or negative charges of ionized groups distributed along the molecular chains.

In these experiments the viscosity could be measured only on the outsides of both maxima, because of the insolubility of polypeptide in the neutral region. The lower viscosity in an alkaline region than that of an acidic region may be due to sodium iodide, which was present as extra-salt in the alkaline solutions. It has been shown that a small amount of neutral salt caused a considerable decrease in the viscosity of vinyl polyelectrolyte solutions[3].

The change of viscosity on ageing has been observed in cases of the solution of proteins, such as gelatin[9], but the cause remains obscure.

The increase in viscosity as the pH approaches that of the insoluble region from either side and the appearance of turbidity in the alkaline solution, together with the shift of pH of the solution caused by ageing, suggest that the aggregation of the solute polypeptide becomes greater as the solution approaches to its isoelectric point. Likewise, the insolubility of these amphoteric polypeptides in the neutral region may be due to this aggregation.

As one of the factors causing such an inter-molecular association, the formation of inter-molecular salt-linkages, such as have been assumed by Isemura and Hamaguchi[10] on the basis of their studies of the surface viscosity of these polypeptides, might be considered. Further, the role of hydrogen bonds formed between large numbers of carbonyl and imino groups distributed in the polypeptide chains can not be ignored, since these amphoteric polypeptides differ essentially from the vinyl polyampholytes only in having peptide bonds in their skeletal molecular chains. The large intrinsic viscosity of the polypeptide suggests that it had a more-or-less rigid and highly extended structure in solution.

The behavior of the solution of the polypeptide with regard to optical rotation is quite similar to that of natural proteins[11].

All of these properties of the polypeptides in solution, together with their surface chemical properties reported by Isemura and Hamaguchi[10] and by Davies and Llopis[12] show that they are interesting substances, similar in many respects to polypeptides of natural proteins.

Experimental

γ-Benzyl DL-Glutamate.—A mixture of 30 ml. of conc. hydrochloric acid, 12 ml. of water, 30 g. of powdered DL-glutamic acid and 300 ml. of benzyl alcohol was heated to 95°C for 1/2 hr. with stirring.

3) T. Alfrey, Jr. and H. Morawetz, *J. Am. Chem. Soc.*, 74, 436 (1952); T. Alfrey, Jr., R.M. Fuoss, H. Morawetz and H. Pinner, ibid., 74, 438 (1954).

4) E.J. Cohn and J.T. Edsall, "Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions", Reinhold, New York (1943), p. 84.

5) H. Yuki and H. Tani, Unpublished.

6) T. Matsumoto, *Chem. High Polymers, Japan*, 8, 412 (1951).

7) A. Katchalsky and I.R. Miller, *J. Polymer Sci.*, 13, 57 (1954).

8) S. Akabori and S. Mizushima, "Protein Chemistry", Vol. 2, Kyoritsushuppan, Tokyo (1954), p. 390.

9) S. Akabori, "Amino Acids and Proteins", Kyoritsushuppan, Tokyo (1944), p. 463.

10) T. Isemura and K. Hamaguchi, This Bulletin, 27, 339 (1954); K. Hamaguchi and T. Isemura, ibid., 28, 9 (1955).

11) W. Pauli and E. Valko, "Kolloidchemie der Proteine", T. Steinkopff, Dresden (1933).

12) J.T. Davies, *Biochem. J.*, 56, 509 (1954); J.T. Davies and J. Llopis, *Proc. Roy. Soc.*, A 227, 537 (1955).

The resultant clear solution gave no phase-separation on cooling. The cooled solution was neutralized by an aqueous solution of 30 g. of sodium bicarbonate, and 500 ml. of ether was added with stirring. The precipitated crystals were filtered and washed with a small amount of water, ethanol, and ether. Plates, m.p. 163–163.5°C; no change in m.p. was observed by recrystallization from 520 ml. of water. Yield 26 g. (54% of the theor.)

Anal. Found: N, 5.97. Calcd. for $C_{12}H_{15}NO_4$: N, 5.91%.

γ-Benzyl L-Glutamate[13].—A mixture of 14.5 g. of powdered L-glutamic acid, 24 ml. of conc. hydrochloric acid and 150 ml. of benzyl alcohol was treated as above, and the product was recrystallized from 370 ml. of water to give 12.6 g. (53% of the theor.). Plates, m.p. 173°C, $[\alpha]_D^{17}$ 20.7° (glacial acetic acid, *c* 6.76).

Anal. Found: N, 5.85. Calcd. for $C_{12}H_{15}NO_4$: N, 5.91%.

γ-Benzyl N-Carboxy-DL-glutamate Anhydride (I).—Phosgene was passed for one hour into a suspension of 13 g. of powdered γ-benzyl DL-glutamate in 260 ml. of anhydrous dioxane with stirring at room temperature. After excess phosgene was removed by passing dry air through the solution, it was concentrated to a syrup in vacuo at 40°C and finally at 50°C. The ethyl acetate solution of this syrup gave colorless needles on the addition of petroleum ether. Yield 12 g. (77% of the theor.), m.p. 70–83°C.

Recrystallization from ethyl acetate and petroleum ether gave 8.5 g. of crystals (Ia) of m.p. 70–71°C, and the concentrated mother liquor gave 2 g. of crystals (Ib) of m.p. 83–85°C by adding petroleum ether. Ia and Ib are supposed to be different crystallographic modifications of the same substance, because neither of them showed optical rotation in ethyl acetate solution and both gave the same infra-red absorption spectra.

Anal. Found: N, (Ia) 5.35; (Ib) 5.39. Calcd. for $C_{13}H_{13}NO_5$: N, 5.32%.

γ-Benzyl N-Carboxy-L-glutamate Anhydride (IV).—A suspension of 10 g. of powdered γ-benzyl L-glutamate in 300 ml. of dry dioxane was treated with phosgene as described above. The crystalline product was recrystallized from ethyl acetate and petroleum ether to give 10.5 g. (95% of the theor.) of needles, m.p. 91–92.5°C. Further recrystallization from ethyl acetate raised the m.p. to 93.5–94.5°C. $[\alpha]_D^{29}$ −17.0° (ethyl acetate, *c* 3.65).

Anal. Found: N, 5.28. Calcd. for $C_{13}H_{13}NO_5$: N, 5.32%.

α-N-Carboxy-ε-N-carbobenzoxy-DL-lysine Anhydride[1] **(II).**—This compound was prepared from 12 g. of N, N′-dicarbobenzoxy-DL-lysine and 20 ml. of thionyl chloride and recrystallized several times from ethyl acetate and petroleum ether. Yield 2.4 g. (27% of the theor.), m.p. 103°C.

α-N-Carboxy-ε-N-carbobenzoxy-L-lysine Anhydride[14] **(V).**—A solution of 15 g. of crystalline N, N′-dicarbobenzoxy-L-lysine in 20 ml. of dry dioxane and 30 ml. of dry ether was cooled to 0°C and 8.8 g. of phosphorus pentachloride was added. After shaking for 1/2 hr., the supernatant liquid was decanted and concentrated in vacuo at 40°C. The resultant crystalline mass was decolorized by washing with a small amount of dry ether. Yield 10 g. (80% of the theor.). Recrystallization from ethyl acetate and petroleum ether gave crystals of m.p. 100°C, $[\alpha]_D^9$ −24.1° (ethyl acetate, *c* 6.22).

N-Carboxy-DL-phenylalanine Anhydride[15] **(III).**—This compound was prepared from 14 g. of N-carbobenzoxy-DL-phenylalanine and 20 ml. of thionyl chloride, and repeatedly recrystallized from ethyl acetate and petroleum ether. Yield 6 g. (72% of the theor.), m.p. 123°C.

N-Carboxy-L-leucine Anhydride[16] **(VI).**—Phosgene was passed for two hours into a suspension of 5 g. of powdered L-leucine in 200 ml. of dry dioxane at 40°C. The crystals, which were obtained in the same manner as described above, were recrystallized from ethyl acetate and petroleum ether. Yield 4.8 g. (80% of the theor.), m.p. 76.5–77.5°C. $[\alpha]_D^{11}$ −42.2° (benzene, *c* 0.95).

Anal. Found: N, 8.83, Calcd. for $C_7H_{11}NO_3$: N, 8.91%.

The Effect of Solvent on the Copolymerization.—All the solvents were purified by distillation, and benzene, dioxane and tetrahydrofuran were further dried over metallic sodium. Dioxane and tetrahydrofuran were colored by Schiff's reagent.

The mixtures of N-carboxy anhydrides in the ratio shown in Table I were dissolved in each solvent in a sealed glass tube, and heated to polymerize them.

The solutions of dioxane and tetrahydrofuran showed no change, and on evaporating the solvents in vacuo the crystalline residues were obtained. These residues were dissolved in nitrobenzene or chlorobenzene and heated in sealed tubes. They polymerized rapidly and formed transparent gels or viscous solutions.

The polymers (B) listed in Table I were precipitated by adding ether and petroleum ether to each reaction mixture and reprecipitated from chloroform solutions by petroleum ether. The viscosities of 0.1% chloroform solutions were determined at 30±0.1°C using an Ostwald viscometer.

Copolymer of I, II and III (VII).—The copolymerization was carried out for the mixture of 0.413 g. (1.57 m mol.) of I, 0.480 g. (1.57 m mol.) of II, and 0.900 g. (4.71 m mol.) of III in 20 ml. of each of the solvents listed in Table I, (A). The dioxane solution was evaporated off, and the residual crystals were polymerized in nitrobenzene. Each polymer was precipitated by adding petroleum ether and reprecipitated from tetrahydrofuran by petroleum ether and ether. All the polymers, excepting that from benzene solution, were collected and dried; white powder, 4.35 g.

13) W.E. Hanby et al. (*J. Chem. Soc.*, 1950, 3239) obtained this compound with a considerably lower yield, using hydriodic acid instead of hydrochloric acid.

14) M. Bergmann, L. Zervas and W.F. Ross, *J. Biol. Chem.*, **111**, 245 (1935).

15) H. Leuchs and W. Geiger, *Ber.*, **41**, 1721 (1908).

16) A.C. Farthing, *J. Chem. Soc.*, 1950, 3213; D. Coleman, ibid., 1950, 3222.

(100% of the theor.), soluble in dioxane, tetrahydrofuran, hot glacial acetic acid, and hot chloroform.

Anal. Found: C, 68.71; H, 5.99; N, 9.07. Calcd. for $[(C_{12}H_{13}NO_3), (C_{14}H_{18}N_2O_3), 3(C_9H_9NO)]_n$: C, 68.94; H, 6.34; N, 9.11%.

The polymer obtained from benzene solution (N, 9.16%) contained a small amount of material which was insoluble in the above solvents.

Copoly-1: 1: 3-(DL-glutamic acid, DL-lysine, DL-phenylalanine) (VIII).—A mixture of 4 g. of VII and 100 ml. of glacial acetic acid was heated until a complete solution was obtained. Six grams of phosphonium iodide was then added in portions during 4.5 hr. at 60–70°C with passing through dry hydrogen. After cooling, the precipitate formed was separated by decantation and washed with dry ether. Repeated reprecipitation from absolute ethanol and dry ether gave 3.3 g. (95% of the theor.) of polypeptide hydriodide as white powder. This was hygroscopic and was soluble in ethanol, methanol, water, wet dioxane and wet tetrahydrofuran.

This hydriodide was dissolved in 100 ml. of water and dialysed against distilled water. The solution obtained had a pH of 5.5 and an osmotic pressure of about 30 mm-H_2O (*c*, 6 g./l.); it coagulated to a white gelatinous precipitate on warming to 30°C. After evaporating the solution in an evacuated desiccator at room temperature, 1.6 g. (53% based on the polymer) of pale yellow glassy solid was obtained. This was soluble in dilute mineral acids and in dilute alkalis, but insoluble in water and conc. hydrochloric acid. It was also insoluble in methanol, ethanol and dioxane, but readily soluble in any of these containing hydrochloric acid. It was also soluble in hot, but not in cold, aqueous pyridine. It gave a positive biuret reaction in aqueous solution and foamed even in dilute solution.

Anal. Found*: C, 62.38; H, 6.62; N, 11.12; amino-N, 1.87; I, 3.70. Calcd. for $[(C_5H_7NO_3), (C_6H_{12}N_2O), 3(C_9H_9NO), 0.21\ HI]_n$: C, 62.90; H, 6.40; N, 11.59; amino-N, 1.93; I, 3.68%.

The polymer obtained from benzene solution was reduced and treated in the same manner as described above, but the product contained a small amount of matter which was insoluble in dilute alkali.

Copolymer of IV, V and VI (IX).—A solution of 2.63 g. (0.01 mol.) of IV, 3.06 g. (0.01 mol.) of V and 2.98 g. (0.019 mol.) of VI in 100 ml. of chlorobenzene was sealed in a glass tube and heated for several hours at 80°C and then for 135 hr. at 60°C. The polymer was precipitated from the clear viscous solution by ether and petroleum ether and then reprecipitated from acetone and petroleum ether. The dried white powder weighed 6.90 g. (99% of the theor.). This polymer was easily soluble in chloroform and dioxane, and soluble in tetrahydrofuran, ethyl acetate, acetone and hot glacial acetic acid.

Anal. Found: C, 64.24; H, 7.18; N, 9.96. Calcd. for $[(C_{12}H_{13}NO_3), (C_{14}H_{18}N_2O_3), 1.9(C_6H_{11}NO)]_n$; C, 64.51; H, 7.52; N, 9.86%.

Copoly-1: 1: 1.9-(L-glutamic acid, L-lysine, L-leucine) Hydriodide (X).—As described for the preparation of VIII the solution of 2 g. of IX in 75 ml. of glacial acetic acid was treated with 4.2 g. of phosphonium iodide (with stirring), and the resultant precipitate was repeatedly reprecipitated from absolute ethanol and ether, and dried. The polypeptide hydriodide was obtained as a pale yellow powder. Yield 1.66 g. (96% of the theor.). Its solubility was similar to that of the hydriodide of VIII described above.

Anal. Found*: C, 43.56; H, 6.55; N, 10.62; amino-N, 2.32; I, 22.7. Calcd. for $[(C_5H_5NO_3), (C_6H_{12}N_2O\cdot HI), 1.9(C_6H_{11}NO)]_n$: C, 44.80; H, 6.87; N, 11.43; amino-N, 2.33; I, 21.2%.

Copoly-1: 1: 1.9-(L-glutamic acid, L-lysine, L-leucine) (XI).—To the solution of 1.4 g. of X in 15 ml. of methanol, 0.75 g. of triethylamine was added. The precipitated polypeptide was washed with methanol repeatedly until no halogen was detectable, then washed with ether and dried. The white powder weighed 1.04 g. (94% of the theor.). It was slightly soluble in water and methanol, and considerably soluble in hot water. In dilute mineral acids and in dilute alkalis, it gave a very foamy and viscous solution and gave a positive biuret reaction.

Anal. Found*: C, 56.16; H, 8.03; N, 14.42; amino-N, 3.03. Calcd. for $[(C_5H_7NO_3), (C_6H_{12}N_2O), 1.9(C_6H_{11}NO)]_n$: C, 56.94; H, 8.52; N, 14.53; amino-N, 2.97%.

Dialysis of the Solution of X.—A solution of 1.3 g. of X in 50 ml. of water was dialysed against distilled water for four days. This was concentrated in an evacuated desiccator at room temperature to 65 ml. and used in the following experiments as the "original solution". The concentration of this solution was determined using 5 ml.; making the correction of the content of hydrogen iodide, the concentration of free polypeptide was 8.07 mg./ml. and its yield was 51% of the theoretical value. The dried substance was transparent glassy solid, soluble in dilute mineral acids or in dilute alkalis, but not in water.

Anal. Found*: C, 52.96; H, 8.11; N, 13.69; amino-N, 2.78; I, 6.8. Calcd. for $[(C_5H_7NO_?), (C_6H_{12}N_2O), 1.9(C_6H_{11}NO), 0.27\ HI]_n$: C, 53.05; H, 7.99; N, 13.54; amino-N, 2.76; I, 6.79%.

Electrometric Titrations.—Titrations were made on solutions containing a known weight of the sample (*c*, ca. 10 mg./ml.) dissolved in a known amount of hydrochloric acid with 0.1 N NaOH using a Beckman G pH meter. During the titrations, insoluble precipitates appeared at pH 4.0–10.8 and 4.5–10.2 for VIII and XI. Blank titrations were made under the same conditions. The hydrogen ion and hydroxyl ion combining capacities were estimated from the differences between the measurements for the unknowns (VIII and XI) and those for the blanks[17] (Fig. 1). Curve II in Fig. 2 represents the titration of a dry sample obtained from the "original solution," and curves I and I′ in Fig. 2 are the titration curves.

* The analytical samples were dried over phosphorus pentoxide for several hours at 108°/3 mmHg.

17) T. V. Parke and W. W. Davis, *Anal. Chem*, **26**, 642 (1954).

of the "original solution" with 0.1 N HCl and 0.1 N NaOH respectively.

The Solution Used for Measurements.—Twenty ml. of the "original solution" was diluted to 50 ml.; this was designated as "solution-N". Each 0.25 m mol. of hydrochloric acid and sodium hydroxide were added to each 10 ml. of the "original solution" and these were diluted with water to 25 ml., giving "solution-A" and "solution-B", respectively. By mixing these solutions the following eleven solutions were made. The numbers inserted in Figs. 2, 4, and 7 represent these solution numbers.

Solution No.	1,	2,	3,	4,	5,	5′,	5″,	6,	7,	8,	9
Solution A (ml.)	11	6	5	3	0	3	2	0	0	0	0
Solution N (〃)	0	2	5	9	10	0	4	9	5	2	0
Solution B (〃)	0	0	0	0	0	3	2	3	5	6	11

In solution No. 6, precipitation occurred.

Measurement of Viscosity.—The measurements were carried out at 25±0.01°C using an Ostwald viscometer. As viscosity changes with time, the time intervals between the measurements and the preparation of the solution were noted.

(1) The effect of concentration.—The measurements were carried out on the "original solution," solution No. 5, and its diluted solution 17–24 hr. after their preparation.

(2) The effect of the time.—Solutions No. 1 and 9 were placed, respectively, in the viscometers held in a thermostat, and the measurements were made at appropriate intervals.

(3) The effect of pH.—The measurements were carried out twice on solutions No. 1–9 at 17–52 hr. and at 305–355 hr.

In Fig. 2 the points numbered 1–9 represent the pH of each solution about 400 hr. after their preparations.

Measurement of Optical Rotation.—The measurements were made 43–48 hr. after the preparation of solution.

Measurement of Osmotic Pressure.—The measurement was carried out on solution No. 5 (c, 3.23 mg./ml.) using a Bull and Currie type osmometer at 25±0.01°C. The observed osmotic pressure was 22.8 mm-H_2O**.

Summary

1. Two linear highmolecular amphoteric polypeptides were synthesized.

2. The pH-dependences of viscosity and optical rotation were measured. Some of these properties resembled that of vinyl polyampholytes and some proteins.

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Department of Chemistry, Faculty of Science, Osaka University, Osaka

** Using van't Hoff's equation and neglecting the presence of hydrogen iodide, this corresponds to a molecular weight of about 3.6×10^4. Davies et al.[12] reported the molecular weight of this substance being 35,000 from their measurements of the surface pressure.