[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND THE HARVARD MEDICAL SCHOOL]

The Synthesis and the Conformation of Poly-L-serine and Poly-O-acetyl-L-serine[1,2]

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Poly-L-serine and poly-O-acetyl-L-serine have been synthesized with degrees of polymerization (DP) slightly above 100. The deacetylation of poly-O-acetyl-L-serine to poly-L-serine using sodium methoxide does not result in chain cleavage. Conformational studies on poly-O-acetyl-L-serine by means of infrared spectroscopy and optical rotatory dispersion indicate that this polymer exists in either a β (extended) or random conformation depending on the solvent. No evidence for an α-helical form has been found. A reversible random $\rightarrow$ β-conformational change has been observed with poly-O-acetyl-L-serine accompanied by an optical rotation change of approximately 100° at λ_{546}. Poly-L-serine exists in random conformation in aqueous solution and in the solid state. No evidence has been found for an α-helical conformation with this DP of poly-L-serine.

I. Introduction

Synthetic high molecular weight polypeptides (derived from α-amino acids) have proved to be useful models for the investigation of protein conformation, but heretofore the only water-soluble polymers that have been available for such studies have had one of the following disadvantages: low molecular weight, ionic side groups or have been derived from imino acids.[3a,b,c] In this paper we describe the synthesis of a non-ionic, water-soluble polypeptide, poly-L-serine (and poly-O-acetyl-L-serine), of molecular weight sufficiently high to stabilize the α-helical conformation in water-insoluble polypeptides. The work recorded in this paper also describes investigations of the conformation of these polymers by means of infrared spectroscopy and optical rotatory dispersion.[3d]

Poly-L-serine is the simplest water-soluble, hydroxyl-containing poly-α-amino acid. The importance that serine plays in protein chemistry has been recognized since Lipmann[4] in 1932 isolated phosphoserine from casein and vitellin hydrolysates. More recently Sanger and Williams[5] have isolated serine phosphopeptides (SerP) from these sources. Peptides containing six units, (SerP)$_6$, from phosvitin and three units from casein have been separated from their respective hydrolysates. Perlmann[6] has investigated the types of phospho–ester bonds in proteins, and has suggested that serine plays an important role in such structures. In addition serine has been implicated in the active sites of several enzymes.[7] A protein containing 40.7% serine has been isolated from the green lacewing fly *Chrysopa*.[8a]

The synthesis of poly-DL-serine was reported by Frankel, *et al.*,[9] in 1952 from the polymerization of O-acetyl-DL-serine-N-carboxyanhydride. However, the N-carboxyanhydride (NCA) used was not crystalline and only low molecular weight polymers were obtained. O-Acetyl-DL-serine-NCA was obtained crystalline by MacDonald and Tullock[10] and polymerized, but the complete deacetylation of the resulting polymer was not achieved. Earlier the synthesis of poly-O-carbobenzoxy-DL-serine with degree of polymerization (DP) of 5 was reported.[11] The blocking group was removed, but the polymer was not characterized. Poly-O-methyl-DL-serine also has been synthesized.[12a]

II. Synthesis

Poly-L-serine and poly-DL-serine have been synthesized by two different methods. First, polymerization of O-acetyl-serine-NCA of the respective L- or DL-amino acids, followed by deacetylation gave the desired polymer. Second, direct polymerization of the L- or DL-serine-NCA yielded poly-L-serine and poly-DL-serine, respectively. The preparation of the crystalline free hydroxy NCA's of the monomers for this second synthesis is described in this paper. A note describing the preparation of DL-serine-NCA has appeared recently.[12b] The polymerization of these anhydrides and the structure of the polymers is the subject of a subsequent communication.[13]

L-Serine and DL-serine were O-acetylated by the Sheehan method[14] followed by reaction with phosgene in either dioxane or ethyl acetate. In both cases the crystalline anhydrides were obtained from benzene, ether or ethyl acetate–hexane solutions. Polymerization of O-acetyl-DL-serine and O-acetyl-L-serine-NCA was carried out in the following solvents: dioxane, nitrobenzene, dimethylformamide, pyridine and methylene dichloride. The polymerizations were initiated with either sodium methoxide, triethylamine or diethylamine

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(1) This is Polypeptides XXVII. For the preceding paper in this series see E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci.*, **45**, 1591 (1959). Alternate address of E. R. Blout: Chemical Research Laboratory, Polaroid Corp., Cambridge 39, Mass.

(2) This work has been supported by the Office of the Surgeon General, Department of the Army.

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(12) (a) W. E. Hanby and Yates in "Synthetic Polypeptides," by C. H. Bamford, A. Elliott and W. E. Hanby, Academic Press, Inc., New York, N. Y., 1956; (b) A. Berger, J. Kurtz, T. Sadeh, A. Yaron, R. Arnon and Y. Lapidoth, *Bull. Res. Counc. of Israel*, **7A**, 98 (1958).

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at anhydride:initiator ratios (A/I) which varied between 1 and 200. The polymer is insoluble in most organic solvents, is slightly soluble in dimethylformamide and soluble in dichloroacetic acid, trifluoroacetic acid, acetic acid or mixtures of these acids with chloroform or ethylene dichloride. The poly-O-acetyl-serines were deacetylated in nitrobenzene or dimethylformamide solutions using sodium methoxide to yield water-soluble polyserines. Some physical constants of poly-O-acetyl-L-serine as prepared by this method and of poly-L-serine are shown in Table I.

TABLE I

PHYSICAL PROPERTIES OF POLY-O-ACETYL-L-SERINE AND POLY-L-SERINE

Poly-O-acetyl-L-serine[a]		Poly-L-serine[b]		
[η]	$[\alpha]^{25}_{546}$	[η]	$[\alpha]^{25}_{546}$[j]	Solvent
0.19	+31[f]		− 9[g]	DCA
		0.10	−29[h]	H_2O
		.08	−25[f]	8 *M* urea
		.06	− 7[f]	10 *M* LiBr
			−14[i]	H_2O–dioxane (1:2)
MW_n	DP_n	MW_n	DP_n	
15,800[c]	122	10,600[c]	122	
		8,300[d]	95	
MW_w	DP_w	MW_w	DP_w	
16,800[e]	130	11,300[e]	130	

[a] Sample #G-17; other samples used in this study GF-E-39-2, $\eta_{sp/c}$ 0.14, 0.2% dichloroacetic acid (DCA): GF-5-33, $\eta_{sp/c}$ 0.14 (0.2% DCA). [b] Sample G-17-2. [c] End group titration with perchloric acid; M. Sela and A. Berger, THIS JOURNAL, **77**, 1893 (1955). [d] Carboxyl group titration with sodium methoxide using thymol blue as indicator. [e] Estimated from viscosity data from P. Doty, J. H. Bradbury and A. M. Holtzer, THIS JOURNAL, **78**, 947 (1956). [f] Concn. 1%. [g] Concn. 0.4%. [h] Concn. 0.3%. [i] Concn. 0.6%. [j] When these values were corrected for the refractive index of the solvents used, the corrections were so small that this correction has been omitted.

The molecular weights obtained were in the range 10,000 to 15,000, that is, a degree of polymerization (DP) of approximately 125. Although many polymerizations were run using the techniques previously found suitable for obtaining molecular weights of the order of 100,000 to 1,000,000 with other α-amino acid N-carboxyanhydrides[15] (DP > 1000) no higher molecular weight polymers than those indicated were obtained. One postulate that can be offered to explain this situation is that an O→N shift[16] of the acetyl group takes place under the basic conditions used and that this shift with the concomitant formation of an amide group acted as an effective terminator of the polymerization reaction. The fact that the molecular weight of these polymers could not be increased by the carbodiimide method[17] favors this explanation. When triethylamine was used as initiator, which would not be expected to cause the O → N shift, no higher molecular weights were obtained. The possibility that the L-serine used in these experiments was contaminated by traces of D-serine cannot be eliminated, since traces of an optical isomer of the opposite optical configuration has been shown to greatly decrease the molecular weight of the resulting polymer;[18] this explanation may be a valid one.

One additional hypothesis has been considered to explain the relatively low molecular weights obtained in the polymerization of serine-NCA. This is based on the finding (see below) that poly-O-acetyl-L-serine exists in the β-conformation. It has been shown that the rate of polymerization of polypeptides in the β-conformation is very much slower than that of such polypeptides when in the helical conformation.[18] If, as shown below, poly-O-acetyl-L-serine exists only in a β or in a random conformation, this may be the explanation of the lower molecular weights obtained with this amino acid compared with others which exist as helices in the polymerizing medium.

That no degradation took place during the deacetylation with sodium methoxide is evident from the titration data (Table I) where the degree of polymerization was found to be approximately the same by perchloric acid titration of the polymer before and after the deacetylation. The degree of polymerization of the poly-L-serine by both amino end groups and carboxyl end groups also gave relatively good agreement. Since there are approximately the same amount of carboxyl and amino groups, these data may be cited as evidence that very little O → N shift of the acetyl group takes place during the polymerization, and this is probably not the cause of the relatively low molecular weight of the polymers.

III. Experimental[18a]

Infrared Measurements.—All infrared measurements were performed on a Perkin–Elmer model 21 double beam spectrometer using a sodium chloride prism. The samples for infrared spectral measurements were prepared either as (a) films cast on silver chloride plates; oriented films were prepared by unidirectional stroking of a viscous solution on silver chloride plates until it was dry; (b) as dispersion in KBr disks under 120,000 p.s.i. pressure; or (c) solutions with polymer concentrations about 2%.

Optical Rotation Measurements.—Optical rotatory dispersion measurements were made with a Rudolf high precision photoelectric polarimeter model 80S, using a General Electric H100-A4 mercury lamp as light source. All measurements were made at 25.0 ± 0.1°. Concentrations were usually of the order 0.4%.

O-Acetyl-L-serine-N-carboxyanhydride.—O-Acetyl-L-serine·HCl[19] (1 g.) was suspended in dry dioxane[20] (30 cc.), stirred and phosgene bubbled in for 0.5 hour, maintaining the temperature at 65°. Nitrogen then was bubbled through the clear solution until free of phosgene. The solvent was removed at reduced pressure, the temperature maintained at 40°, leaving a clear oil. The oil was dissolved in anhydrous ethyl acetate (25 cc.), dry hexane (40 cc.) added until opalescence, the solution filtered, more hexane (165 cc.) added and then the solution was left at −30°. On standing overnight, crystallization had begun and more hexane (150 cc.) was added. The crystals were filtered, yielding 0.90 g., 96% theoretical. Recrystallization from anhydrous ethyl acetate (25 cc.) and *n*-hexane (290 cc.) gave 0.73 g. of crystals, 78%, m.p. 50.0–51.0°. *Anal.* Calcd. for $C_6H_7NO_5$: C, 41.6; H, 4.08; N, 8.09. Found: C, 41.7; H, 4.1; N, 8.0.

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(16) Y. Shalitin, Ph.D. Thesis, Hebrew University, Jerusalem, Israel, 1958.

(17) E. R. Blout and M. E. DesRoches, THIS JOURNAL, **81**, 370 (1959).

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(18a) All melting points corrected.

(19) J. C. Sheehan, M. Goodman and G. P. Hess, *ibid.*, **78**, 1367 (1956).

(20) L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., Boston, Mass., 1941, p. 361.

O-Acetyl-DL-serine·HCl.—DL-Serine (7 g.) was dissolved in glacial acetic acid (300 cc.) and the solution saturated with HCl at 0°. After standing 15 hours at room temperature, the solvent was removed at reduced pressure. This procedure was repeated and the resulting crystals were recrystallized from ethyl alcohol (60 cc.) and ether (60 cc.); yield 10 g., 89%, m.p. 158–162° dec.

Anal. Calcd. for $C_5H_9NO_4 \cdot HCl$: C, 32.6; H, 5.48; N, 7.62. Found: C, 32.7; H, 5.7; N, 7.6.

O-Acetyl-DL-serine-N-carboxyanhydride.—O-Acetyl-DL-serine·HCl was treated in the same manner as O-acetyl-L-serine·HCl. The recrystallized product gave an 80% yield, m.p. 62.5–63.5°. *Anal.* Calcd. for $C_6H_7NO_5$: C, 41.6; H, 4.08; N, 8.09. Found: C, 41.5; H, 4.0; N, 8.1.

L-Serine-N-carboxyanhydride.—L-Serine (4 g.) was suspended, by stirring, in anhydrous ethyl acetate (400 cc.), and phosgene bubbled in for 2.5 hours while the solvent was refluxed. Nitrogen was passed through the solution until it was phosgene free. The solvent was evaporated under reduced pressure, keeping the temperature below 40°. The resulting oil was dissolved in anhydrous ethyl acetate (125 cc.), *n*-hexane was added to opalescence (10 cc.) and the solution filtered and placed at −30°: an amphorous precipitate resulted. The solvent was removed at reduced pressure, the temperature kept below 40°. The residue was extracted four times with anhydrous benzene at 60° (100 cc. each). The benzene extract yielded crystals at 10°. The extracted oil crystallized on standing at −30°; combined yield 4.85 g., 97%, m.p. 115° dec. *Anal.* Calcd. for $C_4H_5NO_4$: C, 36.6; H, 3.8; N, 10.7. Found: C, 36.4; H, 3.8; N, 10.2.

DL-Serine-N-carboxyanhydride.—DL-Serine (10 g.) was suspended in dry dioxane[20] by stirring and phosgene was bubbled in for 3 hours, maintaining the temperature at 65°. Nitrogen was passed through the solution until it was phosgene free. The dioxane was removed under reduced pressure with the temperature kept below 40°. The resulting oil was dissolved in anhydrous ethyl acetate (100 cc.), and *n*-hexane (250 cc.) was added and crystallization began at −30°. The crystals were filtered yielding 4.3 g., 34%, m.p. 82–83°. The material was recrystallized from anhydrous ethyl acetate and hexane for analysis. It crystallized with half a mole of ethyl acetate. *Anal.* Calcd. for $C_4H_5NO_4 \cdot {}^1/_2C_4H_8O_2$: C, 40.9; H, 5.15; N, 7.96. Found: C, 41.1; H, 5.2; N, 7.9. The anhydride is soluble in dioxane, dimethylformamide, ethyl acetate and water, and insoluble in nitrobenzene.

Poly-O-acetyl-L-serine.—O-Acetyl-L-serine-N-carboxyanhydride (2 g.) was dissolved in dry nitrobenzene (50 cc.) and initiated with triethylamine (1.12 cc. of 0.4 *M* in benzene). The polymerization was allowed to proceed for two days. The solution was then poured into anhydrous ether (300 cc.) with stirring, causing the polymer to precipitate. The mixture was centrifuged and washed three times with ether. The polymer was suspended in dioxane and lyophilized, yielding a white powdery solid; weight, 1.1 g., 75% yield.

The viscosity was measured in 0.2% dichloroacetic acid using material dried at 100° *in vacuo*, $\eta_{sp/c}$ 0.19. The polymer was insoluble in most organic solvents. It was soluble in dichloroacetic acid, trifluoroacetic acid, and mixtures of these acids and other organic solvents such as chloroform, and partially soluble in nitrobenzene.

Poly-L-serine.—Poly-O-acetyl-L-serine (1 g.) was partially dissolved in nitrobenzene (100 cc.) by stirring and 1.2 moles of sodium methoxide (22 cc. of 0.426 *N* $NaOCH_3$) added causing immediate precipitation. The solution was stirred overnight and then poured into anhydrous ether (500 cc.) to isolate the polymer. The solution was centrifuged and the polymer washed three times with anhydrous ether. The solid was dissolved in water (5 cc.), giving a yellowish solution, dialyzed *versus* water until it became colorless, and lyophilized yielding a white fluffy powder, weight 0.35 g., 55%, specific viscosity, $\eta_{sp/c}$ 0.10 (*c* 0.2% in H_2O). The material requires drying to remove the water completely. The polymer is soluble in: H_2O, dichloroacetic acid, trifluoroacetic acid, hydrazine and ethylene chlorohydrin. Triethylamine does not deacetylate O-acetyl-L-serine or poly-O-acetyl-L-serine. If the dialysis step is omitted a hygroscopic material is obtained.

Poly-O-acetyl-DL-serine.—O-Acetyl-DL-serine-N-carboxyanhydride (5.0 g.) was dissolved in nitrobenzene (125 cc.) and initiated with sodium methoxide (1.36 cc. of 0.426 *N* $NaOCH_3$). The solution gelled within a few minutes and was allowed to stand overnight. It was then poured into anhydrous ether with stirring causing a heavy precipitate to deposit. The solution was centrifuged, and the precipitate washed three times with ether. The polymer was suspended in dioxane and lyophilized, yielding a white fluffy solid, weight 3.4 g., 91%, $\eta_{sp/c}$ 0.11 (*c* 0.2% in dichloroacetic acid). The polymer was soluble in dimethylformamide as well as the solvents which dissolved poly-O-acetyl-L-serine.

Poly-DL-Serine.—Poly-O-acetyl-DL-serine was dissolved in dimethylformamide and 1.2 mole equivalent of sodium methoxide (0.426 *N*) was added with stirring, causing precipitation of the polymer. The polymer was worked up as above for poly-L-serine.

IV. Conformational Studies

In order to determine the conformations of poly-O-acetyl-L-serine and poly-L-serine in the solid state and in solution, the methods of infrared spectroscopy and optical rotatory dispersion have been used. In addition X-ray diffraction has provided confirmation of some of the conclusions from the infrared and optical rotation data.

A. Poly-O-acetyl-L-serine.—Infrared Results.—The infrared spectra of poly-O-acetyl-L-serine were obtained in the solid state from KBr disks and oriented films. Solutions were studied in the solvents listed in Table II which summarizes some of the infrared data obtained on this polymer.

The infrared spectra of an oriented film of poly-O-acetyl-L-serine are shown in Fig. 1; this polypeptide shows amide I (amide carbonyl) absorption at 1637 cm.$^{-1}$ with perpendicular dichroism, and amide II absorption at 1517 cm.$^{-1}$ with parallel dichroism. The above infrared data indicate that poly-O-acetyl-L-serine exists in the β (extended) conformation in the solid state. In addition to the strong evidence provided by the dichroism of the oriented films, further evidence is provided for the existence of an anti-parallel β-structure by the presence of the band of 1705 cm.$^{-1}$.[21] In addition, preliminary X-ray diffraction results indicate that fibers of poly-O-acetyl-L-serine exist as a crossed β conformation.[22] This structure was also reported for a protein containing 40.7% serine.[8b] The fact

TABLE II

INFRARED DATA ON POLY-O-ACETYL-L-SERINE[d]

Sample state	Frequency, cm.$^{-1}$ Amide 1	Frequency, cm.$^{-1}$ Amide 11
Solid, KBr disk[a]	1633	1512
	1650(s)	1545(s)
Solid, oriented (from TFA)[b]	1637 ⊥	1517‖
	1705(s)‖	1545(s)
Soln., 27% DCA–73% EDC[c]	1627	1514
		1545(s)
Soln., 25% DCA–75% $CHCl_3$[c]	1630	1520
	1655(s)	1550(s)
Soln., 40% TFA–60% EDC[c]	1642–1645	1531
		1547(s)
Soln., 55% TFA–45% EDC[c]	1650–1640	1530
	(split)	1545(s)
Soln., 55% DCA–45% EDC[c]	1615	1525

[a] Sample no. 5-25. [b] Sample no. G-31. [c] Sample no. G-17. [d] Symbols used: s = shoulder, TFA = trifluoroacetic acid, DCA = dichloroacetic acid, EDC = ethylenedichloride. ⊥ = perpendicular dichroism, ‖ = parallel dichroism.

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(21) T. Miyazawa, *J. Chem. Phys.* in press.

(22) C. Johnson and C. Cohen, private communication.

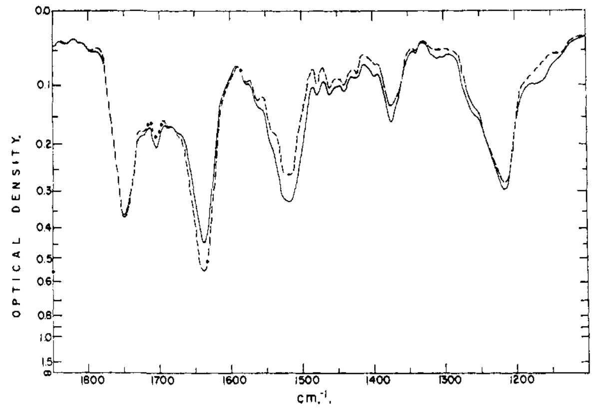

Fig. 1.—The infrared spectra of an oriented film of poly-O-acetyl-L-serine. The film was oriented by unidirectional rubbing of a concentrated solution of the polypeptide in trifluoroacetic acid: ——, electric vibration direction parallel to orientation direction; ------, electric vibration direction perpendicular to orientation direction. Note presence of perpendicular amide I absorption at 1637 cm.$^{-1}$ and parallel amide II absorption at 1517 cm.$^{-1}$.

that poly-O-acetyl-L-serine is not soluble in weakly hydrogen bond-forming organic solvents such as chloroform, but requires the addition of strongly hydrogen bond-forming organic solvents such as dichloroacetic acid or trifluoroacetic acid to promote solubility, by breaking interchain hydrogen bonding, suggests that this polymer exists as a highly hydrogen-bonded structure in the solid state. This is consistent with the assignment of a β-structure. In addition, the fact that the principal amide I frequency of the solid polymer (in KBr disks) lies at 1633 cm.$^{-1}$ provides further evidence for the assignment of a β-conformation and evidence against the existence of a helical conformation, in which the amide I frequency usually lies in the region 1650 to 1655 cm.$^{-1}$.[23]

Less extensive infrared measurements have been carried out on poly-O-acetyl-DL-serine, but the data obtained are similar to those obtained with poly-O-acetyl-L-serine.

Optical Rotatory Dispersion Data.—As indicated above, the insolubility of this polymer in most organic solvents limits the possible optical rotatory dispersion studies. In DCA solution poly-O-acetyl-L-serine shows simple rotatory dispersion, that is, the data fit the one-term Drude equation. The results in this solvent and in mixtures of this solvent with chloroform and ethylene dichloride are shown in Table III.

In all solutions listed above, the data fit a simple one-term Drude equation, and when plotted in the manner suggested by Moffitt[24] using $\lambda_0 = 212$, the coefficient of the second term is approximately zero, indicating the absence of anomalous dispersion of the type associated with the α-helical conformation. We therefore conclude that in the solvents studied no helical conformation is present. It will be noted that the value of λ_0 shows considerable variation as the solvent composition is changed.

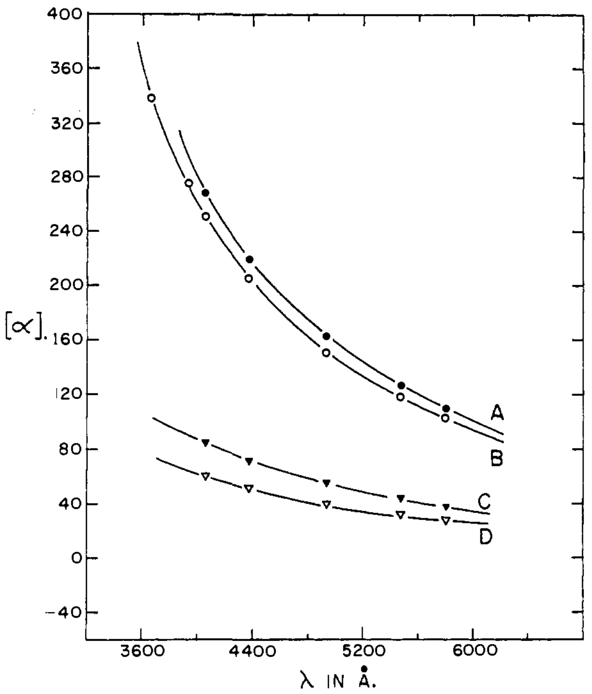

Fig. 2.—The rotatory dispersions of poly-O-acetyl-L-serine in various solvent compositions: A, dichloroacetic acid (DCA): ethylene dichloride (EDC) (1:3); B, DCA: $CHCl_3$ (1:3); C, DCA: EDC (1:1); D, DCA: $CHCl_3$ (3:1). The data for 100% DCA were practically identical with curve D.

The optical rotatory data for poly-O-acetyl-L-serine in DCA–chloroform solution and in DCA–ethylene dichloride solution at various solvent compositions are shown in Fig. 2, where $[\alpha]_{546}$ is plotted as a function of wave length. Over the wave length range studied simple optical dispersion is observed in these solvents.

TABLE III

ROTATORY DISPERSION RESULTS FROM POLY-O-ACETYL-L-SERINE (G-17)

Solvent				
% DCA	% other solvent	$[\alpha]^{25}_{546}$	λ_c	$b_0(\lambda_0 = 212)$
100		+ 31	120	−80
50	50 $CHCl_3$	+ 36	120	−80
50	50 EDC	+ 44	144	Non-linear plot
25	75 $CHCl_3$	+119	214	0
25	75 EDC	+126	214	0
15	85 $CHCl_3$	+127	[c]	[c]
10	90 EDC	+159	[c]	[c]

λ_0 is derived by the modified Drude plot[a] and b_0 from the Moffitt equation.[b] [a] J. T. Yang and P. Doty, THIS JOURNAL, **79**, 761 (1957). [b] W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956). [c] Solution gelled and no dispersion measurements were made.

However, from the values of $[\alpha]_{546}$ listed in Table III, it is seen that there is a large change in optical rotation as a function of solvent composition. DCA solutions of poly-O-acetyl-L-serine have been titrated with chloroform and the data are plotted in Fig. 3. The change in $[\alpha]_{546}$ is approximately 100°—more than twice that observed previously with polybenzyl-L-glutamate when the same solvent compositions were examined.[25] However, as noted

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(23) H. Lenormant, A. Baudras and E. R. Blout, THIS JOURNAL, **80**, 6191 (1958).

(24) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).

(25) E. R. Blout, P. Doty and J. T. Yang, THIS JOURNAL, **79**, 749 1957).

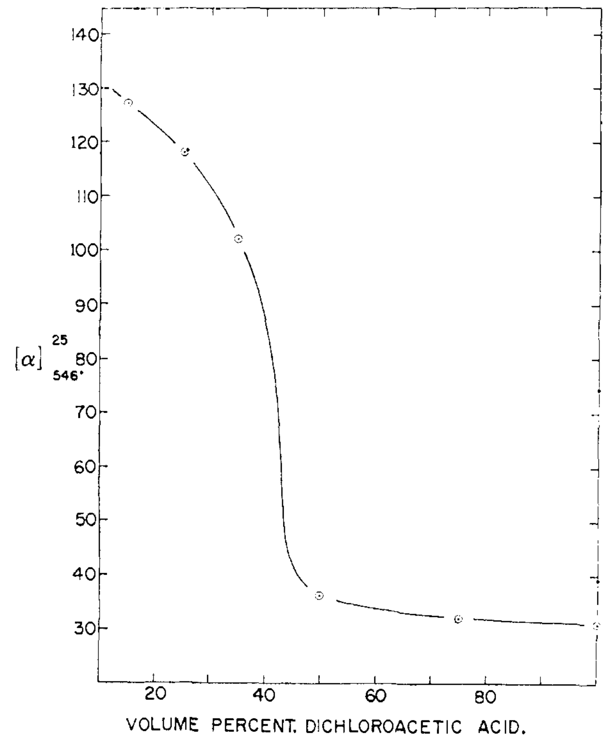

Fig. 3.—The optical rotation of poly-O-acetyl-L-serine in dichloroacetic acid–chloroform mixtures.

above, in contrast to the behavior of polybenzyl-L-glutamate, there is no evidence of anomalous rotatory dispersion and hence the change in $[\alpha]_{546}$ cannot be associated with a helix → random coil transformation. It should be noted that the titration of poly-O-acetyl-L-serine performed in this manner is also completely reversible. The titration curve was obtained with 0.4% polymer solution made by dilution of DCA with chloroform. It was found that gelation occurred on standing with solutions which contained less than about 30% DCA, but this was dependent on polymer concentration. The gels were thixotropic. The solutions were examined for the presence of micelles and liquid crystals, using polarized radiation, and both were found to be absent in the concentrations used in these studies.

Mixtures of TFA and ethylene dichloride (polymer concentration of 0.5%) yielded precipitates at TFA concentrations of 14%, no gelation being noted. This precipitate could be dissolved on addition of TFA, but on further standing reprecipitated. This phenomenon occurred until a 25% TFA concentration was reached, at which point no precipitation occurred.

Optical rotation measurements of poly-O-acetyl-L-serine in 90% EDC–10% DCA and in 75% EDC–25% DCA were carried out over the temperature range 25–55°. In each case there was a slight (approximately 15°) decrease in the optical rotation at 546 mμ, upon heating to 55°. This change was reversed upon lowering the temperature to 25°.

The optical rotation results indicate that there is a conformational change upon the addition of chlorocarbons to DCA solutions of poly-O-acetyl-L-serine. That this change does not involve a helical conformation is indicated by the absence of anomalous rotatory dispersion. Since it was shown by infrared spectroscopy that the β-conformation exists in the solid state and in solution, the rotatory data indicate that the observed change in rotation on going from DCA → chlorocarbon–DCA solvents is associated with a random coil → β-transformation. This large change of $[\alpha]_{546}$ in mixed solvents is the first reported for a random coil → β-conformation transition for a synthetic polypeptide with DP about 100. Although the origin of this change in rotation is not known, it is clear that the β-form must involve orientation in an asymmetric manner. A likely postulate is that this orientation involves the vicinal groups about the α-carbon atoms, but the arrangement of these groups in the β-form which produces the observed optical rotation is not known.

B. **Poly-L-serine.**—As expected, poly-L-serine is soluble in water and water-containing solvents, and is insoluble in organic solvents except hydrazine and strong organic acids such as dichloroacetic acid and trifluoroacetic acid.

Infrared Results.—In the solid state poly-L-serine shows absorption in the amide I region between 1650 and 1660 cm.$^{-1}$ and amide II absorption between 1530 and 1535 cm.$^{-1}$. These frequencies are consistent with either a helical or a random conformation. However, when poly-L-serine is dissolved in D_2O and the spectra measured after complete exchange of the amide hydrogens with deuterium, the amide I frequency is found at 1658 cm.$^{-1}$. Carbonyl frequencies of deuterated peptide groups, when in the random conformation, lie in this region, whereas when they are in the helical conformation the amide I frequency has a maximum between 1640 and 1645 cm.$^{-1}$.[23] This experiment strongly suggests that poly-L-serine exists in the random conformation in aqueous solution.

Furthermore, the rate of exchange of the amide hydrogen with deuterium is very fast compared to that observed with poly-L-glutamic acid in a helical conformation.[26] With poly-L-serine the exchange occurs within 5 minutes whereas it takes approximately 100 hours for the amide hydrogens of helical polyglutamic acid to exchange completely with deuterium.[26] In addition, we have not yet been able to obtain oriented films of poly-L-serine, whereas with other polymers in the α-helical conformation oriented films are readily obtained. Therefore the infrared data provide evidence that poly-L-serine exists as a random coil in solution and in the solid state.

Optical Rotatory Dispersion Results.—Aqueous solutions of poly-L-serine show simple optical rotatory dispersion over the wave length range 365 to 589 mμ. Some data are shown in Fig. 4 and are summarized in Table IV. From Fig. 4 it is seen that the dispersion curves are of the simple negative type, as compared with the simple positive type observed for poly-O-acetyl-L-serine. In all cases the data fit a one-term Drude equation; the λ_c's are listed in Table IV. If the data are plotted in the Moffitt[24] manner, b_0 is zero in all cases, using

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(26) E. R. Blout and A. A. Ferguson, to be published.

TABLE IV

OPTICAL ROTATORY DISPERSION DATA ON POLY-L-SERINE

Solvent	$[\alpha]_{546}$	$[\alpha]_{405}$	λ_c[c]	$b_0(\lambda_0 = 212)$
H_2O	−22.4	−61.8	212	0
10M LiBr[a]	− 7.3	−16.4	290	≅0
8M Urea[a]	−25.2	−56.4	250	≅0
Dioxane–H_2O (70:30)[a]	−13.8	−30.9	206	0
Hydrazine[b]	− 9.3	−19.2	206	0
DCA[a]	− 9.7	−32.4	335	−97

[a] Sample G-39-2. [b] Sample G-17-2. [c] Because of the relatively low rotations we estimate these values of λ_c to be accurate to ±15 mμ.

$\lambda_0 = 212$; this is evidence for the absence of the anomalous rotatory dispersion typical of a helical conformation. It is interesting to note that the addition of both lithium bromide and urea to aqueous solutions of poly-L-serine increase λ_0 in contradistinction to the data reported by Schellman and Harrington[27] on proteins. Attempts to alter the conformation of poly-L-serine by the addition of water-miscible solvents have so far proved unsuccessful.

V. Summary and Conclusions

1. Poly-O-acetyl-L-serine and poly-L-serine have been synthesized with degree of polymerization slightly above 100. 2. Poly-O-acetyl-L-serine exists in a β-conformation in the solid state and in solutions containing large amounts of chlorocarbon solvents. 3. Poly-O-acetyl-L-serine may be transformed to the random conformation in solution by strongly hydrogen bond forming solvents such as dichloroacetic acid and trifluoroacetic acid. 4. A reversible random → β-conformational change has been observed with poly-O-acetyl-L-serine. This is the first instance of such a change being observed in a synthetic polypeptide with DP about 100, and demonstrates that large changes in optical rotation may arise from conformational transitions other than those involving the α-helix. 5. Poly-L-serine (DP ~ 100) exists in a random conformation in solution and in the solid state, as evidenced by its infrared spectra and optical rotatory behavior. 6. Both LiBr and urea were shown to raise the value of the dispersion constant, λ_c, for aqueous solutions of poly-L-serine although the conformation (random coil) is presumably unchanged, as measured by b_0.

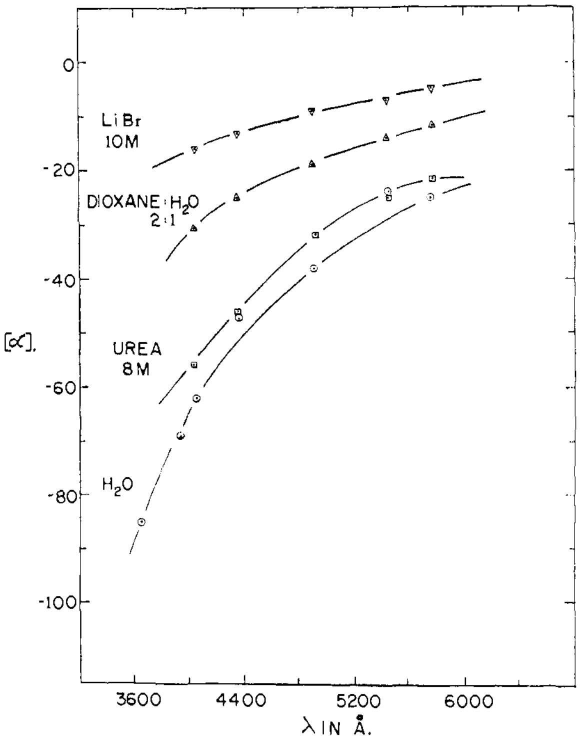

Fig. 4.—The rotatory dispersions of poly-L-serine in aqueous solution with various added solutes. The solutes are indicated in the figure.

Numerous investigations of the optical rotatory properties of polypeptides and proteins have indicated that the large changes in these properties are due to helix → random coil transitions. It is now clear, however, as a result of the data reported in this paper for poly-O-acetyl-L-serine, that large changes in [α] may be caused by a β → random coil transition. Since more than one type of conformational change may produce significant differences in optical properties, it is appropriate to use the term "unfolding," as suggested by Schellman,[28] when dealing with proteins when the exact physical nature of a conformational change is unknown. It is now apparent that the effects of helical, β and random conformations must be considered in the interpretation of the observed changes in optical properties of proteins.

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BOSTON 15, MASS.

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