Syntheses of Photosensitive Poly(amino acids)

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New poly(amino acids)(L-glutamic acid, L-tyrosine and L-lysine) containing photosensitive acryloyl, methacryloyl, and cinnamoyl groups in their side chains were synthesized from the corresponding amino acid NCAs. Their photosensitivities under UV irradiation were also investigated as to kind of functional group and the skeleton portion of the poly(amino acids). As regards the effect of the former, the sensitivities were in the order: acryloyl≃methacryloyl>cinnamoyl; as regards the effect of the latter, the sensitivities were in the order: poly-glutamate>poly-lysine>poly-tyrosine.

Many basic studies of the syntheses and properties of poly (amino acids) have been reported. Furthermore, these polymers have been used as synthetic fibers with characterstics similar to those of natural polypeptide fibers, leather, and surface-coating agents, and have also been utilized as models for natural peptides in the field of biochemistry. In recent years, some functinal polymers containing the naphthalene,[1] carbazole,[2] and cinnamyl[3] groups at the γ-position of poly-glutamate, and photochromic groups[4] at the ω-position of poly-tyrosine have been synthesized with the intention of keeping the steric regularities of poly(amino acids). This paper will describe the syntheses of characteristic photo resins carrying photosensitive groups at the ω-positions of the side chains of the poly(amino acids) in the light of the properties of poly(amino acid) films, which possess a high vapor permeability and thermal stability and which are edible.

The polymers were derived from the following L-amino acid derivatives previously synthesized: γ-acryloyloxyethyl-L-glutamate (**1a**), γ-methacryloyloxyethyl-L-glutamate (**2a**), γ-cinnamoyloxyethyl-L-glutamate (**3a**), *O*-acryloyl-L-tyrosine (**4a**), *O*-methacryloyl-L-tyrosine (**5a**), *O*-cinnamoyl-L-tyrosine (**6a**), ε-*N*-acryloyl-L-lysine (**7a**), ε-*N*-methacryloyl-L-lysine (**8a**), and ε-*N*-cinnamoyl-L-lysine (**9a**). These derivatives were transformed into the corresponding amino acid *N*-carboanhydrides by treatment with phosgene, followed by polymerization with tertiary amine in dichloromethane. The routes of the syntheses were as follows:

$$\mathrm{RCOOCH_2CH_2OH} + \text{L-glutamic acid} \xrightarrow{H_2SO_4}$$

$$(\mathbf{1a}\text{—}\mathbf{3a}) \xrightarrow{COCl_2} \text{NCA } (\mathbf{1b}\text{—}\mathbf{3b}) \xrightarrow{Bu_3N}$$

$$\left[\mathrm{RCOOCH_2CH_2OOC(CH_2)_2CH}\begin{matrix}\diagup\mathrm{CO}\\ \diagdown\mathrm{NH}\end{matrix}\right]_n \quad (\mathbf{1c}\text{—}\mathbf{3c})$$

$$\mathrm{RCO}\diagdown \\ \quad \mathrm{O} + \text{O-Na-L-tyrosine-Cu} \longrightarrow (\mathbf{4a}\text{—}\mathbf{6a})\text{-Cu} \\ \mathrm{RCO}\diagup$$

$$\xrightarrow{H_2S} (\mathbf{4a}\text{—}\mathbf{6a}) \xrightarrow{COCl_2} \text{NCA } (\mathbf{4b}\text{—}\mathbf{6b}) \xrightarrow{Bu_3N}$$

$$\left[\mathrm{RCOO}\text{-}\langle\bigcirc\rangle\text{-}\mathrm{CH_2CH}\begin{matrix}\diagup\mathrm{CO}\\ \diagdown\mathrm{NH}\end{matrix}\right]_n \quad (\mathbf{4c}\text{—}\mathbf{6c})$$

$$\mathrm{RCO}\diagdown \\ \quad \mathrm{O} + \text{L-lysine-Cu} \longrightarrow (\mathbf{7a}\text{—}\mathbf{9a})\text{-Cu} \\ \mathrm{RCO}\diagup$$

$$\xrightarrow{H_2S} (\mathbf{7a}\text{—}\mathbf{9a}) \xrightarrow{COCl_2} \text{NCA } (\mathbf{7b}\text{—}\mathbf{9b}) \xrightarrow{Bu_3N}$$

$$\left[\mathrm{RCONH(CH_2)_4CH}\begin{matrix}\diagup\mathrm{CO}\\ \diagdown\mathrm{NH}\end{matrix}\right]_n \quad (\mathbf{7c}\text{—}\mathbf{9c})$$

R; CH_2=CH- (**1, 4, 7**), CH_2=C(CH$_3$)- (**2, 5, 8**),  -CH=CH- (**3, 6, 9**)

The films made of the resulting photosensitive poly(amino acids) were crosslinked by irradiation from a UV lamp, the solubilities of the films being compared as to the kind of functional group and amino acid.

Experimental

The IR spectra were measured on a Hitachi EPI-G spectrophotometer. The elemental analyses were carried out by the use of a Perkin-Elmer 240 apparatus. The melting points were measured by a capillary method and were not corrected. The double-bond contents of the polymers were determined by the pyridine-sulfate dibromide method.[5] Materials of a reagent grade were used without further purification; all the solvent were of the highest purity commercially available and were used after dehydration over a molecular sieve.

γ-Acryloyl(methacryloyl)oxyethyl-L-glutamate (1a, 2a). Into a mixture of 8.5 g (58 mM) of L-glutamic acid, 25 ml (232 mM) of 2-hydroxyethyl acrylate, and 0.2 g of hydroquinone, concentrated sulfuric acid was dropped with stirring at 25 °C until the mixture was in solution. After then standing for five days, the reaction mixture was vigorously stirred into 200 ml of ether. The ether layer was discarded, the remaining viscous crude ester-sulfate being washed once with ether and dissolved in 150 ml of ethanol. The solution was then neutralized with *n*-tributylamine. The crude ester was isolated by filtration, washed with ethanol, and dried *in vacuo*. The product was then dissolved in 50 ml of water and then stored in a refrigerator overnight. The remaining L-glutamic acid thus crystallized was filtered; the filtrate was precipitated into a large quantity of ethanol, and then dried *in vacuo*. **1a**: Yield, 52.6%. Mp 145—147 °C. Found: C, 47.95; H, 6.39; N, 5.71%. Calcd for $C_{10}H_{15}NO_6$: C, 48.98; H, 6.12; N, 5.71%. **2a**: Yield, 55.6%. Mp 152 °C. Found: C, 49.80; H, 6.89; N, 5.67%. Calcd for $C_{11}H_{17}NO_6$: C, 50.88; H, 6.56; N, 5.40%.

γ-Cinnamoyloxyethyl-L-glutamate (3a). 2-Hydroxyethylcinnamate was prepared from cinnamic acid and ethyleneglycol in the presence of *p*-toluenesulfonic acid; bp 130—131 °C/0.5 mmHg (lit,[6] 186—189 °C/10 mmHg). A mixture of 3.53 g (24 mM) of L-glutamic acid, 23 g (120 mM) of 2-

hydroxyethylcinnamate, and 4 ml of conc. sulfuric acid was stirred at 90 °C for 10 min. After then standing for two days at 25 °C, the reaction mixture was warmed up to 40 °C over a 4 hr period and then vigorously stirred into 100 ml of ether. The ether layer was discarded, and the remaining viscous crude ester-sulfate was washed with ether and dissolved in 50 ml of water. The solution was then neutralized with dil. aqueous ammonia and cooled in a refrigerator overnight. The ester thus crystallized was filtered, washed with cold water, and dried *in vacuo*. Yield, 10.2%. Mp 167—168 °C. Found: C, 59.27; H, 5.85; N, 4.13%. Calcd for $C_{16}H_{19}NO_6$: C, 59.81; H, 5.91; N, 4.36%.

O-Acryloyl(methacryloyl)-L-tyrosine (4a, 5a). Acrylic (methacrylic) anhydride was prepared from the corresponding acid with triethylamine and phosgene by a conventional method.[7] Compounds **4a** and **5a** were prepared from acid anhydrides and L-tyrosine Cu-complex as in the case of the preparation of *O*-acetyl-L-tyrosine in an alkali solution.[8] **4a**: Yield, 67.5%. Mp 211—214 °C (dec.). Found: C, 61.38; H, 5.94; N, 5.48%. Calcd for $C_{12}H_{13}NO_4$: C, 61.21; H, 5.53; N, 5.95%. **5a**: Yield, 56.7%. Mp 228—230 °C (dec.). Found: C, 62.62; H, 6.09; N, 5.65%. Calcd for $C_{13}H_{15}NO_4$: C, 62.58; H, 6.02; N, 5.62%.

O-Cinnamoyl-L-tyrosine (6a). L-Tyrosine (4.5 g, 25 mM) was dissolved in 50 ml of 1 M sodium hydroxide, followed by the addition of 3.43 g (10% excess) of cupric sulfate. The solution was stirred at 60 °C for 30 min, then cooled to 30 °C and filtered. To the filtrate, 13.92 g (50 mM) of cinnamic anhydride in 100 ml of DMF was added dropwise. The mixture was stirred at 50 °C for 3 hr, and then cooled to room temperature, filtered, and washed with water. The *O*-cinnamoyl-L-tyrosine-Cu thus obtained was washed with hot benzene. The finely powdered **6a**-Cu was decomposed with hydrogen sulfide in 0.5 M hydrochloric acid, followed by filtration. The filtrate was neutralized with dil. aqueous ammonia and cooled overnight. The product thus crystallized was isolated by filtration, washed with cold water and then with ethanol, and dried *in vacuo*. Yield, 57.0%. Mp 238—241 °C (dec.). Found: C, 69.32; H, 5.51; N, 4.50%. Calcd for $C_{18}H_{17}NO_4$: C, 69.38; H, 5.46; N, 4.50%.

ε-N-Acryloyl (methacryloyl)-L-lysine (7a, 8a). L-Lysine-Cu (4.63 g, 13 mM) was suspended in 80% THF containing 2.82 g (30 mM) of sodium acrylate; 3.93 g (31.2 mM) of acrylic anhydride was then added dropwise to the mixture in an ice bath. The mixture was stirred in an ice bath for 2 hr, then stirred at 25 °C overnight and refluxed for 4 hr. The product (**7a**-Cu) was isolated by filtration and washed with cold water. The **7a**-Cu and **8a**-Cu thus obtained were decomposed with hydrogen sulfide in 50 ml of water and then filtered. The filtrates were concentrated *in vacuo* below 50 °C. The residues were recrystallized from 90% ethanol. **7a**: Yield, 45.8%. Mp 213—216 °C (dec.). Found: C, 53.99; H, 7.95; N, 13.72%. Calcd for $C_9H_{16}N_2O_3$: C, 53.92; H, 7.99; N, 13.98%. **8a**: Yield, 42.6%. Mp 218—225 °C (dec.). Found: C, 55.66; H, 8.49; N, 13.98%. Calcd for $C_{10}H_{18}N_2O_3$: C, 55.98; H, 8.40; N, 13.07%.

ε-N-Cinnamoyl-L-lysine (9a). The molar ratio of L-lysine-Cu, cinnamic anhydride, and sodium cinnamate was equal to that of the above preparation. A mixture of the three compounds in 100 ml of 80% THF was refluxed for 12 hr with stirring, filtered while still hot, and washed with water. The **9a**-Cu thus obtained was decomposed with hydrogen sulfide in 100 ml of warm 0.5 M hydrochloric acid, and then filtered. The filtrate was neutralized with dil. aqueous ammonia and cooled in a refrigerator. The resulting solid was isolated by filtration, washed with cold water, and dried *in vacuo*. Yield, 38.5%. Mp 225—228 °C (dec.). Found: C, 65.31; H, 7.29; Calcd for $C_{15}H_{20}N_2O_3$: C, 65.31; H, 7.24; N, 10.13%.

Preparation of NCA and Polymerization. A dry and finely powdered amino acid derivative (4 mM) was suspended in 25 ml of abs. THF and stirred at 50—55 °C. A 12 mM portion of phosgene (30% in carbon tetrachloride) was then added dropwise to the mixture, and the mixture was stirred at that temperature for 1 hr. The unreacted phosgene was taken off by passing through dry nitrogen gas. The reaction mixture was concentrated to a 2—3 ml volume *in vacuo*, followed by precipitation into dry petroleum-ether cooled in a dry ice methanol bath. The crude amino acid NCA thus obtained was subjected to chromatographic purification with 10 g of a molecular sieve (type 3A) column[9] in dichloromethane. The NCA solution was then concentrated to 5 ml and precipitated into petroleum ether. NCA (2 mM) was dissolved in 15 ml of dichloromethane to afford a 2—3% (wt/vol) solution, which was then polymerized with 0.4 mM of dry *n*-tributylamine at 25 °C with stirring. The solution was stirred for 3 hr, allowed to stand for three days, and then precipitated into ether to isolate a solid polymer. Polymer films were made from dichloromethane solution on cover glass plates so as to afford 30—35 mg of the polymer (by weight), left overnight, and then dried *in vacuo* for 2 hr. The polymer films were crosslinked upon irradiation for 15 min by the use of a mercury lamp (Toshiba SHL 100UV-2) placed at a distance of 15 cm; the films crosslinked being immersed in chloroform overnight; they were then dried *in vacuo* for 2 hr to determine their weight loss.

Results and Discussion

Syntheses of ω-Derivatives of L-Amino Acid. Ester (**1a**—**3a**) were synthesized using sulfuric acid as the esterification catalyst. The yield of **3a** was lower than those of the others (**1a** and **2a**) because of the gelation of the reaction mixture at room temperature. When the reaction is prolonged at 90 °C, the ester and double bond of 2-hydroxyethyl cinnamate might be broken. With other acidic catalysts such as *p*-toluenesulfonic acid, however, the yield was very little, so, for this direct esterification, the method with sulfuric acid was adopted. The IR spectra indicated the NH-bond at 3350 cm^{-1}, $COO^-NH_4^+$ at 2600—3200 cm^{-1}, the C=O band of the ester at 1740 cm^{-1}, NH_4^+ at 1610 cm^{-1}, COO^- at 1590 cm^{-1} and the double bond of the corresponding ester at 900—1000 cm^{-1}. Compounds **1a** and **2a** were soluble in water and 50% ethanol; **3a** was slightly soluble in hot water and hot ethanol. *O*-Acetyl-L-tyrosine was directly prepared from L-tyrosine and acetyl chloride without the protection of the α-amino group of the former.[10] In this experiment, however, drastic conditions are unsuitable since double bonds are present in the reaction mixture and should be affected by the reaction; the direct use of acid chloride would cause low yield. In the synthesis of **6a**, the reaction mixture gradually turned brown, presumably due to the decomposition of a certain amount of the Cu-complex. The IR spectra indicated the phenyl group of tyrosine at 1900 and 840—850 cm^{-1} besides the absorption noted above. Compounds **4a** and **5a** were freely soluble in water and were slightly soluble in hot ethanol. Compound **6a** was slightly soluble only in hot water, but its hydrochloride was freely soluble in water

and ethanol. ε-*N*-Derivatives of L-lysine were prepared by the so-called aminolysis of L-lysine with the methyl ester in conc. aqueous ammonia.[11] With an acrylic or methacrylic ester, however, the possibilities of the formation of α-derivatives of L-lysine and the occurrence of a Michael reaction are present, so aminolysis is not suitable. The yield of the product prepared with L-lysine-Cu and acid anhydride in an aqueous solution was about 20% lower than those of tyrosine derivatives (**4a**—**6a**). Sodium salt was used to prevent the decomposition of the Cu-complex under basic conditions,[12] although a long reaction time at a high temperature as in the case of the preparation of **9a** caused a certain amount of decomposition. The IR spectra indicated amide I at 1650 and amide II at 1510 cm^{-1}, besides the absorption of amino acid and the double bond noted above. Compounds **7a** and **8a** were soluble only in water; **9a** was slightly soluble in hot water.

Syntheses of Polymer from ω-Derivatives of Amino Acid. Amino acid *N*-carboanhydrides (NCA) were prepared by a general method with phosgene from the corresponding ω-derivatives of amino acid, and were purified by reprecipitation and by column chromatography carried out with a 3A sieve. The chlorine contents and the purity were not determined. The NCAs (**1b**—**3b**) were poorly crystallized because of the presence of two ester bonds; they melted between 55 and 70 °C and were soluble in conventional organic solvents except petroleum ether. The solubility in dichloromethane was decreased in the order of the *O*-derivatives of tyrosine NCA and the ε-*N*-derivatives of lysine NCA. The melting points of the NCAs were about 100 °C lower than those of the preceding amino acid ω-derivatives: **4b**, 98 °C; **5b**, 111 °C; **6b**, 118 °C; **7b**, 98—100 °C; **8b**, 110—115 °C; **9b**, 120—123 °C. The IR spectra in dichloromethane indicated the absorption of NCA at 1860—1870 and 1790 cm^{-1} besides the absorption of the functional groups in the ω-position and the double bonds described above. Amino-acid NCAs, even without drying, were polymerized with *n*-tributylamine in dichloromethane. When the solubility of NCA in dichloromethane was low, an α-helix solvent such as DMF was added until cleanness was reached. Table 1 shows the results of the analysis of the polymer thus prepared. The values of the double-bond content less than 100% may be accounted for by the some precipitates were formed during the pyridine-sulfate-dibromide titrations of the polymer solutions in dichloroacetic acid (DCA), thereby causing errors in analysis due to heterogeneity. The degrees of polymerization were low in the case of the polymers carrying sterically large cinnamoyl groups at the ω-position. The solubility in an α-helix solvent was large when polymers contained two ester groups as in the case of **1c**—**3c**, and decreased in the order of substituted poly-lysine and poly-tyrosine. The IR spectra indicated NH at 3280 cm^{-1}, the ester at 1720—1740 cm^{-1}, amide I and amide II at 1650 and 1530 cm^{-1} respectively (**7c**—**9c** overlapped), and the double bonds in the side chains at 900—1000 cm^{-1}. Table 2 shows the results obtained in the crosslinking experiments effected for polymer films. To compare the effect of the kind of sensitizer, the weightloss by solvent extraction was determined for **2c**; none, 74.8%; methylene blue, 66.1%; 1% chlorophyll, 67.0%; 1% methylene blue and 5% benzoyl peroxide (BPO), 14.0% and 1% chlorophyll and 5% BPO, 4.3%. The last combination was used for the crosslinking experiments shown in Table 2. As regards the effects of functional groups in polymers, the degree of crossliking was in the order: acryloyl≃methacryloyl>cinnamoyl. No differences in effect were observed between the acryloyl and the methacryloyl groups. The photodimerizable cinnamoyl groups caused a very low degree of crosslinking, presumably because of steric hindrance.

Table 1. The results of analysis of ploy(amino acid) ω-derivatives

Polymer No.	Yield[a] %	$[\eta]$[b] dl/g	Double bond[c]	Elemental analysis Found (Calcd), % C	H	N	Solubility[d] Soluble	Insoluble
1c	53.7	0.302	81.2	51.53 (52.86)	6.02 (5.72)	6.26 (6.16)	DCA, DCM, DMF, THF, CF, warm EtOH, AC	Et_2O, W
2c	55.7	0.313	88.9	54.26 (54.77)	6.47 (6.22)	5.95 (5.81)	DCA, DCM, DMF, THF, CF, warm EtOH, AC	Et_2O, W
3c	50.2	0.204	94.0	64.48 (63.36)	6.07 (5.61)	4.40 (4.62)	DCA, DCM, DMF, CF, warm EtOH	MeOH, Et_2O, W
4c	45.0	0.280	79.0	66.84 (66.26)	5.78 (5.06)	5.86 (6.44)	DCA, warm DCM, DMF, CF	EtOH, MeOH, Et_2O, AC, W
5c	42.1	0.359	87.7	67.23 (67.44)	5.67 (5.62)	6.17 (6.05)	DCA, warm DCM, CMF, CF	EtOH, MeOH, Et_2O, AC, W
6c	38.8	0.178	77.7	74.41 (73.32)	5.33 (5.12)	4.11 (4.79)	DCA, DCM, DMF, CF, warm AC	MeOH, Et_2O, W
7c	57.3	0.292	83.2	60.91 (59.24)	7.61 (7.68)	14.72 (15.35)	DCA, DCM, DMF, CF slightly EtOH, AC	MeOH, Et_2O, W
8c	72.0	0.235	91.2	61.83 (61.13)	8.29 (8.15)	14.05 (14.26)	DCA, DCM, DMF, CF slightly EtOH, AC	MeOH, Et_2O, W
9c	38.7	0.208	81.0	70.39 (69.66)	6.96 (6.79)	10.10 (10.84)	DCA, DCM, DMF, CF, warm EtOH, AC	MeOH, Et_2O, W

a) Based on amino acid ω-derivatives. b) **1c**—**3c** in DMF at 25 °C, **4c**—**9c** in DCA at 25 °C. c) mol % based on theory by the method of pyridine-sulfate-dibromide. d) Determined woithut drying of polymers, DCM, dichloromethane; CF, chloroform; AC, acetone; W, water.

Table 2. Crosslinking of polymer films[a,b]

Polymer	Sensitizer None	Present[c] I	Present[c] II	Appearance[d]
1c	64.5	2.4	2.7	Swelling
2c	74.8	4.3	10.9	Swelling
3c	74.7	24.8		Swelling
4c	73.5	46.8	29.3	Swelling
5c	59.2	28.5	9.7	Swelling
6c	90.1	83.1	83.5	Almost dissolved
7c	68.8	11.6	26.3	Swelling
8c	61.6	28.1	39.1	Swelling
9c	86.1	69.8	66.8	Almost dissolved

a) 15 min-irradiation at 15 cm from Hg lamp. b) Weight loss % due to the immersion in $CHCl_3$ overnight after irradiation. c) 1% chlorophyll and 5% BPO based on polymer. I, weightloss in $CHCl_3$; II, after immersion in $CHCl_3$ (I), weightloss in DCA determined in the same manner as in I. d) For the irradiated polymer films containing the sensitizers.

As regards the effect of the skeleton portion of poly amino acid, the degree of crosslinking was in the order: poly-glutamate>poly-lysine>poly-tyrosine. The substituted poly-tyrosine have a sterically large phenyl groups adjacent to the ester group, thereby causing a low extent of crosslinking; while poly-glutamates carrying photosensitive functional groups at the ends of long flexible side chains were crosslinked to a large extent.

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