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Poly(*S*-alkyl-L-cysteines) containing long aliphatic side chains

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INTRODUCTION

Conformational studies of branched polymers containing long aliphatic side chains have been investigated.

It has been reported that the *S*-lower alkyl derivatives of poly(L-cysteine), such as *S*-methyl, *S*-benzyl, and *S*-benzyloxycarbonyl, are hardly soluble in most of the common organic solvents, and form a stable β-structure: in coil-promoting solvents, such as dichloroacetic acid (DCA) and trifluoroacetic acid (TFA), they are in a random coil[1–4].

In a previous paper, we have reported that poly(*S*-menthyloxycarbonylmethyl-L-cysteine)[5] and poly(*S*-benzyl-L-penicillamine)[6] which are soluble in many organic solvents, are in the α-helical or the ω-helical conformation. It seems interesting to study the synthesis and conformational analysis of poly(*S*-alkyl-L-cysteines) which are easily soluble in many organic solvents.

In the present paper, we report the results of structural investigations in solution and in the solid state on polypeptides with long alkyl side chains, such as poly(*S*-lauryl-L-cysteine) (I), poly(*S*-myristyl-L-cysteine) (II), and poly(*S*-stearyl-L-cysteine) (III). These higher side-chain homologues of *S*-alkyl-L-cysteine were prepared by a reaction of L-cysteine and the corresponding higher alkyl bromides. The cysteine derivatives were then polymerized to the above polymers by the *N*-carboxyanhydride (NCA) method. These polymers were soluble in chloroform, hot monochlorobenzene, DCA, and TFA. In order to investigate the effect of the side-chain lengths on the polypeptide structure, the conformation of the polymers were studied by means of the infra-red (i.r.) spectra, X-ray diffraction, and optical rotatory dispersions (o.r.d.) in the solid state and in solution.

EXPERIMENTAL

Materials

The physical and analytical data of the materials are shown in *Tables 1* and *2*.

Table 1 The physical properties and elemental analyses of monomers

					Calculated			Found		
Sample	Yield (%)	M_p* (°C)	$[\alpha]_D$†	Molecular formula	C (%)	H (%)	N (%)	C (%)	H (%)	N (%)
S-lauryl-L-cys	69.2	209	–2.09	$C_{15}H_{31}O_2NS$	62.24	10.79	4.84	62.35	10.56	4.83
S-myristyl-L-cys	46.0	201	–0.36	$C_{17}H_{35}O_2NS$	64.30	11.11	4.41	64.61	10.94	4.36
S-stearyl-L-cys	36.4	196	–0.62	$C_{21}H_{43}O_2NS$	67.51	11.60	3.75	67.16	11.60	3.67
S-lauryl-L-cys NCA	68.8	70		$C_{16}H_{29}O_3NS$	60.92	9.27	4.44	60.94	9.31	4.37
S-myristyl-L-cys NCA	78.6	77		$C_{18}H_{33}O_3NS$	62.70	9.65	4.06	62.41	9.65	4.06
S-stearyl-*L*-cys NCA	58.5	83		$C_{22}H_{41}O_3NS$	66.11	10.34	3.50	66.09	10.56	3.54

* All melting points were determined on a Yamato MP-1 melting-point apparatus.
† Optical rotation: all samples were measured at 20°–25°C in DCA

Table 2 The physical properties and elemental analyses of polymers

					Calculated			Found		
Polymer	Yield (%)	D_p*	$[\eta]$† (dl/g)	Molecular formula	C (%)	H (%)	N (%)	C (%)	H (%)	N (%)
Poly(*S*-lauryl-L-cys)	58.1	20	0.140	$(C_{15}H_{29}ONS)_n$	66.37	10.77	5.16	66.53	10.71	5.13
Poly(*S*-myristyl-L-cys)	79.6	22	0.152	$(C_{17}H_{33}ONS)_n$	68.40	10.81	4.69	68.29	10.58	4.60
Poly(*S*-stearyl-L-cys)	56.2	15	0.138	$(C_{21}H_{41}ONS)_n$	70.93	11.62	3.94	70.60	11.35	3.94

* Degree of polymerization (D_p): all samples were determined from the amino end-group titration using crystal violet as an indicator
† Intrinsic viscosity: all samples were measured at 25°C in DCA using an Ubbelohde viscometer

S-alkyl-L-cysteines. S-lauryl-L-cysteine, S-myristyl-L-cysteine, and S-stearyl-L-cysteine were prepared from L-cysteine and the corresponding alkyl bromides by the same procedure as described by Frankel *et al.*[7]. The products were recrystallized from glacial acetic acid.

S-alkyl-L-cysteine NCAs. S-alkyl-L-cysteine NCAs were prepared by the usual procedure using phosgene. The NCAs were recrystallized from ethyl acetate–n-hexane.

Poly(S-alkyl-L-cysteines). The above NCAs were dissolved in five-fold monochlorobenzene, and triethylamine was added as an initiator ($A/I = 100$). The mixture was polymerized at 50°C for a few days and yielded a semi-solid gel. The polymer was washed successively with ether, methanol, water, and acetone, and dried.

Methods

O.r.d. and i.r. spectra measurements were performed on a ORD/UV 5 and IR-301 instruments, both made by the Japan Spectroscopic Co. Ltd. Solution i.r. measurements were carried out in a tube of polyethylene film. X-ray diffraction photographs were taken with a Rigaku–Denki Geigerflex, using a Cu target. The value of the parameter b_0 derived from the Moffitt–Yang equation[8] was determined from the o.r.d. curve of the solution.

RESULTS AND DISCUSSION

Infra-red studies and X-ray analyses

Poly(S-alkyl-L-cysteine) (I, II and III) showed i.r. absorption bands of the polypeptides both in the solid state and in solution. The absorption bands of amide I (0,π), amide I (π,0), and amide II are listed in *Table 3*. The absorption bands at about 1635 cm^{-1} (amide I) and 1533 cm^{-1} (amide II) in the solid state and in chloroform solution suggest that the polymers have the β-conformation. The frequency of amide I (0,π) band appeared at 1695 cm^{-1}, indicating that the polymer conformation is an antiparallel β-conformation. In a chloroform/DCA 90/10 (v/v) mixture, the amide I band at 1645–1655 cm^{-1} assumed to be a random coil structure. These phenomena may be explained by the change from the β-form to the random coil conformation of the peptide chain. The X-ray diffraction photographs of the polymers showed a backbone spacing of 4.6–4.7 Å (β conformation), which coincided well with the results of the i.r. studies.

Optical rotatory dispersion and β-coil transition

The o.r.d. above 350 nm for the polymers was fitted with the Moffitt–Yang equation[8]. The b_0 values for polymers in chloroform were −100 for polymer I, −150 for polymer II, and −240 for polymer III, respectively. The negative value of b_0 increases with increasing methylene group in the side chains. The b_0 values of the three poly(S-alkyl-L-cysteines) are similar to that of the β-conformation of poly(L-lysine) as has been reported by Sarkar *et al.*[9] and Davidson *et al.*[10]. In 100% DCA, the b_0 values were nearly zero, suggesting that the polymers exist in a random coil.

The stability of the β-form and the effect of side-chain length of the polymers were studied. The plots of the $[\alpha]_{400}$ values *versus* solvent composition of the polymers in a chloroform/DCA mixture are shown in *Figure 1*. All polymers cause a conformational change at a few per cent DCA and the specific rotaton reverses from positive to negative. The β-coil transition was observed at 4, 3, and 1.5% DCA, respectively, for polymers I, II and III. These results indicate that the order of stability of the β-conformation of these polymers in a chloroform/DCA mixture decreases with increasing side-chain length. The β-stability of the three polymers are lower than that of poly(S-lower alkyl-L-cysteine)[1–4]. This could be interpreted as indicating that the introduction of the bulkier side chain made the conformation more unstable.

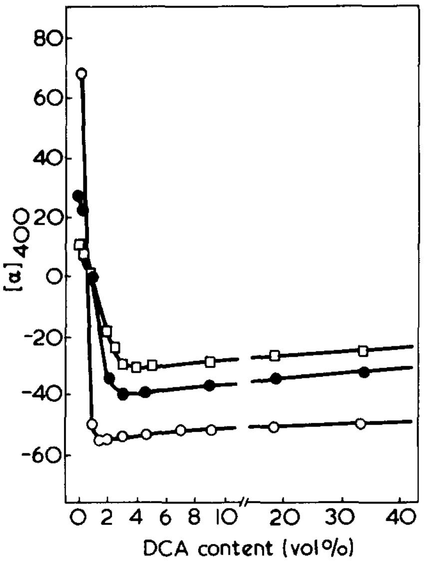

Figure 1 β-coil transition of poly(S-alkyl-L-cysteines) in chloroform/DCA mixtures at 22°C: □, poly(S-lauryl-L-cysteine); ●, poly(S-myristyl-L-cysteine); ○, poly(S-stearyl-L-cysteine)

Table 3 Characteristic absorption bands of polymers

	Wavenumber (cm^{-1})				
	Solid (KBr-discs)			Solution	
Polymer	Amide I (0,π)	Amide I (π,0)	Amide II	Amide I in $\mathrm{CHCl_3}$	Amide I in $\mathrm{CHCl_3}$/DCA (90/10)
Poly(S-lauryl-L-cys)	1695	1635	1533	1636	1655
Poly(S-myristyl-L-cys)	1695	1635	1535	1634	1650
Poly(S-stearyl-L-cys)	1695	1632	1532	1633	1645

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