Syntheses and Conformational Studies of Poly(S-aminoalkyl-L-cysteines) and Their Benzyloxycarbonyl Derivatives

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ABSTRACT: Poly(S-2-benzyloxycarbonylaminoethyl-L-cysteine), poly(S-3-benzyloxy-carbonylaminopropyl-L-cysteine), poly(S-L-2-benzyloxycarbonylaminopropyl-L-cysteine), and poly(S-D-2-benzyloxycarbonylaminopropyl-L-cysteine) were prepared by the *N*-carboxyanhydride method. The protected polymers were converted into poly(S-2-amino-ethyl-L-cysteine), poly(S-3-aminopropyl-L-cysteine), poly(S-L-2-aminopropyl-L-cysteine), and poly(S-D-2-aminopropyl-L-cysteine) through decarbobenzoxylation with hydrogen bromide. The conformation of the above polypeptides was studied by means of their infrared (IR) spectra, X-ray diffractions, optical rotatory dispersions (ORD), and circular dichroisms (CD). By means of the IR spectra and X-ray diffractions, the polymers are found to be in the β-conformation in the solid state. The results of the ORD and the CD suggest that the β—coil transition of these protected polymers occurs at 3—15-% DCA in a chloroform—DCA mixture. In the pH range 3.0—8.0 in an aqueous solution, the deprotected polymers are in a random coil structure. However, when the pH is increased to about 9, these polymers change into the β-conformation. The results of IR measurements of these polypeptides in D_2O also indicated that they had the β-conformation.

KEY WORDS   Poly(S-benzyloxycarbonylaminoalkyl-L-cysteines) /
Poly(S-aminoalkyl-L-cysteines) / Synthesis / β—Coil Transition /
Infrared Spectra / Optical Rotatory Dispersion / Circular Dichroism /

It has been reported that poly(S-2-aminoethyl-L-cysteine) is hydrolyzed by trypsin in a manner closely resembling the hydrolysis of poly(L-lysine).[1] This observation suggests the possibility that the geometrical relationship between the side chain amino group and the peptide bond is practically identical in peptides of L-lysine and of S-2-aminoethyl-L-cysteine. The S-alkyl derivatives of poly(L-cysteine), such as S-methyl, S-benzyl, and S-benzyloxycarbonyl, have been reported to exist in the stable β-conformation in the solid state and in solution.[2-5] It has also been reported that poly(S-carboxyalkyl-L-cysteines) and poly(S-2-aminoethyl-L-cysteine) can undergo a β—coil transition in aqueous media if there is a change in pH.[6-9]

In this paper, poly(S-2-benzyloxycarbonyl-aminoethyl-L-cysteine) (Z-I), poly(S-3-benzyloxy-carbonylaminopropyl-L-cysteine) (Z-II), poly(S-L-2-benzyloxycarbonylaminopropyl-L-cysteine) (Z-L-III), poly(S-D-2-benzyloxycarbonylaminopropyl-L-cysteine) (Z-D-III), poly(S-2-aminoethyl-L-cysteine) (I), poly(S-3-aminopropyl-L-cysteine) (II), poly(S-L-2-aminopropyl-L-cysteine) (L-III), and poly(S-D-2-aminopropyl-L-cysteine) (D-III) were synthesized. The secondary structures of these polymers were studied in order to investigate the effect of the contribution of side chains with optically active and inactive groups to the polypeptide structure by means of the ORD, CD, IR spectra, and X-ray diffractions.

Synthesis and conformational aspects of I have already been reported by Stokrova and coworkers.[9] Other homologous polypeptides of L-cysteine derivatives were prepared in the almost same way as I.

The catalytic hydrolyses of poly(S-aminoalkyl-L-cysteine) by trypsin will be studied later.

EXPERIMENTAL

Materials

The physical and analytical data of materials

Table I. The physical property and elemental analysis of monomers

Sample[a]	Yield, %	mp, °C	$[\alpha]_D^{22}$	Molecular formula	Calcd			Found		
					C, %	H, %	N, %	C, %	H, %	N, %
3-Aminopropylbromide·HBr	78.8	172	—	$C_3H_9NBr_2$	16.46	4.14	6.40	16.49	4.20	6.27
L-2-Aminopropylbromide·HBr	91.2	53—61	+12.4	$C_3H_9NBr_2$	16.46	4.14	6.40	16.62	4.30	6.37
D-2-Aminopropylbromide·HBr	92.0	Oil	—	$C_3H_9NBr_2$	16.46	4.14	6.40	—	—	—
3-Z-Aminopropylbromide	93.3	Oil	—	$C_{11}H_{14}NO_2Br$	48.55	5.18	5.15	48.73	5.13	4.83
L-2-Z-Aminopropylbromide	42.9	48—48.5	−5.7	$C_{11}H_{14}NO_2Br$	48.55	5.18	5.15	48.78	4.98	5.07
D-2-Z-Aminopropylbromide	39.8	46—47.5	+2.2	$C_{11}H_{14}NO_2Br$	48.55	5.18	5.15	49.17	5.11	5.41
S-3-Z-Aminopropyl-L-Cys	54.5	197—198	−4.8	$C_{14}H_{20}N_2O_4S$	53.83	6.45	8.97	54.01	6.46	8.90
S-L-2-Z-Aminopropyl-L-Cys	69.0	197	+27.1	$C_{14}H_{20}N_2O_4S$	53.83	6.45	8.97	53.51	6.32	8.71
S-D-2-Z-Aminopropyl-L-Cys	57.9	178—181	−32.8	$C_{14}H_{20}N_2O_4S$	53.83	6.45	8.97	53.61	6.41	8.64
S-3-Z-Aminopropyl-L-Cys NCA	98.5	Oil	—	$C_{15}H_{18}N_2O_5S$	53.24	5.36	8.28	—	—	—
S-L-2-Z-Aminopropyl-L-Cys NCA	94.5	141—142	—	$C_{15}H_{18}N_2O_5S$	53.24	5.36	8.28	53.39	5.50	8.27
S-D-2-Z-Aminopropyl-L-Cys NCA	94.5	114—115	—	$C_{15}H_{18}N_2O_5S$	53.24	5.36	8.28	52.94	5.58	7.92

[a] Z represents benzyloxycarbonyl.

Table II. The physical property and elemental analysis of polymers

Polypeptide	Yield, %	DP[a]	$[\eta]$,[b] dl/g	Molecular formula	Calcd			Found		
					C, %	H, %	N, %	C, %	H, %	N, %
Z-I	68.0	31	0.210	$(C_{13}H_{16}N_2O_3S)_n$	55.70	5.75	9.99	55.14	5.85	9.69
Z-II	72.7	22	0.135	$(C_{14}H_{18}N_2O_3S)_n$	57.12	6.16	9.52	57.21	6.24	9.31
Z-L-III	79.5	15	0.120	$(C_{14}H_{18}N_2O_3S)_n$	57.12	6.16	9.52	57.04	5.83	9.51
Z-D-III	78.1	25	0.170	$(C_{14}H_{18}N_2O_3S)_n$	57.12	6.16	9.52	56.80	6.12	9.48
I	71.6	—	—	$(C_5H_{11}N_2OSBr)_n$	26.44	4.88	12.33	26.49	4.61	12.12
II	85.4	—	—	$(C_6H_{13}N_2OSBr)_n$	29.88	5.43	11.62	29.38	5.32	11.72
L-III	85.5	—	—	$(C_6H_{13}N_2OSBr)_n$	29.88	5.43	11.62	29.24	5.35	11.27
D-III	78.4	—	—	$(C_6H_{13}N_2OSBr)_n$	29.88	5.43	11.62	29.54	5.36	11.43

[a] Determined from the amino end-group titration.
[b] In dichloroacetic acid at 25°C.

are shown in Tables I and II.

2-Aminoethylbromide Hydrobromide. 2-Aminoethylbromide hydrobromide was prepared from ethanolamine and hydrobromic acid as described by Lindley.[10] By the same procedure as above, 3-aminopropyl-, L-2-aminopropyl-, and D-2-aminopropylbromide were prepared from the corresponding alaninols and hydrobromic acid.

Benzyloxycarbonylaminoethylbromide. Benzyloxycarbonylaminoethylbromide was prepared from aminoethylbromide hydrobromide and benzyloxycarbonylchloride as described by Lindley.[10] 3-Benzyloxycarbonylaminopropylbromide, L-2-benzyloxycarbonylaminopropylbromide, and D-2-benzyloxycarbonylaminopropylbromide were prepared from the corresponding aminoalkylbromide hydrobromides and benzyloxycarbonylchloride by the same procedure as the above method.

S-2-Benzyloxycarbonylaminoethyl-L-cysteine. S-2-Benzyloxycarbonylaminoethyl-L-cysteine was prepared from 2-benzyloxycarbonylaminoethylbromide and L-cysteine as described by Lindley.[10] *S*-3-Benzyloxycarbonylaminopropyl-L-cysteine, *S*-L-2-benzyloxycarbonylaminopropyl-L-cysteine, and *S*-D-2-benzyloxycarbonylaminopropyl-L-cysteine were prepared from the corresponding benzyloxycarbonylaminoalkylbromides and L-cysteine.

Preparation of N-Carboxyamino Acid Anhydrides (NCA). The *S*-benzyloxycarbonylaminoalkyl-L-cysteine NCAs were prepared by the usual procedure.

Preparation of Protected Polymers. The above NCAs were polymerized in a chlorobenzene—dimethylformamide (3 : 1) mixture.

Preparation of Poly(S-aminoalkyl-L-cysteine hydrobromides). The polypeptide hydrobromides were prepared from the protected polymers by the usual procedure using hydrogen bromide in glacial acetic acid.

Methods

IR spectra were measured on a JASCO Model DS-301 spectrophotometer at 22°C. IR spectra of the solutions of polymers were measured in a tube of polyethylene film. X-ray diffraction photographs were taken with a Rigakudenki Geigerflex, using a Cu-target. CD and ORD were measured on a JASCO ORD/UV-5 spectropolarimeter with a CD attachment at 22°C. The measurements were performed with 0.1- and 1-mm cells from 260 to 200 nm, and with 10- and 50-mm cells for visible regions. The concentration of the samples were in the 0.1—1.0-% range. CD and ORD are expressed by the residual ellipticity, $[\theta]$, and the reduced residual rotation, $[m']$, respectively. The amino end-groups were determined by titration of the polymer in *m*-cresol with $HClO_4$/acetic acid using crystal violet as the indicator. The viscosities were measured at 25°C using an Ubbelohde viscometer.

RESULTS AND DISCUSSIONS

Conformation of the Polymers in the Solid State
 IR Studies and X-Ray Analyses. The protected polymers (Z-I, Z-II, Z-L-III, and Z-D-III) showed

Table III. Characteristic absorption bands of polypeptides (KBr disks)

Assignment	Wave number, cm^{-1a}			
	Z-I	Z-II	Z-L-III	Z-D-III
Amide A	3310 (vs)	3300 (vs)	3318 (vs)	3315 (vs)
Amide B	3080 (w)	3080 (w)	3080 (w)	3080 (w)
Amide I $(0, \pi)$	1695 (sh)	1695 (sh)	1695 (sh)	1695 (sh)
Amide I $(\pi, 0)$	1635 (vs)	1637 (vs)	1635 (vs)	1635 (vs)
Amide II	1530 (vs)	1528 (vs)	1530 (vs)	1530 (vs)
Amide V	690 (m)	690 (s)	690 (s)	690 (s)

[a] vs, very strong; s, strong; m, medium; w, weak; sh, shoulder.

IR characteristic absorption bands at about 1635 cm^{-1} (Amide I) and 1530 cm^{-1} (Amide II), suggesting that the polymer could have the β-conformation (Table III). The frequencies of amide I $(0, \pi)$ and amide V bands appeared at 1695 and 690 cm^{-1}, respectively, indicating that the polymer conformation is an antiparallel β-conformation. The deprotected polymers (I, II, L-III, and D-III) also have the β-conformation.

The X-ray diffraction photographs of the protected polymers and the deprotected polymers showed a backbone spacing of about 4.6 Å (β-conformation), which coincided well with the results of the IR studies.

Conformation of the Polymers in Solutions.

Optical Rotation and Infrared Absorption of Protected Polymers. The plots of the $[\alpha]_{400}$ values *vs.* solvent composition of these protected polymers in a chloroform—DCA mixture are shown in Figure 1. All polymers cause a conformational change at 3—15-% DCA. The levorotation of Z-I decreases at 4-% DCA. The levorotation of Z-II increases until the DCA content reached 4%, but it decreases markedly at a DCA content of less than 3%. On the other hand, Z-L-III and Z-D-III with an optically active side chain, show increased levorotation at about 10-% DCA. This result can be considered to mean that the chromophore in the side chain causes an extraordinary optical rotation. In order to elucidate the conforma-
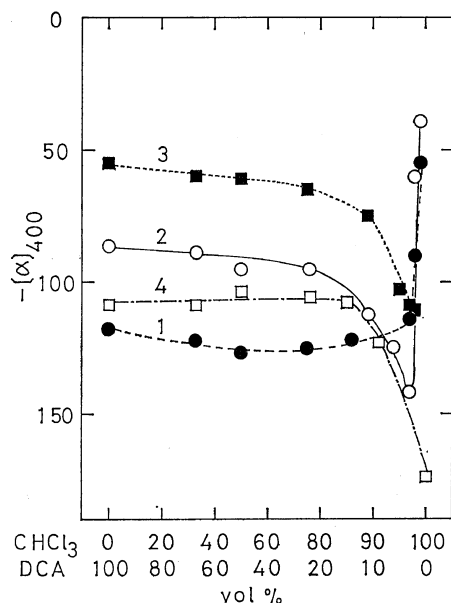

Figure 1. Specific rotation $[\alpha]_{400}$ values for the protected polymers as a function of varing solvent compositions at 22°C: 1, Z-I; 2, Z-II; 3, Z-L-III; 4, Z-D-III.

Table IV. Characteristic absorption bands of polypeptides in a chloroform—DCA mixture at 22°C[a]

Poly-peptide	Solvent composition, % DCA content	Amide I, cm^{-1}	Amide II, cm^{-1}	Confor-mation[b]
Z-I	2	1638	1528	β
	5	1649	1535	R
Z-II	1	1638	1520	β
	5	1658	1535	R
Z-L-III	1	1638	1522	β
	3	1648	1535	R
Z-D-III	0	1638	1520	β
	5	1660	1535	R

[a] Polymer concentration, 0.5 g/d*l*.
[b] β, β-conformation; R, random coil.

tional change, IR solution measurements were carried out. The results are listed in Table IV. With a low DCA content (0—3%) the amide I band at $1638 \, cm^{-1}$ shows the β-conformation and in a chloroform—DCA (95 : 5) mixture the amide I band at 1648—$1660 \, cm^{-1}$ shows random coiling. This clearly indicates that the conformational change is the β-conformation—random coil transition.

ORD and CD of Deprotected Polymers. Conformational studies of the deprotected polymers in aqueous solutions were carried out. The ORD curves of the polymers in aqueous solutions are shown in Figures 2 and 3. At pH 7.0, the polymers exhibit a trough at about 233 nm

with $[m']_{233} = -2000$——$3000 \, deg \, cm^2/dm$, a 220—223-nm peak, and a trough at 206 nm with $[m']_{206} = -6000$——$10000 \, deg \, cm^2/dm$. However, when the pH is increased above 9.5, a remarkable change in the ORD spectra is observed: the trough at 206 nm disappears and a peak appears at 210—215 nm. The trough at 233 nm shifts to 236 nm. This behavior is close to the β-conformation—random coil transition of poly(S-carboxyalkyl-L-cysteine)[6-8] and poly(L-thialysine).[9] Consequently, the polymer is in the β-conformation at basic pH. In Table V, the position and magnitude of the observed
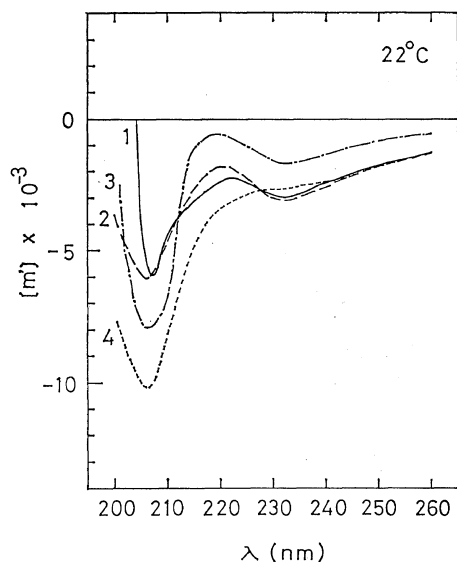

Figure 2. ORD of the deprotected polymers in aqueous solutions at pH 7.0: 1, I; 2, II; 3, L-III; 4, D-III.
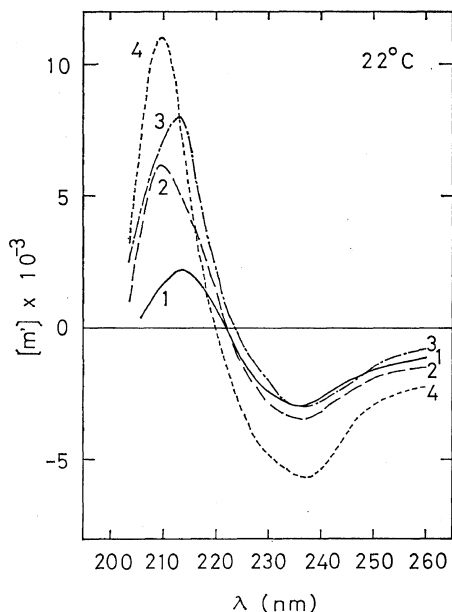

Figure 3. ORD of the deprotected polymers in aqueous solutions at pH 9.5; symbols the same as in Figure 2.

Table V. Cotton effects of polypeptides in aqueous solutions at 22°C

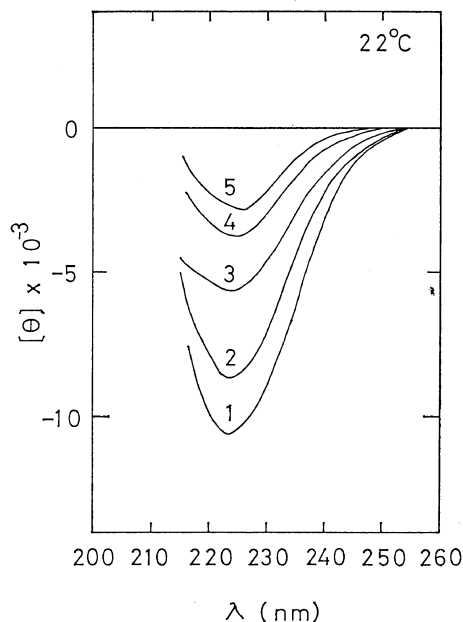| Conformation	λ, nm		$[m']$			
			I	II	L-III	D-III
Random coil	233	Trough	−3000	−3000	−1700	−2600
	206	Trough	−6000	−6000	−8000	−10200
β-Form	236	Trough	−3000	−3500	−3000	−5600
		Cross-over point, nm	222	222	223	220
	210—213	Peak	2200	6100	8000	11000

Figure 4. CD of poly(*S*-3-aminopropyl-ʟ-cysteine) (II) as a function of pH in aqueous media: 1, pH 10.02; 2, pH 9.03; 3, pH 8.80; 4, pH 7.25; 5, pH 3.85.

Table VI. Circular dichroic bands of polypeptides in aqueous solutions at 22°C

Polypeptide	Conformation	λ, nm	$[\theta]$	λ, nm	$[\theta]$
I	R	226	-2700		
	β	223	-9100	<205	$+$
II	R	223	-4500		
	β	220	-8200	<205	$+$
ʟ-III	R	225	-2800		
	β	223	-10600	<205	$+$
ᴅ-III	R	230	-900		
	β	221	-15100	<205	$+$

Cotton effects of the polymers are listed. The peaks and troughs of these polymers are found approximately in the same position, but their absolute magnitudes differ from each other. This may be due to the influence of the side chain in the polymer.

The CD curves of ʟ-III are shown in Figure 4. A negative dichroic band at 226 nm with $[\theta]_{226}=-2800$ was observed at pH 3.85. The
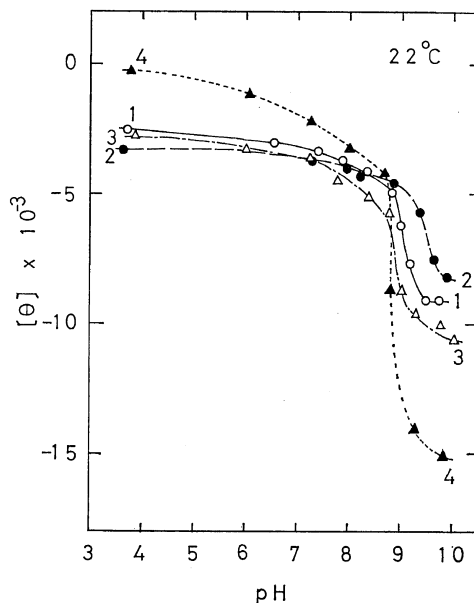

Figure 5. β—Coil transition of the deprotected polymers in aqueous media: 1, I ($[\theta]_{223}$); 2, II ($[\theta]_{220}$); 3, ʟ-III ($[\theta]_{223}$); 4, ᴅ-III ($[\theta]_{221}$).

band at 226 nm is assigned to the n—π^* peptide electronic transition. When the pH is increased, the negative band at 226 nm shifts to 223 nm, and the magnitude of the $[\theta]_{223}$ value enlarges gradually. At pH 10.02, the polymer has the negative band at 223 nm with $[\theta]_{223}=-10600$. This is similar to the β-conformation—random-coil transition of poly(*S*-carboxyalkyl-ʟ-cysteine)[6-8] and poly(ʟ-thialysine),[9] and coincides well with the results of the ORD. In Table VI, the position and magnitude of the CD are listed. The pH-induced transition of these polymers in aqueous solutions is shown in Figure 5. ʟ-III and ᴅ-III showed a sharp transition at about pH 8.8, while, I and II gave it at about pH 9.3. In the β-conformation and the random coil region, the $[\theta]_{220-226}$ values of each polymers are different. From this result it may be assumed that the magnitude of the n—π^* transition depends on the nature of the side chain of the polypeptide. Furthermore, in the case of ʟ-III and ᴅ-III with the optically active side chain, the changes of the $[\theta]_{220-226}$ values is especially remarkable.

Infrared Spectra of the Polymers in D_2O

The IR spectra of the polypeptides were

T. Hayakawa, Y. Kondo, and Y. Murakami

Table VII. Characteristic absorption bands of polypeptides in D_2O solutions at 22°C[a]

Poly-peptide	pD	Amide I $(0, \pi)$, cm^{-1}	Amide I $(\pi, 0)$, cm^{-1}	Confor-mation
I	3.7		1658	R
	9.5	1695	1623	β
II	3.7		1665	R
	9.5	1690	1625	β
L-III	3.7		1655	R
	9.5	1690	1625	β
D-III	3.7		1665	R
	9.5	1695	1625	β

[a] Polymer concentration, 0.5 g/dl.

determined in D_2O. The frequencies of the amide I bands are listed in Table VII. At pD 3.7, they have an amide I band $(\pi, 0)$ at about 1660 cm^{-1}, which can be assigned to the random coil. However, the amide I band $(\pi, 0)$ shifts to 1625—1635 cm^{-1} at pD 9.5, suggesting the formation of a β-conformation. The amide I band $(0, \pi)$ is located around 1690 to 1695 cm^{-1}. This clearly indicates that the polypeptide is in the β-conformation with an antiparallel arrangement.

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