Worthington Biochemical Co., Freehold, New Jersey, and used in these studies. The results obtained with this preparation did not differ from those obtained after the enzyme was exhaustively dialyzed at 4° against 7×10^{-4} *M* HCl. Protein concentrations were determined by spectrophotometric measurements at 282 mμ, using a molar extinction coefficient of 50,000.[18] The molecular weight of α-chymotrypsin was taken as 25,000.[7]

DIP-CT was prepared as described by Jansen.[19] The samples were exhaustively dialyzed at 3° against 7×10^{-4} *M* HCl. Phosphorous analysis by the method of Sumner[20] and the difference spectrum of DIP-CT *versus* CT[8,9] were used to check the preparation.

All other chemicals were reagent grade unless otherwise indicated. Diisopropylphosphorofluoridate was a gift of Dr. B. J. Jandorf, Biochemical Research Division, Army Chemical Center, Maryland. Eastman guanidine hydrochloride was purified according to the method of Kolthoff, *et al.*,[21] dried for 8 hr. at 40° and kept in a vacuum dessicator over phosphorous pentoxide. Analytical reagent grade urea (Mallinckrodt) with correct melting point was used. The GU solution was prepared by direct weighing. Baker Chemical Co. HCl and Mallinckrodt KCl and KOH were also used. The base was prepared CO_2 free according to the method of Kolthoff.[22] The CO_2-free KOH was standardized against potassium acid phthalate (A.C.S. Reagent Grade B&A Co). The HCl was diluted and standardized against the base.

The solutions used to standardize the *p*H meter at 16° were Beckman *p*H 7.03 buffer and 0.01 *M* borax (*p*H 9.26 at 16°) prepared as described by Bates.[23]

All solutions were prepared with conductivity water.

Instruments.—Ultraviolet absorption measurements were made with a Beckman model DU spectrophotometer using 1 cm. silica cells covered with plastic stoppers to avoid absorption of CO_2. Temperature in the cell compartment was maintained at 16° by thermostatting.

*p*H determinations were made with a Beckman model G *p*H meter, using a general purpose glass electrode (No. 40498). The solutions were placed in a small cell through which constant temperature water was circulated. The instrument was checked against Beckman *p*H 7.03 buffer and 0.01 *M* borax, *p*H 9.26 at 16° after every three measurements.

All essential volumetric glass ware was calibrated by weighing with water.

Spectrophotometric Titrations.—Appropriate amounts of KCl and KOH were added to a series of 10 ml. volumetric flasks. In the experiments in denaturing solvent, appropriate amounts of GU were weighed into each flask before addition of the protein solution. Before the spectrophotometric measurements, appropriate amounts of KOH were added. The protein concentration was 0.025 to 0.040 g./100 ml. For the experiments in aqueous solution water was used in the reference cell. For the experiments in denaturing solvent, the reference cell contained CT or DIP-CT in GU at *p*H 7.0. Since the optical density of GU is *p*H dependent, the ultraviolet absorption spectrum of the tyrosyl groups was obtained by subtracting the optical density of the blank GU solution at various *p*H's from that of the protein solutions in GU at the corresponding *p*H's. Measurements were made at two different wave lengths, 295 and 290 mμ. The data for relative absorption at the two wave lengths agreed within the experimental error. *p*H measurements of the solutions were made before and after the spectrophotometric measurements. The reversibility of the titrations was checked by addition of appropriate amounts of standard HCl to selected test solutions at high *p*H.

Acknowledgments.—This work was aided by grants from the National Science Foundation and the Office of Naval Research.

---

[18] G. H. Dixon and H. Neurath, *J. Biol. Chem.*, **225**, 1049 (1957).

[19] E. F. Jansen, M. D. F. Nutting, R. Jang and A. K. Balls, *ibid.*, **179**, 189 (1949).

[20] J. B. Sumner, *Science*, **100**, 413 (1944); J. B. Sumner and G. F. Sommers, in "Laboratory Experiments in Biological Chemistry," Academic Press, Inc., New York, N. Y., 1949, p. 71.

[21] I. M. Kolthoff, A. Anastasi, W. Stricks, B. H. Tan and G. S. Deshmukh, *J. Am. Chem. Soc.*, **79**, 5102 (1957).

[22] I. M. Kolthoff, *Z. Anal. Chem.*, **61**, 48 (1922).

[23] R. G. Bates, "Electrometric *p*H Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954, pp. 118–121.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL, AND FROM THE ROCKEFELLER INSTITUTE, NEW YORK]

Conformation of Poly-L-methionine and Some of its Derivatives in Solution[1,2]

BY GERTRUDE E. PERLMANN[3] AND EPHRAIM KATCHALSKI

RECEIVED MAY 20, 1961

Poly-L-methionine (PM). poly-L-methionine S-methylsulfonium bromide (PMS) and poly-L-methionine S-carboxymethylthetin (PMT) of average degree of polymerization, DP = 210, were synthesized. To evaluate the configuration of these polymers, their optical rotatory dispersion and viscosity under different conditions were investigated. PM was found to possess in chloroform an asymmetric helical conformation as deduced from the position of the infrared absorption bands at 3330, 1660, 1555 cm.$^{-1}$ and an anomalous rotatory dispersion ($[\alpha]_{546} = 24.4$, $b_0 = -650$). In dichloroacetic acid the rotatory behavior resembles that in chloroform ($[\alpha]_{546} = 16.1$, $b_0 = -550$), indicating that the molecular forces that stabilize the PM-helix are stronger than those prevailing in other polypeptides. The lower value of b_0 in dichloroacetic acid suggests that the PM-helix in this solvent is somewhat less stable than in chloroform. The optical rotatory dispersion of PM in trifluoroacetic acid can be represented by a one-term Drude equation ($[\alpha]_{546} = -115.3$. $\lambda_c = 222 \pm 1$ mμ) thus predicting a random coil in this solvent. A helix-random coil transition occurs in chloroform-trifluoroacetic acid mixtures of 50:50 v./v. but already at 80:20 v./v. in dichloroacetic acid–trifluoroacetic acid. The changes in η_{sp}/c during the transition that occur in the two solvent mixtures parallel those of the specific rotations, $[\alpha]_{589}$. PMS shows in aqueous solution typical polyelectrolyte behavior. The molecules are extended at low ionic strength (η_{sp}/c exceeds 10 at $c = 0.1$ g. per dl.) but contract when the ionic strength is increased ($\eta_{sp}/c = 0.6$ at $\Gamma/2 = 1.1$). The optical rotatory properties follow a one-term Drude equation with a $\lambda_c = 190 \pm 1$ mμ. The optical rotatory dispersion of PMT also follows a one-term Drude equation. $\lambda_c = 186 \pm 1$ mμ remains unchanged in the *p*H range of 1 to 12 and is independent of ionic strength, suggesting that the zwitterionic polypeptide retains a random coil conformation.

Work on the behavior of poly-α-amino acids in solution, using optical and hydrodynamic techniques, has shown that these polymers may attain in weakly interacting solvents a definite secondary

---

(1) A preliminary report was presented at the Meetings of the Biophysical Society, St. Louis, February 1961.

(2) This investigation was supported by Grant A-3083 of the National Institutes of Health, U. S. A. Public Health Service.

(3) On sabbatical leave from the Rockefeller Institute, New York. Expenses were covered by Grant G-8618 of the National Science Foundation and by Grant A-2449 of the National Institutes of Health, U. S. A. Public Health Service.

structure[4–6] for which an α-helical conformation has been postulated. In strongly interacting solvents the polymers exhibit a random form. Recent analyses of the physico-chemical properties of different polyamino acids in solution reveal that the nature of the amino acid residues of a synthetic polypeptide may have a marked effect on the stability and the sense of twist of the helix formed.[6] Since polyamino acids are used as model compounds for some of the characteristics of the secondary structure of proteins, it seems desirable to investigate the properties and conformation of as many different polyamino acids as possible. Poly-L-methionine was chosen since optically pure preparations of high average molecular weight may be prepared with ease and this polypeptide can readily be transformed into a water-soluble base, poly-L-methionine S-methylsulfonium bromide (PMS), or a polyampholyte, poly-L-methionine S-carboxymethylthetin (PMT). The results obtained indicate that poly-L-methionine in chloroform exists in an α-helix conformation of similar nature to that of poly-γ-benzyl-L-glutamate.[7–10] The helical structure in solution is extremely stable and is disrupted only by a strongly interacting solvent such as trifluoroacetic acid but not as is the case with most polyamino acids, by dichloroacetic acid. The optical rotatory properties of the polybase (PMS) and the polyampholyte (PMT) show that the water-soluble polymers do not attain an asymmetric macromolecular conformation under the various experimental conditions investigated.

Experimental

Materials.—Poly-L-methionine was prepared by the polymerization of L-methionine N-carboxy anhydride by a procedure analogous to that given for poly-DL-methionine.[11] The details of the synthesis will be published elsewhere. Two preparations, PM 8 and PM 10, of poly-L-methionine with η_{sp}/c = 1.72 and 2.25, respectively, at c = 1.0 g. per 100 ml. dichloroacetic acid were used. The poly-L-methionine S-methylsulfonium bromides, PMS 8 and PMS 10, and the poly-L-methionine S-carboxymethylthetin were derived from the corresponding poly-L-methionine samples.[12a]

The concentrations of poly-L-methionine in the various solutions studied are based on the weight of the polymer. Since PMS and PMT are hygroscopic, their concentration in solution was estimated by nitrogen analysis (micro-Kjeldahl). Unless stated otherwise, the concentrations recorded for PMS, given in g./ml. or g./100 ml.. refer to the polysulfonium ion and not to the polysulfonium salt. The molecular weights listed below, however, are those of the polysulfonium chloride.

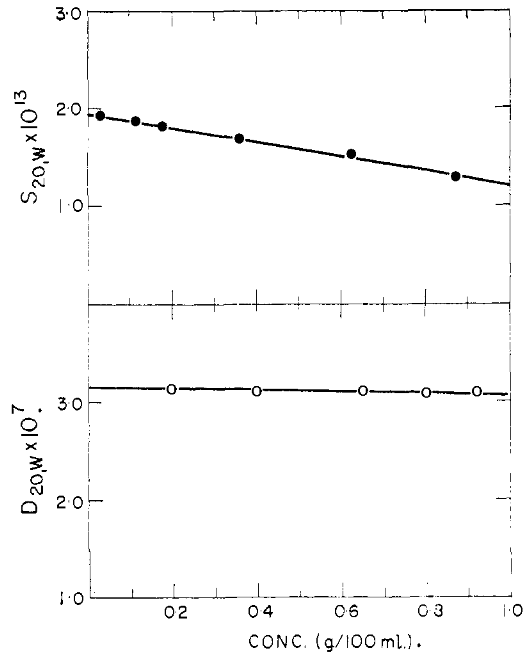

Fig. 1.—Concentration dependence of $S_{20,w}$ (●), and $D_{20,w}$ (O) of poly-L-methionine S-methylsulfonium bromide, PMS 10, in 1.0 M NaCl–0.0139 M KH_2PO_4–0.0282 M K_2HPO_4 pH 6.7 and 1.1 $\Gamma/2$.

The sedimentation and diffusion coefficients of PMS 10 in aqueous solution as a function of concentration in 1.0 M NaCl–0.0139 M KH_2PO_4–0.0282 M K_2HPO_4 buffer of pH 6.7 and 1.1 ionic strength are given in Fig. 1. The high ionic strength was chosen to minimize the charge effect of the polycationic sulfonium salt. The sedimentation velocities recorded in 0.5 M NaCl–0.0139 M KH_2PO_4–0.0282 M K_2HPO_4 buffer of pH 6.7 and 0.6 ionic strength were close to those found at the higher ionic strength. From the extrapolated sedimentation and diffusion constants at c = 0, $S^0_{20,w}$ = 1.95 S., D = 3.26 × 10^{-7}, and the partial specific volume, $\bar{V}$ = 0.590, determined for the poly-L-methionine S-methylsulfonium chloride, a molecular weight of 38,000, corresponding to an average degree of polymerization DP = 210, was calculated. For the preparation PMS 8, we obtained under identical conditions $S^0_{20,w}$ = 1.80 S., D = 3.90 × 10^{-7}, a molecular weight of 32,000, corresponding to a degree of polymerization DP = 176. Since the chemical procedure adopted for the preparation of PMS and PMT from poly-L-methionine should not cause any or only a slight degradation of peptide bonds, it can be assumed that the average degree of polymerization of the parent poly-L-methionine preparations (PM 8 and PM 10) is similar to that of the corresponding polysulfonium salts (PMS 8 and PMS 10) and to the poly-L-methionine S-carboxymethylthetins (PMT 8 and PMT 10).[12b]

N-carbobenzoxy-L-methionine amide (m.p. 125°) was synthesized according to Dekker, *et al.*[13a] N-carbobenzoxy-L-methionine amide S-methylsulfonium bromide and N-carbobenzoxy-L-methionine S-carboxymethylthetin hydrobromide were prepared from carbobenzoxy-L-methionine amide by treatment with methylbromide and bromoacetic acid, respectively.[13b]

Methods.—Viscosity measurements were made with a 10 ml. volume Ostwald–Fenske viscometer at temperatures of 30 ± 0.03° and 1 ± 0.03°, respectively.

---

(4) S. J. Leach, *Revs. Pure and Appl. Chem.* (*Australia*), **9**, 33 (1959).

(5) E. M. Shooter, *Prog. in Biophys.*, **10**, 195 (1960).

(6) E. Katchalski and I. Z. Steinberg, *Ann. Rev. Phys. Chem.*, 1961, in press.

(7) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

(8) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, **42**, 596 (1956).

(9) P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **78**, 498 (1956).

(10) E. R. Blout, P. Doty and J. T. Yang, *ibid.*, **79**, 749 (1957).

(11) A. C. Farthing, *J. Chem. Soc.*, 3213 (1950); R. N. MacDonald, U. S. Patent 2,650,314 (1953), *Chem. Abstr.*, **48**, 1064 (1954).

(12) (a) T. Sadeh and A. Berger, *Bull. Research Council Israel*, **7A**, 97 (1958). (b) Lawson, *et al.*, have shown recently that in the case of ethyl N-acetylmethionylglycinate about 3.6 of peptide cleavage occurred on heating a solution of the peptide in 0.1 M citrate buffer, pH 3.0, with methyliodide at 100° for 1 hr. Since the preparation of poly-L-methionine S-methylsulfonium salt was carried out in a non-aqueous solution (formic acid) at 0°, a much smaller cleavage or none at all should occur. The correct average degree of polymerization of poly-L-methionine is either that derived for the poly-L-methionine S-methylsulfonium salt or slightly higher than that given above. W. B. Lawson, E. Gross, C. M. Foltz and B. Witkop, *J. Am. Chem. Soc.*, **83**, 1509 (1961).

(13) (a) Ch. A. Dekker, St. P. Taylor and J. S. Fruton, *J. Biol. Chem.*, **180**, 155 (1949). (b) The synthesis of these compounds will be described in detail elsewhere by T. Sadeh, A. Berger and E. Katchalski.

Sedimentation velocity experiments were carried out in a Spinco Model-E analytical ultracentrifuge at 56,100 r.p.m. at 20°, in a cell of 12 mm. thickness. The temperature was controlled within ± 0.5° by means of the RTIC unit provided with the instrument. The runs at low concentration were made in the Anal. B rotor at 50,740 r.p.m. using a 30 mm. cell. The sedimentation coefficients were derived from enlarged tracings of the patterns accordings to the conventional procedure.[14]

Diffusion measurements were performed in a Spinco Model-E ultracentrifuge. The boundary between the solvent and the solution was obtained using a synthetic boundary cell and operating at 8.766 r.p.m. At this speed the sedimentation of the polymer investigated is practically negligible. The diffusion constant was computed by the area-maximum height method from enlarged tracings of the Schlieren patterns.[14]

Partial specific volume was determined at 25° with the aid of a pycnometer of a volume of 19.5 ml.

Prior to the ultracentrifugation, diffusion and density analyses that were carried out on the poly-L-methionine S-methylsulfonium bromide, the polymer solutions were dialyzed at 5° for 24 hr. against large volumes of the appropriate buffers.

Optical rotations were measured at 25° in the wave length range of 350 to 600 mμ with the Rudolph precision ultraviolet polarimeter, model 80, equipped with a photoelectric detector and an oscillating polarizer prism. Most of the measurements were made with the mercury arc lamp, Hanovia Quartz Type S-100, as a light source. In some experiments a sodium lamp and a Xenon Arc Lamp were used.

Since the indices of refraction of most of the solvents used differ considerably from water, all specific rotations given in this paper are corrected for the corresponding values in water with the aid of the equation

$$[\alpha]^{25}_{w}\,(\text{corr.}) = [\alpha]^{25}_{s} \times \frac{n^2_w + 2}{n^2_s + 2}$$

where $[\alpha]^{25}_s$ is the specific optical rotation of the solution, $[\alpha]^{25}_w$ (corr.) is the expected specific optical rotation in water, n_w is the index of refraction of water and n_s is that of the solvent. The refractive index of the solvent was measured in each case with an Abbe-type refractometer.

Results and Discussion

Poly-L-methionine.—The infrared absorption spectrum of poly-L-methionine in chloroform has characteristic absorption bands at 1660, 1555 and 3330 cm.$^{-1}$. Those at 1660 cm.$^{-1}$ and 1555 cm.$^{-1}$ may be attributed to amide I (C=O stretching) and amide II (NH deformation), respectively, while that at 3330 cm.$^{-1}$ corresponds to NH stretching. The location of the absorption bands for amide I and amide II are characteristic of peptide bonds in the α-helix configuration[15] and are similar to those recorded for poly-γ-benzyl-L-glutamate in chloroform.[16,17] The existence of the poly-L-methionine molecules in a helix configuration was further supported by optical rotatory dispersion measurements. Because of the fact that poly-L-methionine dissolves readily in chloroform–dichloroacetic acid mixtures and the finding that the specific rotations, $[\alpha]_{589}$ and $[\alpha]_{546}$, in chloroform are identical with those in the chloroform–dichloroacetic acid mixtures of 96:4 and 90:10 v./v., precise optical rotatory dispersion measurements were carried out in the last mentioned solvent.

The optical rotatory dispersion of poly-L-methionine in the chloroform–dichloroacetic acid mixture 90:10 (v./v.) is given in Table I. These data could not be fitted by a one-term Drude equation. However, when $[\alpha].(\lambda^2 - \lambda^2_0)$ was plotted *versus* $(\lambda^2 - \lambda_0^2)^{-1}$, according to Moffitt,[18,19] assuming $\lambda_0 = 212$ mμ, a straight line was obtained. From the slope of this line, a value of −650 was calculated for the constant b_0. Furthermore, it should be noted that the specific optical rotation at 579 mμ is somewhat lower than at 546 mμ, a result obtained in all chloroform–dichloroacetic acid mixtures and which at present cannot be explained.

TABLE I

OPTICAL ROTATORY DISPERSION OF POLY-L-METHIONINE

Wave length, mμ	Specific optical rotation,[a] $[\alpha]^{24}$, corr. in Chloroform–dichloroacetic acid (90:10 v./v.)	Dichloacetic acid	Trifluoroacetic acid
579	21.3	16.1	−113.4
546	24.4	16.8	−115.3
492	24.0	16.6	−152.8
435	20.9	6.8_6	−208.9
405	12.1	1.2	−252.3
365.8	−19.2	−14.1	−350.9

[a] The concentration of poly-L-methionine was 1.0 g./100 ml.

Poly-L-methionine in its anomalous optical rotatory behavior resembles poly-γ-benzyl-L-glutamate[7,9] and poly-ε-carbobenzoxy-L-lysine[20] which, in weakly interacting solvents, have an optical activity that can also be described by the Moffitt equation with b_0 values of −600 to −650. If the anomalous rotatory dispersion of these polyamino acids is correlated with their asymmetric helical conformation in solution, one can assume by analogy that poly-L-methionine in the chloroform–dichloroacetic acid mixture (90:10, v./v.) possesses a helical configuration with a right-handed twist similar to that of poly-γ-benzyl-L-glutamate.[7] In strongly interacting solvents, such as trifluoroacetic acid, polypeptides give a normal optical rotatory dispersion ($b_0 = 0$ in the Moffitt equation[18,19]). The data for poly-L-methionine in this solvent (see Table I) could similarly be fitted by a one-term Drude equation with a $\lambda_c = 222$ mμ. Poly-L-methionine, therefore, seems to have a random-coil conformation in trifluoroacetic acid.

The specific optical rotations, $[\alpha]_{589}$, of poly-L-methionine in different chloroform-trifluoroacetic mixtures are given in Fig. 2. The marked decrease in the optical rotation on increasing the amount of trifluoroacetic acid from 50 to 60% by volume reflects the transition from helix to random coil. The fact that the specific optical rotation of the randomly coiled molecules is more levorotatory than that of the helical ones is in agreement with the conclusion drawn above that

---

(14) H. K. Schachman, "Methods in Enzymology," Vol. IV, edited by S. P. Colowick and N. O. Kaplan, Academic Press, Inc., New York, N. Y., 1957, p. 32.

(15) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956.

(16) G. R. Bird and E. R. Blout, *J. Am. Chem. Soc.*, **81**, 2499 (1959).

(17) A helical conformation was recently attributed to poly-L-methionine in the solid state with the aid of infrared absorption and dichroism of oriented films of this peptide. E. R. Blout, C. de Lozé, S. M. Bloom and G. D. Fasman, *J. Am. Chem. Soc.*, **82**, 3787 (1960); S. M. Bloom, G. D. Fasman, C. de Lozé and E. R. Blout, *ibid.*, **84**, 458 (1962).

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

(19) E. R. Blout, in "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., New York, N. Y., 1960, Chapter 17.

(20) J. Applequist, Doctoral Thesis, Harvard University, Cambridge (1959); G. D. Fasman, M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 709 (1961).

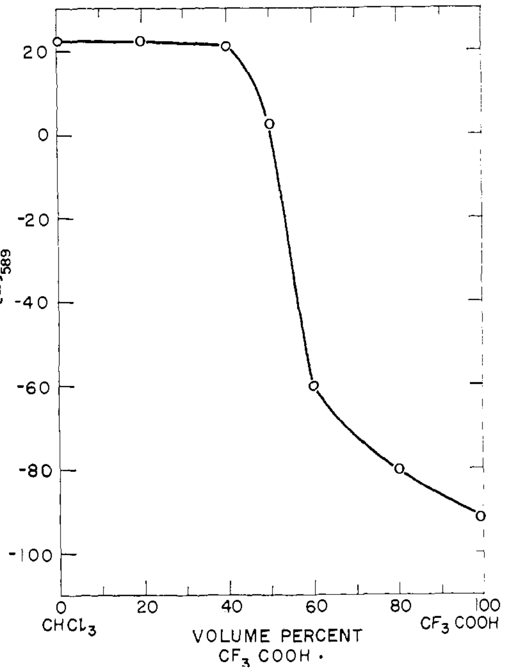

Fig. 2.—Specific optical rotation $[\alpha]^{24}_{589}$, of poly-L-methionine, PM 10 in mixtures of CHCl$_3$–CF$_3$COOH.

the sense of the helix of poly-L-methionine is similar to that of poly-γ-benzyl-L-glutamate.[7] It is, however, opposite to that found for poly-β-benzyl-L-aspartate which gives in chloroform a positive value of b_0 (b_0 = +630).[21] Furthermore, the decrease in the methionine residue rotation, ΔR = 140°, accompanying the helix-coil transition is of the same order of magnitude as that recorded for the corresponding decrease in the residue rotations of poly-γ-benzyl-L-glutamate, poly-L-alanine, poly-L-leucine, poly-L-glutamic acid and poly-L-lysine.[19]

The optical rotatory dispersion of poly-L-methionine in dichloroacetic acid resembles that in chloroform (see Table I) and can also be represented by the Moffitt equation with λ_c = 212 mμ and b_0 = −550. The specific rotations, $[\alpha]_{546}$, and the characteristic constants b_0 for different chloroform–dichloroacetic acid mixtures are given in Fig. 3. From the data presented it follows that poly-L-methionine retains a helical conformation in dichloroacetic acid. Since, however, most polyamino acids exist in dichloroacetic acid as random coils,[6] it is clear that the intramolecular forces stabilizing the helix of poly-L-methionine are stronger than those of the other polypeptides. Uncoiling of the helix structure of poly-L-methionine in dichloroacetic acid may, therefore, again be effected only in a most strongly interacting solvent, such as trifluoroacetic acid. The fact that the value of b_0 = −550 for poly-L-methionine in dichloroacetic acid is less negative than that in chloroform suggests that the helix in the acid solvent is less stable. This assumption is further strengthened by the finding that the helix-coil transition in dichloroacetic acid–trifluoroacetic acid mixtures occurs at a concentration of 20% by volume (see Fig. 4), whereas in the chloroform–trifluoroacetic acid system, 50% by volume of the acid is required (Fig. 2).

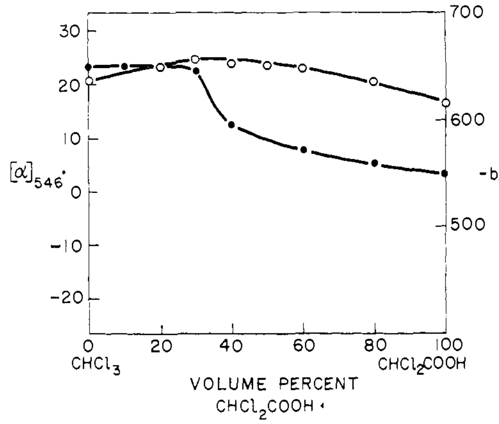

Fig. 3.—Specific optical rotation $[\alpha]^{24}_{546}$, (○), and calculated values of b_0, (●), of poly-L-methionine, PM 10, in mixtures of CHCl$_3$ and CHCl$_2$COOH.

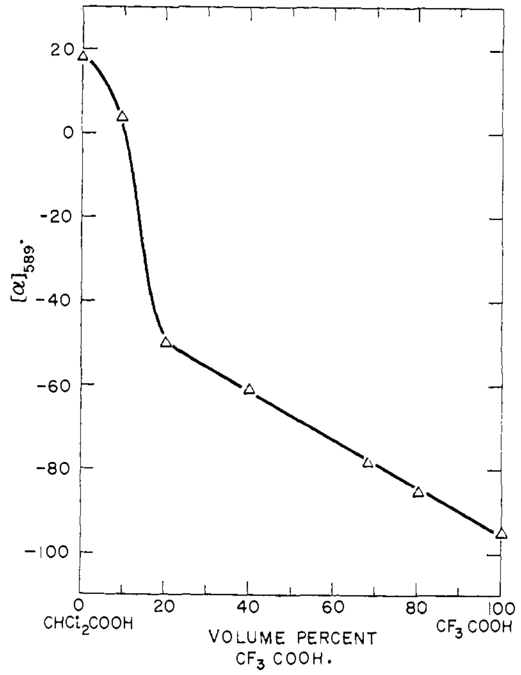

Fig. 4.—Specific optical rotation $[\alpha]^{24}_{589}$, of poly-L-methionine, PM 10, in mixtures of CHCl$_2$COOH and CF$_3$COOH.

The poly-L-methionine sample PM 10 has the relatively high degree of polymerization, DP = 210. It could, therefore, be predicted that the intrinsic viscosity of this preparation in its α-helix configuration should be considerably higher than that in the random coil form.[22] Indeed, it was found that PM 10 gives an η_{sp}/c = 2.2 dl./g. (c = 0.5 g.) in a chloroform–trifluoroacetic acid mixture of 80 to 20 (v./v.) and η_{sp}/c = 1.3 dl./g. (c = 0.5 g) in trifluoroacetic acid. The relative viscosity of PM 10 as a function of solvent composition is shown in Fig. 5. A marked drop

---

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

[22] For a comparison of the relative viscosities of various poly-γ-benzyl-L-glutamate samples with different average molecular weights in the α-helix or random coil conformation see: P. Doty, J. H. Bradbury and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).

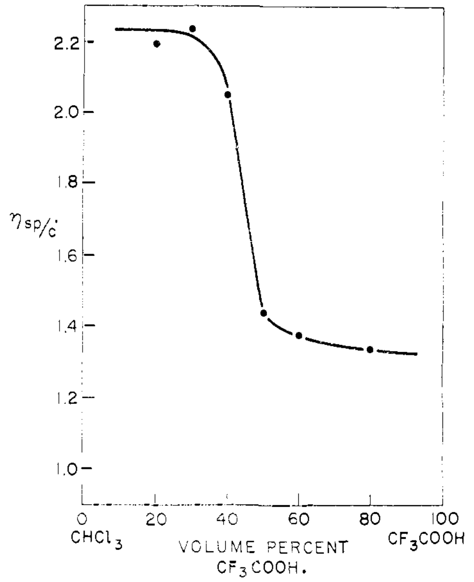

Fig. 5.—Specific viscosity of poly-L-methionine, PM 10, at 0.9° in mixtures of $CHCl_3$ and CF_3COOH (c = 0.5 g./100 ml.)

in the relative viscosity occurs in the range of chloroform–trifluoroacetic acid of 60:40 to 40:60 (v./v.). This observation is in agreement with the changes in the poly-L-methionine conformation in the same solvents when followed by optical activity measurements (Fig. 2). It should be noted, however, that no precise comparison can be made between the two sets of data since the optical rotation measurements were carried out at 25° whereas the results listed in Fig. 5 were obtained at 0.9°. The lower temperature for the viscosity measurements was chosen to reduce the volatility of the solvent.

The relative viscosity of PM 10 in dichloroacetic acid (η_{sp}/c = 1.9 dl./g., c = 0.5) exceeds that in trifluoroacetic acid (η_{sp}/c = 1.3 dl./g., c = 0.5) but is lower than in chloroform–trifluoroacetic acid (80:20, v./v.) (η_{sp}/c = 2.2 dl/g., c = 0.5). A study of the change of the relative viscosity of PM 10 as a function of solvent composition at different dichloroacetic acid–trifluoroacetic acid mixtures at 0.9 ± 0.1° reveals that, analogous to the optical rotation measurements, the transition in this system occurs already at trifluoroacetic acid concentrations of 10 to 15% by volume. This finding further supports the view that the helical molecules of poly-L-methionine in dichloroacetic acid are considerably less stable than in chloroform.

Poly-L-methionine S-Methylsulfonium Bromide.—The viscosity of poly-L-methionine S-methylsulfonium bromide (PMS 10) in aqueous solution as a function of concentration, at different ionic strengths, is shown in Fig. 6. The high specific viscosities at low ionic strength and the relatively low values at high salt concentrations indicate a characteristic polyelectrolyte behavior. The polypeptide molecules thus seem to attain a stretched configuration at low ionic strength as a result of electrostatic repulsion between the positively charged sulfonium groups. At high salt concentrations the screening effect of the small ions reduces the intramolecular repulsion forces and facilitates a free movement of the segments of the polypeptide chains.

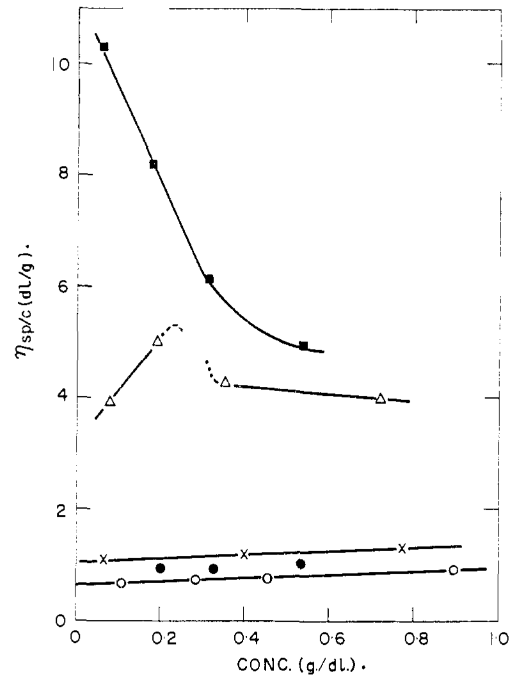

Fig. 6.—Intrinsic viscosity of poly-L-methionine S-methylsulfonium bromide, PMS 10 at 30° in H_2O (■) and in NaCl–K phosphate buffers of pH 6.7 and Γ/2 0.006 (△), 0.1 (×), 0.3 (●) and 0.6 (○). The buffers were prepared by dilution of the buffer 1.0 M NaCl–0.0139 M KH_2PO_4–0.0282 M K_2HPO_4, pH 6.7, 1.1 Γ/2.

The optical rotatory dispersion of PMS 8 in aqueous solution can be represented by a one-term Drude equation, with a dispersion constant of λ_c = 190 ± 1 mμ, and is independent of salt concentration in the range of 0.06 to 1.1 ionic strength. The plot of $[\alpha]\lambda^2$ *versus* $[\alpha]$ for some representative data is given in Fig. 7. The normal optical rotatory dispersion of PMS and its independence of salt concentration indicate that charge screening in this polyamino acid is not accompanied by the formation of an optical macromolecular asymmetric conformation.

A normal optical rotatory dispersion is also found for PMS 8 in aqueous lithium bromide solutions. The increase in the dispersion constant, λ_c, shown in Table II, on increasing the lithium bromide concentration from 0 to 10 M is in accord with the findings for ribonuclease of Harrington and Schellman.[23] The decrease in water activity, caused by the high concentrations of lithium bromide, most likely induces intramolecular hydrogen bonding between some segments of the polypeptide chain. Helical regions may thus be formed and their presence is indicated by the relatively high dispersion constants.

Optically active polyamino acids possessing ionizable side chains, such as poly-L-glutamic acid and poly-L-lysine behave as typical polyelectrolytes when ionized but can be transformed into α-

---

[23] W. F. Harrington and J. A. Schellman, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **30**, No. 12 (1957).

helices on uncharging.[20,24,25] It could, therefore, be expected that poly-L-methionine S-methylsulfonium bromide will have similar properties. No attempt was made, however, to uncharge this polypeptide because of the strong basicity of the positively charged sulfonium groups and the relative instability of the polysulfonium salt in strong alkali.

TABLE II

OPTICAL ROTATORY PROPERTIES OF POLY-L-METHIONINE S-METHYLSULFONIUM BROMIDE IN AQUEOUS LITHIUM BROMIDE

Lithium bromide, mole/l.	Specific optical rotation[a] $-[\alpha]^{24}_{546}$	$-[\alpha]^{24}_{405}$	λ_c, mμ
0	73.4	150.5	191
2.0	68.6	140.0	189
5.0	43.0	94.0	218
10.0	38.6	85.5	244

[a] The concentration of the polysulfonium ion was 0.7 g./100 ml.

Poly-L-methionine S-Carboxymethylthetin.—The optical rotatory dispersion of PMT 10 can also be described by a one-term Drude equation. The optical rotatory dispersion constant, λ_c = 186 ± 1 mμ, and the specific rotation at 546 mμ, $[\alpha]_{546} = -44° \pm 1$ are the same in dilute hydrochloric acid of *p*H 1.1 to 2.0, in an acetate buffer of *p*H 4.6 and 0.1 ionic strength, and in dilute alkali of *p*H 10 to 12. Furthermore, the optical dispersion does not change when the ionic strength of the potassium phosphate–sodium chloride buffer of *p*H 6.7 is varied from 0.1 to 1.1. At neutral *p*H poly-L-methionine S-carboxymethylthetin carries a dipolar ion at each of its side chains, composed of a positively charged sulfonium group and a negatively charged carboxylate ion. It is, therefore, of interest that this poly-L-methionine derivative does not attain a helical conformation despite its over-all zero net charge. It is not unlikely that dipole-dipole interaction prevents the formation of a helical structure in this case.

Low Molecular Weight L-Methionine Derivatives.—In an attempt to explore the effect of macromolecular conformation on the optical activity of the methionine residue, the optical rotations of the following low-molecular weight compounds, N-carbobenzoxy-L-methionine amide, N-carbobenzoxy-L-methionine amide S-methylsulfonium bromide and N-carbobenzoxy-L-methionine amide S-carboxymethylthetin hydrobromide, were compared with those of the corresponding poly-α-amino acids. A normal optical rotatory dispersion was found for N-carbobenzoxy-L-methionine amide in chloroform ($[\alpha]_{546} = -5.2°$, λ_c = 257 ± 1 mμ), in dichloroacetic acid ($[\alpha]_{546} = -3.3$, λ_c = 258 ± 1 mμ) and in trifluoroacetic acid ($[\alpha]_{546} = -14°$). The finding that the specific optical rotation at 546 mμ of this compound in trifluoroacetic acid does not differ significantly from that in chloroform or dichloroacetic acid, whereas that of poly-L-methionine in trifluoroacetic acid ($[\alpha]_{546} = -115°$) is by 135 to 140° more levorotatory than in chloroform ($[\alpha]_{546} = +24.4°$) or in dichloroacetic acid ($[\alpha]_{546} = 16.8°$) further supports the conclusion drawn that the macromolecular conformation of poly-L-methionine in trifluoroacetic acid differs from that in chloroform or dichloroacetic acid. For comparison of the absolute values of the optical rotation of N-carbobenzoxy-L-methionine amide and poly-L-methionine, the residue rotations, $[R']$,[19] should obviously be used. Thus the methionine residue rotation of the low-molecular weight compound in trifluoroacetic acid ($[R']_{546} = -28°$) markedly differs from that of the polymer in the same solvent ($[R']_{546} = -108°$). Since the poly-L-methionine molecules exist in this solvent as random coils, one must conclude that the polypeptide chain even in its random conformation affects the optical rotatory properties of each of the methionine residues. In this connection it is pertinent to note that the methionine sulfonium residue rotation of N-carbobenzoxy-L-methionine amide S-methylsulfonium bromide and of N-carbobenzoxy-L-methylthetin sulfonium bromide also differ from those of the corresponding polymers.

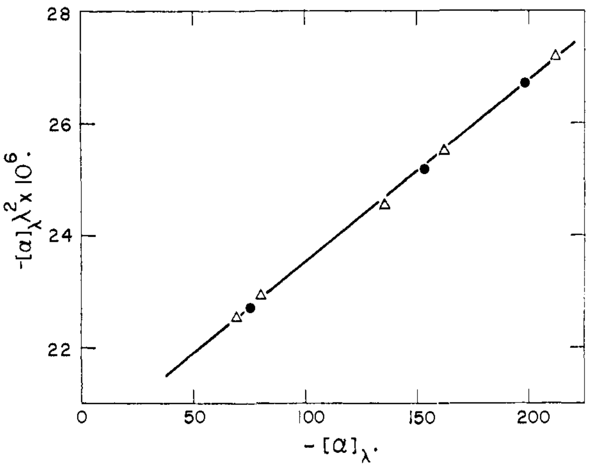

Fig. 7.—Modified plot of dispersion data for poly-L-methionine S-methylsulfonium salt, PMS 8, in 0.05 *M* NaCl–0.0007 *M* KH_2PO_4–0.00141 *M* K_2HPO_4, *p*H 6.7, Γ/2 0.06 (Δ) and in 0.25 *M* NaCl–0.0035 *M* KH_2PO_4, *p*H 6.7, Γ/2 0.3 (●).

Acknowledgments.—We wish to thank Dr. J. Kurtz and Mr. K. Jacobson for help in the synthesis of the polypeptides. Our thanks go also to Mrs. S. Ehrlich Rogozinski and Miss Elizabeth S. Lacock.

---

[24] P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

[25] L. Goldstein and E. Katchalski, *Bull. Research Council Israel*, **9A**, 138 (1960).