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The Effect of Amino Acid Composition on the Conformations of Synthetic Polypeptides, Polymers and Copolymers of L-Methionine S-Methyl-L-cysteine and L-Valine[1,2]

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The synthesis and conformational studies of polypeptide polymers and copolymers of L-methionine, L-valine and S-methyl-L-cysteine are reported. Poly-S-methyl-L-cysteine exists in the β conformation in contrast to poly-L-methionine, the next higher methylene homolog, which exists in the α-helical conformation. The effect of the "β-forming" amino acids, S-methyl-L-cysteine and L-valine, on the stability of the α-helix of poly-L-methionine is described for both the solid state and solution. The results obtained from the synthetic polypeptides studied give credence to the postulate that the α-amino acid composition is of significance in determining the conformation of proteins.

Introduction

Recently we reported evidence for the dependence of the conformation of synthetic poly-α-amino acids on the nature of the side chains.[4] Determination of the infrared dichroism of films cast from a series of polypeptides of comparable molecular weights suggested the occurrence of two classes of α-amino acids, (I) those which form helical (α) structures and (II) those which form either random or extended (β) structures. The non-helix forming polypeptides (II) are of two types, those due to steric factors caused by disubstitution of other than hydrogen on the β-carbon atom (IIa) and those non-helix forming amino acids which have a hetero atom (oxygen or sulfur) attached to the β-carbon (IIb). It is also known that L-proline and hydroxy-L-proline form helical structures[5] other than the α-helix, and it has been suggested they destroy or prevent α-helix formation when incorporated in polypeptides and proteins.[6]

Poly-L-methionine is a polypeptide of class I, poly-L-valine belongs to class IIa and poly-S-methyl-L-cysteine belongs to class IIb. In this paper we report the results of conformational studies on these homopolymers as well as copolymers of L-methionine with both L-valine and S-methyl-L-cysteine. These copolymers were made to investigate the effect of the incorporation of both types of "non-helix formers" into an α-helix forming polypeptide. The data obtained reenforced and extended our observations on the significance of the α-amino acid side chains on the conformational behavior of synthetic polypeptides.

Experimental

All melting points are corrected. The reduced specific viscosity and elemental analyses for all the polymers prepared below are recorded in Table II and III. All polymers were dried under high vacuum at 90° for 2 hr.

L-Methionine-N-Carboxyanhydride.—L-Methionine (18.6 g.) was suspended in dry ethyl acetate (250 ml.) and phosgene bubbled in for 1.5 hr. while reflux temperature was maintained. The ethyl acetate was removed by bubbling a nitrogen stream through the solution. Two portions of dry ethyl acetate were added to the reaction mixture and removed as before. The phosgene-free oil remaining was diluted to about 40 ml. with dry ethyl acetate, filtered through Filter-Cel and dry hexane added to the cloud point. L-Methionine-N-carboxyanhydride, m.p. 42–44° dec., (12.8 g., 56%) crystallized out on storage overnight at −30°. The compound was twice recrystallized from ethyl acetate–hexane, m.p. 44° dec. (8 g.).

Anal. Calcd. for $C_6H_9NO_3S$: C, 41.13; H, 5.17; N, 7.99; S, 18.30. Found: C, 41.4; H, 5.2; N, 8.1; S, 18.2.

Poly-L-methionine. High Molecular Weight.—L-Methionine-N-carboxyanhydride (8.0 g.) was dissolved in dry nitrobenzene (200 ml.) and initiated with 0.556 ml. of 0.392 *N* sodium methoxide (in 25% methanol–75% benzene). (Anhydride/Initiator mole ratio (A/I) = 210.) The polymerization was allowed to proceed for one day during which time the solution gelled. Ethanol (200 ml.) was added to break up the gel. Centrifugation brought down the polymer which was washed with three portions of ethanol (95%) and with dioxane. The polymer was suspended in dioxane and isolated by lyophilization, 6.0 g. 100%.

Low Molecular Weight.—L-Methionine-N-carboxyanhydride (200 mg., 1.14 mmoles) was dissolved in dry nitrobenzene (5 ml.) and hexylamine (7.14 cmm.) added. (A/I = 22). The polymerization was allowed to proceed overnight. The polymer was then isolated as described above.

S-Methyl-L-cysteine-N-carboxyanhydride.[7]—S-Methyl-L-cysteine (10 g.) was suspended in 250 ml. of dry ethyl acetate and phosgene bubbled in for 1.5 hr. while the reflux temperature was maintained. The ethyl acetate was removed by bubbling a nitrogen stream through the solution. Two portions of dry ethyl acetate were added to the reaction mixture and removed as before. The phosgene-free oil remaining was made up to about 40 ml. with dry ethyl acetate, filtered through Filter-Cel, and dry hexane added to the cloud point. The S-methyl-L-cysteine-N-carboxyanhydride, m.p. 73–4° dec., crystallized out on standing at −30° (9.5 g., 79%). The compound was recrystallized twice from ethyl acetate–hexane, m.p. 75° dec.

Anal. Calcd. for $C_5H_7NO_3S$: C, 37.25; H, 4.38; N, 8.69; S, 19.89. Found: C, 37.5; H, 4.4; N, 8.8; S, 20.2.

Poly-S-methyl-L-Cysteine.—S-Methyl-L-cysteine-N-carboxyanhydride (200 mg., 1.42 mmoles) was dissolved in 5 ml. of dry nitrobenzene and initiated with 18.4 cmm. of 0.392 *N* sodium methoxide (A/I = 200). The polymerization was allowed to proceed one day, then the polymer was isolated in the manner described above for poly-L-methionine.

L-Valine-N-carboxyanhydride.[8]—L-Valine (5.0 g.) was suspended in dry dioxane (50 ml.) and phosgene bubbled in for 6 hr. while the temperature was maintained at 65°. The dioxane and excess phosgene were removed *in vacuo* and the residual oil dissolved in chloroform. After filtra-

(1) This is Polypeptides XXXVII. For the previous paper in this series see N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer, and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961). Portions of this work were reported at the Biophysical Society Meeting, St. Louis, Missouri, 1961. Alternate address for E. R. Blout, Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Massachusetts.

(2) This work was supported in part by the Department of the Army, Office of the Surgeon General and in part by U. S. Public Health Service Grant A2558.

(3) Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Massachusetts.

(4) E. R. Blout, C. de Lozé, S. M. Bloom and G. D. Fasman, *J. Am. Chem. Soc.*, **82**, 3787 (1960).

(5) P. M. Cowan and S. McGavin, *Nature*, **176**, 501 (1955).

(6) A. G. Szent-Györgyi and C. Cohen, *Science*, **126**, 697 (1957).

(7) M. Frankel, D. Gertner, H. Jacobson and A. Zilkha, *J. Chem. Soc.*, 1390 (1960).

(8) The L-valine-N-carboxyanhydride used in this study was synthesized by Mr. R. H. Karlson.

tion hexane was added to the cloud point. The product crystallized out after cooling to −30° (3.0 g., 58%). The N-carboxyanhydride was recrystallized from dichloromethane at −30°, m.p. 65°, dec. (lit. 65°[9]).

Copolymers of S-Methyl-L-cysteine and L-Methionine.—The N-Carboxyanhydrides (NCA's) of S-methyl-L-cysteine and L-methionine in mole ratios of 1:9, 2:8; 3:7, and 5:5 were dissolved by warming in nitrobenzene (distilled from P_2O_5) to make 4% solutions. Polymerizations were initiated with a sodium methoxide solution (0.366 *N*) at A/I 200. The solutions gelled immediately and were allowed to polymerize for four days. The gels were broken up and precipitated in 4× volume of 95% ethanol. The polymer was centrifuged down and washed three times with anhydrous ether. The white precipitate was suspended in dioxane and isolated by lyophilization.

Copolymers of L-Valine and L-Methionine.—The NCA's of L-valine and L-methionine in mole ratios of 1:9, 2:8, 3:7 and 5:5 were dissolved by warming in nitrobenzene (distilled from P_2O_5) to make 4% solutions. They were initiated with sodium methoxide solution (0.366 *N*) at A/I = 200. The solutions gelled immediately and were allowed to polymerize for one day. The gels were broken up and precipitated in approximately 4× volume of 95% ethanol. The polymer was centrifuged and washed three times with anhydrous ethyl ether. The white precipitate was suspended in dioxane and isolated by lyophilization.

Optical Rotations and Calculation of Rotatory Data.—The rotations and calculations of b_0 values were made in the manner described previously[10] with the sole exception of the use of a G.E. AH-6 lamp as light source. The values of b_0 reported herein were calculated without the dispersion of refractive index correction in the range 365 to 578 mμ.

Infrared Measurements.—All infrared measurements were performed on a Perkin–Elmer Model 21 double beam spectrometer using a sodium chloride prism. The oriented film samples were prepared from infrared spectral measurements by unidirectional stroking of a viscous chloroform solution on silver chloride plates until dry. To determine the conformations of the polymers in the solid state, the optical densities of the oriented films were taken at the peak of the parallel band for the α portion and at the peak of the perpendicular band for the β portion.[11] Some values were determined by taking the optical densities of the two peaks of non-oriented films and the results were similar, though the resolution of the two bands is not as good. Some error may have arisen from the overlapping of the two bands, but this overlapping was important only at the foot of the bands. As the scale is logarithmic, this represented only a small fraction of the optical density.

Results and Discussion

The syntheses of the desired polymers and copolymers were carried out by base initiation or the N-carboxyanhydrides (NCA's) of the α-amino acids.[12] The N-carboxyanhydrides of L-methionine, S-methyl-L-cysteine and L-valine were made by the phosgene method first described by Farthing[13] and utilized in several laboratories in recent years.[14] High molecular weight polymers (MW_w ~80,000) were obtained from L-methionine NCA and from mixtures containing at least 70% of L-methionine NCA with either S-methyl-L-cysteine NCA or L-valine NCA. Whereas the structure of the homopolymers follows from the method of synthesis, the sequential arrangement of the amino acid residues in the copolymers is unknown. The existence in the copolymers studied as either essentially block copolymers or truly random copolymers does not affect the arguments to be presented below. To insure that conformational differences between poly-S-methyl-L-cysteine and its higher homolog, poly-L-methionine, were not determined by molecular weight, poly-L-methionine of low molecular weight was synthesized by adjusting the anhydride-initiator ratio. The poly-L-methionine so synthesized also exhibited the α-helical conformation (see Table II).

The conformations of the homopolymers, as determined both from infrared dichroism in the solid state and from their b_0 values obtained from Moffitt plots[15] of the rotatory dispersions of solutions, are seen in Table I. The b_0 value of −630° (found for poly-L-methionine) is that attributed to the α-helix and is shown by the anomalous rotatory dispersion of this conformation.[16] It is of interest to note that although poly-S-methyl-L-cysteine has a β structure it shows b_0 values ranging from −150 to −100° depending on solvent composition (Fig. 3). Poly-L-valine was found to exist in the β conformation by means of solid state infrared dichroism determinations. It is too insoluble to be studied in solvents which do not completely destroy any ordered structure.

Optical rotatory dispersion studies were used to investigate the helix to random chain transition of poly-L-methionine.[17] This transition is seen in Fig. 1 where both the b_0 and $[\alpha]_{546}^{25}$ are plotted *vs.* the solvent composition. As a strong hydrogen bonding solvent such as dichloroacetic acid (DCA) or trifluoroacetic acid (TFA) is added to a solution of the polypeptide in a chlorocarbon solvent an abrupt change in $[\alpha]$ and b_0 is observed. This change in b_0 from −630 to zero is a result of a sharp conformational change from helical to random forms.[18]

The determination of b_0 values from poly-α-amino acids and their utilization in determining the helix content of the various polymers has been reviewed recently.[19] The dependence of the helix to random chain transition on the solvent composition has been employed to determine the relative stabilities of poly-γ-benzyl-L-glutamate,[20] poly-β-benzyl-L-aspartate[10] and poly-ε-carbobenzyloxy-L-lysine.[21] While the above listed polymers show b_0 values of zero in 100% DCA and therefore are presumably in the random form, poly-L-methionine is still about 60% helical in this solvent. The complete breakdown (Fig. 1) of the poly-L-methionine helix occurs in about 10% TFA–90% DCA, thus indicating the greater stability of this helix since it requires a stronger hydrogen bond breaking solvent to bring about the transition.

(9) L. Biechowsky-Slomnicki, A. Berger, J. Kurtz and E. Katchalski, *Arch. Biochem. Biophys.*, **65**, 400 (1956).

(10) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).

(11) In as yet unpublished results from this Laboratory the absorption coefficients (ε) of α and β polymers have been shown to be approximately the same.

(12) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).

(13) A. C. Farthing, *J. Chem. Soc.*, 2313 (1950).

(14) E. Katchalski and M. Sela, "Adv. in Protein Chem.," Academic Press, Inc., New York, N. Y., 1958, pp. 243–492.

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

(17) See G. E. Perlmann and E. Katchalski, *J. Am. Chem. Soc.*, **84**, 452 (1962).

(18) See for example G. D. Fasman, M. Idelson and E. R. Blout, *ibid.*, **83**, 709 (1961).

(19) "Polypeptides and Proteins" by E. R. Blout in "Optical Rotatory Dispersion," C. Djerassi, ed., McGraw-Hill Book Company, New York, N. Y., 1960, pp. 238–273.

(20) E. R. Blout, P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **79**, 749 (1957).

(21) G. D. Fasman, M. Idelson and E. R. Blout, *ibid.*, **83**, 709 (1961).

TABLE I

Polymer of	Solution b_0	Solution % Helix	Solid state[a] Amide I Freq.	Amide I Dichroism	Structure	% Helix	% β
L-Methionine	−630	100	1648	∥	α	100	0
S-Methyl-L-cysteine			1630	⊥	β	0	100
L-Valine			1638	⊥	β	0	100
L-Meth:L-val (9:1)	−580	92	1650	∥	$\alpha + \beta$	80	20
			1638	⊥			
L-Meth:L-val (8:2)	−545	86	1650	∥	$\alpha + \beta$	75	25
			1638	⊥			
L-Meth:L-val (7:3)	−460	73	1650	∥	$\alpha + \beta$	65	35
			1638	⊥			
L-Meth:L-val (5:5)			1650	∥	$\alpha + \beta$	55	45
			1638	⊥			
L-Meth-S-methyl-L-cys (9:1)	−600	95	1650	∥	$\alpha + \beta$	87	13
			1630	⊥			
L-Methyl-S-methyl-L-cys (8:2)	−600	95	1650	∥	$\alpha + \beta$	80	20
			1630	⊥			
L-Methyl-S-methyl-L-cys (7:3)	−600	95	1650	∥	$\alpha + \beta$	70	30
			1630	⊥			
L-Meth-S-methyl-L-cys (5:5)	−350	56	1650		$\alpha + \beta$	46[b]	54
			1630				

[a] All films were oriented from chloroform. [b] This figure includes both the per cent. α and random.

TABLE II

COPOLYMERS OF L-VALINE AND L-METHIONINE

Sample no.	Mole ratio of L-valine NCA:L-methionine NCA	$\eta_{sp/c}$[a]	MW_w[b]	DP_w	Analysis C Found	C Calcd.	H Found	H Calcd.	N Found	N Calcd.	S Found	S Calcd.
SMB-293	0:10	0.80	76,000	580	46.0	45.8	7.0	6.9	10.5	10.7	24.6	24.4
SMB-355	0:10	.22	18,000	135								
SMB-373-91	1:9	.85	79,000	620	46.7	46.9	7.2	7.0	11.1	11.0	22.8	22.6
SMB-373-82	2:8	.91	84,000	670	48.0	48.1	7.2	7.3	11.4	11.2	20.3	20.6
SMB-393-73	3:7	.88	78,000	640	49.3	49.4	7.3	7.4	11.5	11.5	18.1	18.5
GF-6-287	5:5	.86	71,000	620	52.0	52.2	7.7	7.9	12.0	12.2	13.6	13.9
GF-6-41	10:0	.29	19,000	190	60.1	60.6	9.3	9.2	13.8	14.1		

[a] c = 0.2% in trifluoroacetic acid. [b] Estimated from the viscosity using the molecular weight calibration for poly-γ-benzyl-L-glutamate from P. Doty, J. H. Bradbury and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).

TABLE III

COPOLYMERS OF S-METHYL-L-CYSTEINE AND L-METHIONINE

Sample no.	Mole ratio of S-methyl-L-cyst.:L-methionine NCA	$\eta_{sp/c}$[a]	MW_w[b]	DP_w	Elementary analysis C Found	C Calcd.	H Found	H Calcd.	N Found	N Calcd.	S Found	S Calcd.
SMB-312	10:0	0.24*	16,000	140	40.7	41.0	6.2	6.0	11.9	12.0	27.5	27.4
SMB-323	10:0	.16*	11,000	90	40.8	41.0	5.9	6.0	11.7	12.0	27.5	27.4
GF-6-289	1:9	.76	71,000	547	45.3	45.4	6.7	6.8	10.9	10.8	25.0	24.8
GF-6-291	2:8	.71	64,500	502	45.1	44.9	6.8	6.8	10.7	10.9	24.8	25.0
GF-6-293	3:7	1.00	94,200	745	44.3	44.4	6.3	6.7	11.3	11.1	25.3	25.3
GF-6-295	5:5	0.31	24,700	199	43.5	43.5	6.4	6.5	11.2	11.3	26.0	25.8

[a] c = 0.2% in trifluoroacetic acid except where (*) indicates c = 0.2% in dichloroacetic acid. [b] Estimated from the viscosity using the molecular weight calibration for poly-γ-benzyl-L-glutamate from P. Doty, J. H. Bradbury and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).

The observed change in b_0 during the transition from −630 to zero and of $[\alpha]_{546}$ from positive to negative parallels the changes recorded for poly-γ-benzyl-L-glutamate.[20] The direction of these changes indicates that poly-L-methionine has the same sense of helix as poly-γ-benzyl-L-glutamate and opposite to that of poly-β-benzyl-L-aspartate.[10]

The greater stability of the poly-L-methionine helix compared to those of other poly-α-amino acids is of special interest. Poly-γ-benzyl-L-glutamate, for example, undergoes the helix→random chain transition at a solvent composition of about 70% DCA–30% $CHCl_3$. The greater stability of poly-L-methionine is explicable in terms of "hydrophobic bonds" postulated by Kauzmann and others.[22] To break down an α-helix, solvent or solute molecules must find their way to the backbone of the polymer and successfully compete in hydrogen bond formation with the amide linkages of the polymer. It is suggested that the solvents used (DCA or TFA) to bring about the helix→random transition have a greater affinity for the relatively polar environment of the side chains of poly-γ-benzyl-L-glutamate or poly-

(22) W. Kauzmann, in "Advances in Protein Chemistry," XIV, Academic Press, Inc., New York, N. Y., 1959, p. 1.

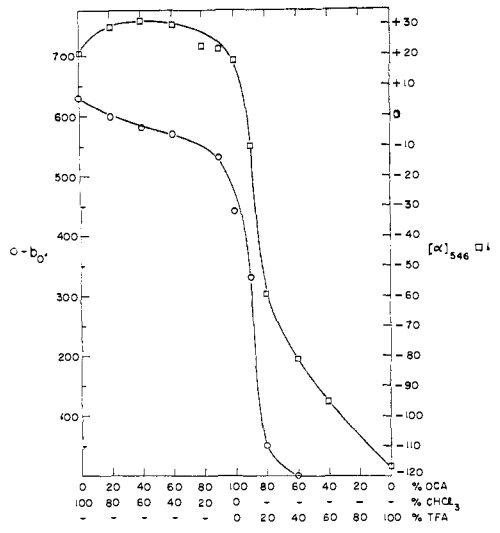

Fig. 1.—The dependence of b_0 values, O–O–O, and $[\alpha]^{546}$, □–□–□, of poly-L-methionine on solvent composition.

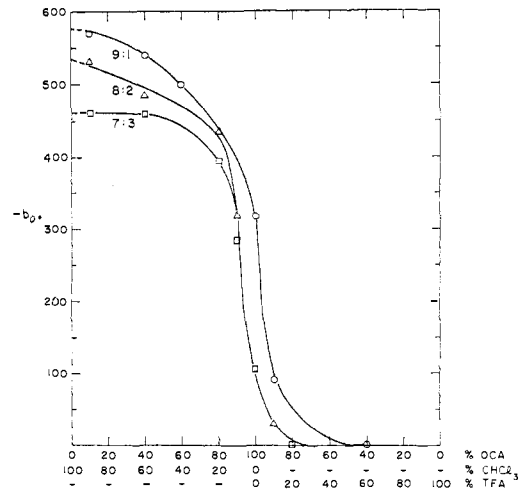

Fig. 2.—The dependence of b_0 values of copolymers of L-methionine and L-valine on solvent composition. The α-amino acid composition of the polymers is shown in the figure.

ε-carbobenzyloxy-L-lysine than for the relatively less polar side chains of poly-L-methionine. Other factors being equal, the amino acids having less polar side chains should provide a better shield for the internally hydrogen bonded α-helical backbone than those having polar side chains and thus should exhibit greater stability.

The observation that poly-L-valine does not exist in an α-helix is rationalized by inspection of space filling molecular models. When such models were used to attempt to build an α-helix from poly-L-valine, the methyl groups on the β-carbon of one valyl residue sterically crowd the methyl groups of a second valyl residue, and it does not appear possible to construct a strainless α-helical structure. Thus it might be expected that either random or β-conformations would be preferred for poly-L-valine.

The observation that poly-L-methionine exists in the helical conformation while the desmethylene homolog, poly-S-methyl-L-cysteine does not form an α-helix is not so easily explained. Molecular models of a right-handed α-helix of S-methyl-L-cysteine residues reveal no significant steric side-chain to side-chain interaction. The explanation necessarily would seem to center on the close proximity of the sulfur atom to the backbone of the polymer. The greater bulk of the sulfur atom, the dipole moment of the carbon-to-sulfur bond or the interaction of the unshared electron pairs of the sulfur atom with the N–H linkages of the backbone all could hinder formation of the internal –C=O---H–N– hydrogen bonds necessary for the α-helix.[23]

Having firmly established the conformations of the homopolymers, a series of copolymers of L-methionine:L-valine and L-methionine:S-methyl-L-cysteine were made. (See Tables II and III for physical characteristics.) Various amounts of either of the non-helix-forming residues, L-valyl and S-methyl-L-cysteinyl, were incorporated in polypeptides derived from L-methionine in order to investigate the effect of amino acid composition on the amount and stability of the poly-L-methionine helix.

Copoly-L-methionine:L-valine.—The rotatory dispersive behavior in solution of copolymers of L-methionine and 10, 20 and 30 mole % valine were studied. Table I shows the b_0 values of the various copolymers. As measured by the b_0 criterion, the copolymer containing 90% L-methionine:10% L-valine is about 90% helical in chloroform solution and undergoes the α-helix→random transition in in about 100% DCA. The copolymers of 20 and 30 mole % of L-valine which are, respectively, about 85 and 75% helical, both undergo the helix→random transition at about 90% DCA–10% $CHCl_3$ (Fig. 2).

Two conclusions may be drawn from these data. First, the percent. α-helix in the L-valine copolymers is nearly proportional to the L-methionine content. Secondly, the stability of the helical regions of any copolymer is essentially independent of the non-helical regions, as all the copolymers undergo the helix→random transition in a relatively narrow range of solvent composition, close to that found for poly-L-methionine.

Typical infrared spectra of oriented films of these copolymers are seen in Fig. 4. The spectra show the presence of two amide I bands. The higher frequency band at approximately 1650 cm.$^{-1}$, found at the same frequency as that of poly-L-methionine, has parallel dichroism and is due to the helical portion of the polymer. The lower frequency band is at 1630 cm.$^{-1}$, the same frequency as found in poly-L-valine. This band has perpendicular dichroism and is due to the β-portion of the polymer.[24]

It has been shown recently that the amide I absorption coefficient is approximately the same for α and β polymers.[11] Therefore, an estimate of the α and β content can be made by taking the

(23) (a) I. M. Klotz and J. M. Urqhart, *J. Am. Chem. Soc.*, **71**, 1597 (1949); (b) R. Cecil, *Biochem. J.*, **47**, 572 (1950); (c) R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **75**, 4367 (1953).

(24) E. R. Blout and A. Asadourian, *J. Am. Chem. Soc.*, **78**, 955 (1956).

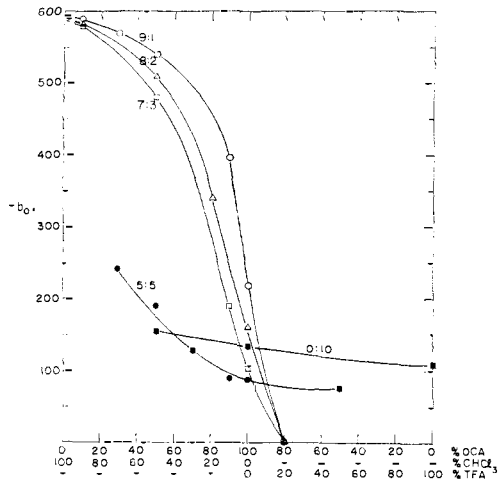

Fig. 3.—The dependence of b_0 values of copolymers of L-methionine and S-methyl-L-cysteine on solvent composition. The α-amino acid composition of the polymers is shown in the figure.

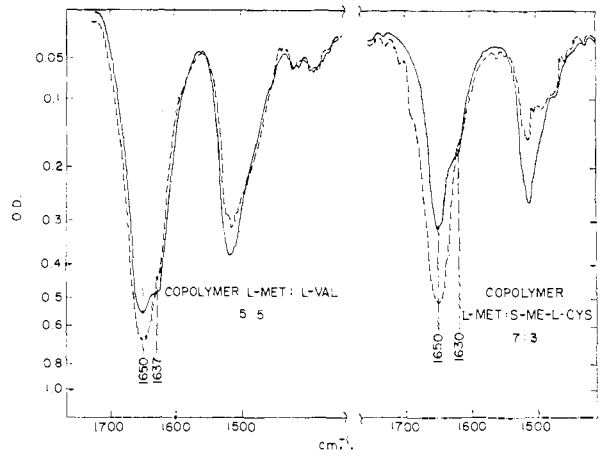

Fig. 4.—Portions of the infrared spectra of two copolymers which had previously been oriented are shown: ———, electric vibration direction perpendicular to orientation direction; ---------, electric vibration direction parallel to orientation direction.

ratio of optical densities of the β and α bands (Table I). The percent. β found is approximately equal to the percent. of valine in the copolymers. Thus it can be concluded that the L-methionine:L-valine copolymers show similar conformations in both the solid state and in solution.

Copoly-L-methionine : S-methyl-L-cysteine.—The conformational behavior of copolymers containing 10, 20, 30 and 50% S-methyl-L-cysteine with L-methionine in solution was investigated. Table I gives the b_0 values for the various copolymers. Figure 3 shows the helix→random transition for these polymers with varying solvent composition. The copolymers containing 10, 20 and 30% S-methyl-L-cysteine all possessed similar b_0 values of about −570° in 90% $CHCl_3$–10% DCA. A 50% S-methyl-L-cysteine:L-methionine copolymer has a b_0 value of −240° in 70% $CHCl_3$–30% DCA. The optical titration curve of the 50% copolymer approaches that of poly-S-methyl-L-cysteine (Fig. 3) and reflects a pronounced change in the helix content of the copolymer. Again two conclusions may be drawn from the data. First, the constancy of the b_0 of solutions of the 9:1, 8:2 and 7:3 copolymers in chloroform–DCA (9:1) solution reflects the unaltering percent. helix in these polymers in the particular solvent compositions used and is in marked contrast to the findings under identical conditions for the L-valine:L-methionine copolymers. Secondly, the helix→random transition for these copolymers is at approximately the same $CHCl_3$–DCA composition as that observed with the L-valine:L-methionine copolymers and again suggests that the stability of the helical region of a polymer is essentially independent of the other regions.

Application of the infrared procedure described above provided the means for estimating the α and β fractions of the copolymers in the solid state. The oriented infrared spectrum of one representative S-methyl-L-cysteine:L-methionine polymer is shown in Fig. 4. Here again two amide I bands are found, the higher, at 1650 cm.$^{-1}$, has parallel dichroism and is due to the helical portion of the polymer. The lower frequency band lies at 1630 cm.$^{-1}$, has perpendicular dichroism and is due to the β portion of the polymer. A special case arises with the 5:5 S-methyl-L-cysteine:L-methionine copolymer where orientation was not satisfactorily accomplished and where the band observed around 1650 cm.$^{-1}$ is due to random material rather than α-helical material.[25] These data are summarized in Table I. The per cent. β in the solid state is proportional to the per cent. incorporation of S-methyl-L-cysteine.

Although the solid state data reflect the "β-forming" character of S-methyl-L-cysteine, an apparent anomaly is observed in solution where the S-methyl-L-cysteine does not exert any "β-forming" influence. The presence of at least 10% dichloroacetic acid in the chloroform media used to dissolve the copolymers lies at the heart of the apparent anomaly. It may be that the small amount of strong acid is sufficient to destroy the influence of the sulfur atom of the S-methyl-L-cysteine, eliminating its "β-forming" character.

Conclusions.—Clearly, the differing behavior of the various polymers and copolymers studied may may be ascribed to their amino acid composition. In our preliminary communication[4] we described a class of helix-forming α-amino acids and two groups of α-amino acids which together constituted a class of non-helix-formers. These conclusions were drawn from an examination of the infrared spectra of oriented films of several poly-α-amino acids and are now supported by similar data on the copolymers of L-methionine with L-valine and with S-methyl-L-cysteine. In the solid state, the per cent. helix of all the copolymers was found to be proportional to the amount of L-methionine in the polypeptides.

In solution, however, the per cent. helix was proportional to the amount of L-methionine in the valine copolymers but was higher than the L-methionine ratio in the S-methyl-L-cysteine copolymers. The optical titration studies on the copolymers of L-

(25) Unpublished results from this Laboratory.

methionine and S-methyl-L-cysteine indicate that any influence the S-methyl-L-cysteine might have on the conformational behavior is not observable in the mixed $CHCl_3$:DCA solvents employed. It is thought that the necessity of using some DCA in the solvent mixtures probably is the cause of the seeming inconsistency of the solution and solid state results for the S-methyl-L-cysteine copolymer. As might have been expected, however, the influence of L-valine was unaltered by the change from solid to solution. One factor which is probably important in conformational stability in solution, as illustrated by the relative greater stability of the α-helix found in poly-L-methionine than in some other poly-α-amino acids, is the interaction of hydrophobic areas which contribute to the stabilization of any ordered conformation. Also significant was the finding that the helix→random transition of all the predominantly helical polymers studied was essentially independent of the non-helical regions.

We suggest that the conclusions derived from the conformational studies on the model polypeptides are applicable to proteins. Amino acid composition and sequence, therefore, should play an important role in determining the conformational states of proteins. Further the sharp change in optical rotation found on denaturation of some globular proteins[26] may be attributed to the breakdown of small helical regions, within the encompassing non-helical regions. Finally, we should like to emphasize that although the application of the concept of "α-forming" and "β-forming" α-amino acids will aid in understanding the conformational behavior of proteins, the importance of intra- and inter-molecular hydrogen bonds, disulfide bridges, salt links, etc., should not be neglected.[22,27]

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Immunochemical Studies on Blood Groups.[1] XXVII. Periodate Oxidation of Blood Group A, B and O(H) Substances

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On exposure to periodate, blood group substances rapidly consume from 2.7 to 3.7 moles per 1000 g. The periodate consumption is essentially complete in one day at 0 to 4° and little or no further uptake is noted even up to 346 hours. The consumption of periodate can, for the most part, be accounted for by the destruction of fucose, galactose and hexosamine and the production of formic acid. Fucose is almost completely destroyed, galactose about 70 to 90% and hexosamine is the least affected. The glucosamine of two human cyst blood group substances is almost completely resistant to periodate. Galactosamine is affected to a varying extent in the five blood group substances and two P1 preparations used, but was only very slightly changed in the human B cyst and the hog O (H) substances. After periodate oxidation and borohydride reduction, the seven preparations were exposed to 1 *N* HCl and dialyzed. The non-dialyzable material contained appreciable amounts of galactose, hexosamine and amino acids. The N content of the non-dialyzable fraction was increased from the 5 to 6% originally present in blood group substances to 8 to 9%. The behavior of blood group substances on periodate oxidation is compatible with a highly-branched structure with a portion resistant to periodate made up either of carbohydrate residues or of amino acids in peptide linkage or both.

Periodate oxidation has been used extensively in investigations of the structures of many polysaccharides.[2-6] However, it has become increasingly evident that results based only on periodate consumption and the release of one-carbon fragments can be extremely misleading. Compounds with sterically hindered *trans*-hydroxyl groups consume less[7] and compounds with active hydrogen atoms consume more[8] periodate than expected. Other types of anomalous behaviour have been reported with hyaluronic acid[9a] and with glycoproteins[9b] in which periodate oxidizes the amino acids cysteine, cystine, methionine, tryptophan and tyrosine. Whenever possible studies should, therefore, include quantitative estimation of products as well as constituents destroyed by or resistant to periodate.[10]

With blood group substances, periodate oxidation data can be correlated with destruction or resistance of fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine and various amino acids.

In the past few years this Laboratory has modified standard periodate methods so that microgram quantities can be analyzed. For example, with a total of 50 to 100 μg. of a disaccharide, 8 points on a periodate uptake curve, a formaldehyde and a formic acid determination may be carried out, 5 to

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