added, and the samples were boiled until colorless and for an additional 10 minutes to destroy any residual peroxide. The samples were then cooled and rinsed into 50 ml. volumetric flasks. Five ml. of 6.6% ammonium molybdate were added and the samples were diluted to approximately 30 ml. Two ml. of 7.5 *N* sulfuric acid were added, the samples were mixed, and finally, 5 ml. of freshly prepared 8% ferrous sulfate containing 2 ml. of 7.5 *N* sulfuric acid per 100 ml. were added. The samples were diluted to volume, mixed and read at 660 mμ in the Bausch and Lomb spectrophotometer.

Phenol Test.—Samples of CT and DIP-CT containing approximately 2.5, 5.0 and 7.5 mg. of enzyme were run in triplicate.[17,18] One part of phenol reagent was diluted with two parts of water before use.

***p*H 8.0.**—To samples in 25 ml. volumetric flasks were added in order: 6.0 ml. of 0.1 *M* NaCl, 4.0 ml. of 0.4 *M* Na_2HPO_4, 3 ml. of diluted phenol reagent and 2 ml. of 0.9 *M* NaOH. The samples were diluted to volume, mixed and incubated for 15 minutes at 37°. They were then cooled for 5 minutes in a water bath, and the optical density was determined at 760 mμ in a Beckman DU spectrophotometer. The final *p*H of the samples was 8.0. Standard curves were determined using L-tyrosine and L-tryptophan at levels between 0.25 and 1.5 mg. per sample.

***p*H 12.0.**—To the samples in 25 ml. volumetric flasks were added in order: 6.0 ml. of 0.1 *M* NaCl, 3.0 ml. of 0.4 *M* Na_2HPO_4, 3.0 ml. of diluted phenol reagent and 4.0 ml. of 0.9 *M* NaOH. The procedure was continued as at *p*H 8.0.

N-Bromosuccinimide Reaction.[21]—Analogous samples of denatured DIP-CT and CT were prepared as discussed above. After the volumetric flasks were allowed to stand in 9 *M* urea, *p*H 7.0 at 37° for 12 hr. the samples were chilled.

Appropriate solutions of N-bromosuccinimide were prepared in acetonitrile. Immediately before use, 0.5 ml. of the acetonitrile solution was mixed with 4.5 ml. of cold 0.4 *M* acetate buffer, *p*H 4.0. Two ml. aliquots of these N-bromosuccinimide solutions were added to each pair of denatured DIP-CT and CT solution. The samples were then diluted to volume and mixed. The final urea concentration was 8 *M*. The reaction with N-bromosuccinimide was allowed to proceed for at least 15 minutes. Appropriate controls containing acetonitrile-acetate buffer in 8 *M* urea were also prepared.

All samples were scanned in the Cary Model 14 spectrophotometer against appropriate reagent blanks. Difference spectra were scanned between analogous samples of DIP-CT and CT.

The amount of tryptophan destroyed was calculated according to the procedure of Witkop, *et al.*[21]

Acknowledgments.—The authors wish to express their appreciation to Professors Hartman and Sproull, Department of Physics, Cornell University, for the use of the Cary Model 14 self-recording spectrophotometer and for the use and renovations of facilities in the Department of Physics where this research was carried out. This research was aided by grants from the National Science Foundation and the Office of Naval Research.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY, ITHACA, NEW YORK]

Characterization of the Difference Spectrum of Diisopropylphosphoryl-α-chymotrypsin *versus* α-Chymotrypsin. III. Spectrophotometric Titration of Tyrosyl Groups

BY BENT H. HAVSTEEN[1] AND GEORGE P. HESS

RECEIVED JUNE 29, 1961

Two out of four tyrosyl groups of α-chymotrypsin (CT) or diisopropylphosphoryl-CT (DIP-CT) titrate reversibly in 0.70 *M* KCl or in 0.14 *M* KCl. After 3 hr. at *p*H 13 and 16° the remaining two tyrosyl groups can be titrated. The apparent *pK*'s of the reversibly titrating tyrosyl groups of CT and DIP-CT are the same. In a denaturing solvent (5 *M* in guanidine hydrochloride and 1.2 *M* in urea) all four tyrosyl groups in CT and DIP-CT titrate normally with the same *pK*. Correction for the electrostatic interaction of the protein (calculated from the amino acid composition of CT) with the protons of the tyrosyl groups indicates that the *pK*'s of these groups are the same as that of phenol in this solvent. Also, the electrostatic interaction parameter *w*, for the tyrosyl titration in both CT and DIP-CT, indicates absence of any significant *p*H-dependent configurational changes in this solvent in the *p*H region 7–12. Unlike the tryptophyl residues, the tyrosyl residues in CT and DIP-CT appear equivalent.

Investigations of the α-chymotrypsin (CT) catalyzed hydrolysis of *p*-nitrophenyl acetate[2] revealed changes in absorption of the enzyme at 290 mμ,[3] which are intimately related to the formation and decomposition of the acyl-enzyme. These absorption changes have been observed[3] in the catalytic reaction of CT with *p*-nitrophenyl acetate and diisopropylphosphorofluoridate (DFP) and in the reaction of trypsin with DFP. The experiments suggested that the spectral changes are due to reversible conformational changes of the enzyme brought about by acylation of its active site. Presumably these structural changes of the enzyme form an important part of the catalytic process. In order to be able ultimately to identify the part of the CT molecule which participates in these reversible structural changes, initial investigations were concerned with the chemical reactivity of tryptophyl residues in CT and DIP-CT. Both chemical and spectroscopic data indicated[3] that these residues are not equivalent in CT and DIP-CT. This investigation is concerned with the hydrogen ion equilibria of tyrosyl residues in CT and DIP-CT.

Spectrophotometric titration of tyrosyl residues in proteins have been used with excellent results and abnormalities in their behavior due to interactions with other groups have been readily observable.[4] In the experiments reported here the spectrophotometric titrations were carried out in 0.7

(1) Fulbright grantee, 1959–1961.

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(4) M. Laskowski, Jr., J. M. Widom, M. L. McFadden and H. A. Scheraga, *Biochem. Biophys. Acta*, **19**, 581 (1956); H. A. Scheraga, *ibid.*, **23**, 196 (1957); C. C. Bigelow and M. Ottesen, *ibid.*, **32**, 574 (1959); O. O. Blumenfeld and M. Levy, *Arch. Biochem. Biophys.*, **76**, 97 (1958); C. Tanford, J. D. Hauenstein and D. G. Rands, *J. Am. Chem. Soc.*, **77**, 6409 (1955); M. Laskowski, Jr., S. J. Leach and H. A. Scheraga, *ibid.*, **82**, 571 (1960).

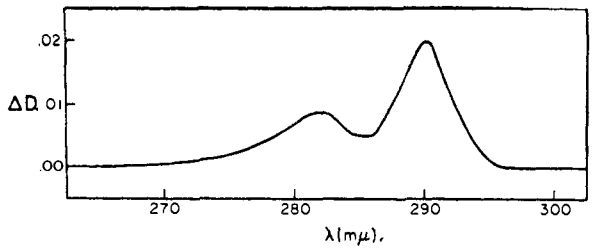

Fig. 1.—Ultraviolet difference spectrum, DIP-CT *versus* CT, *p*H 6.9 (0.033 *M* Tris (hydroxymethyl)-aminomethane-HCl buffer 0.033 *M* $CaCl_2$), 3.6×10^{-5} *M* enzyme, 1.1×10^{-4} *M* DFP 25°. Scanned 3 minutes after addition of DFP to sample cell of a Cary Model 14 self recording spectrophotometer.

M KCl and in 0.14 *M* KCl to conform to the conditions used previously[3] in characterizing the difference spectrum of DIP-CT *versus* CT. The titrations were also carried out in a solvent which would denature the protein. Solutions 5 *M* in guanidine hydrochloride and 1.2 *M* in urea were used. (This solvent is designated as GU.) GU was used rather than 8 *M* urea in order to avoid large solvent effects on the intrinsic *pK*'s (pK'_{intr}). It has been demonstrated previously[5] that the *pK* of acetic acid is the same in GU as it is in 0.15 *M* KCl and that the *pK*'s of imidazole, phenol and *n*-butylamine in GU differ very slightly from the corresponding values in 0.15 *M* KCl. A similar use has been made of GU in structural studies of lysozyme[5] and ribonuclease.[6]

Results

Spectrophotometric Titration.—Spectrophotometric titrations of CT and DIP-CT in 0.70 *M* and 0.14 *M* KCl, and in GU, all at 16°, were carried out in a Beckman model DU spectrophotometer. A molecular weight of 25,000[7] was used in the computations. A plot of log $A^-/(AH - A^-)$ *versus* *p*H[8] was obtained for the titration curves in all solvents over the *p*H range where tyrosyl groups titrate reversibly. A^- corresponds to the moles of ionized tyrosyls and *AH* to the total concentration of tyrosyl residues in the reversible portion of the titration curve. Both the data obtained at 290 mμ and 295 mμ were used for the calculations. The data at 290 mμ were corrected for the 290 mμ peak of DIP-CT (Fig. 1) with a molar extinction coefficient of 565.[9] It has been demonstrated previously[9] that the magnitude of the 290 mμ spectral peak of DIP-CT is independent of *p*H and ionic strength. The data for the reversible portion of the titration curve were obtained within 1 minute after adding calculated amounts of base to analogous samples of CT and DIP-CT. The actual *p*H of the solutions was measured immediately after the spectrophotometric measurements. These values agreed with the initial *p*H of the solutions obtained in parallel experiments. The stability of the DIP group under the conditions of the experiment can be seen in Fig. 2 which gives the data for DIP-CT uncorrected for the 290 mμ peak and demonstrates the presence of this peak throughout the *p*H range. The stability of the DIP group of CT at high *p*H has also been ascertained by direct phosphate analysis.[9]

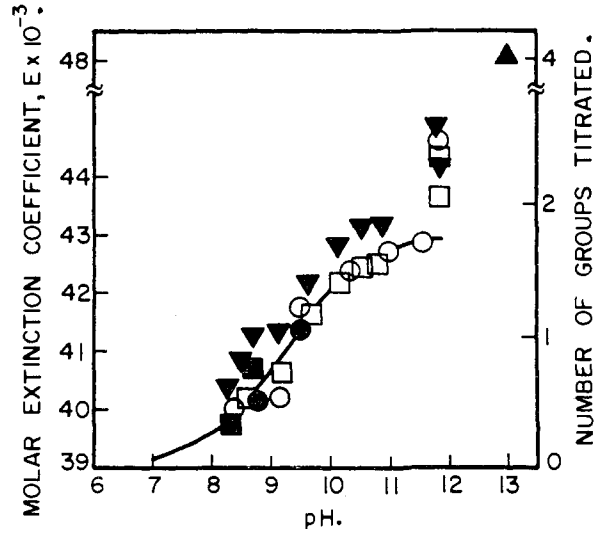

Fig. 2.—Spectrophotometric titration curve of CT and DIP-CT in 0.70 *M* KCl at 16° at a wave length of 290 mμ. The open circles and squares are for the forward titration of CT and DIP-CT, respectively, the filled circles and squares are for the corresponding back titration. The inverted triangles are for the forward titration of DIP-CT uncorrected for the 290 mμ absorption peak (see text). The single triangle indicates the value obtained by both CT and DIP-CT after 3 hr. at *p*H 13°, 16°. The coördinates defining the solid curve were obtained from the line computed by the method of least squares in Fig. 3. This line applies to the reversible portion of the titration curve only.

The spectrophotometric titration curve for the tyrosyl groups in CT and DIP-CT in 0.70 *M* KCl at 16° is shown in Fig. 2. Only 2 out of 4 tyrosyl groups in CT and DIP-CT titrate reversibly in 0.70 *M* KCl at 16°. Above *p*H 11.9 additional tyrosyl groups are liberated due to denaturation of the enzyme. At 16° and *p*H 11.9 0.25 tyrosyl group is liberated in 5 hr. It is therefore unlikely that the contribution of alkaline denaturation to the experimental error is significant below this *p*H. This experiment also indicates that removal of the DIP group does not affect the titration curve. The remaining two tyrosyl groups in CT and DIP-CT can be titrated after 3 hr. at *p*H 13.0. The solid line was obtained from the computed line in Fig. 3.

In order to compare the data for CT and DIP-CT a plot of log $A^-/(AH - A^-)$ *versus* *p*H was obtained separately for CT and DIP-CT. To avoid overcrowding the graph in Fig. 3 the experimental points represent the data at 290 mμ only, both for the forward and back titration. The data for DIP-CT at 290 mμ were corrected for the 290 mμ spectral peak. The line shown in Fig. 3, computed by the method of least squares has the same slope and intercept for both CT and DIP-CT.

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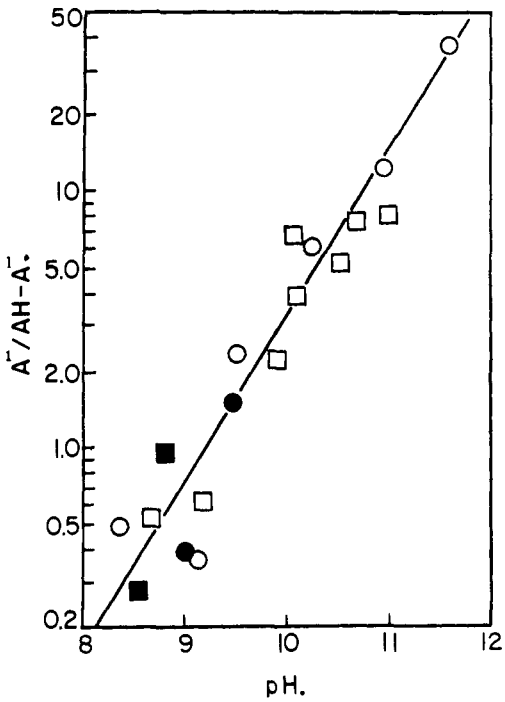

Fig. 3.—A plot of pH *versus* log $A^-/(AH - A^-)$ for the data obtained in Fig. 2. A^- corresponds to the ionized tyrosyl groups, and AH to the total concentrations of reversibly titrating tyrosyl groups (see text). The open circles and squares are for the forward titration of CT and DIP-CT, respectively, the filled circles and squares are for the corresponding back titration.

The apparent pK' for tyrosyl groups titrating reversibly, both in CT and DIP-CT, is 9.3. This value is obtained from the abscissa of the graph in Fig. 3 when $\log A^-(AH - A^-)$ is 1.

The spectrophotometric titration data for CT and DIP-CT in 0.14 M KCl at 16° are shown in Fig. 4. The solid line was obtained from a computed line of a log $A^-/(AH - A^-)$ *versus* pH plot. The apparent pK for the reversibly titrating tyrosyl groups in both CT and DIP-CT is 9.1. As in 0.70 M KCl about 2 out of 4 tyrosyl groups titrate reversibly below pH 11.6.

The change in molar extinction coefficient E at 295 mμ in 0.14 M KCl is 9,670 for 4 tyrosyl groups or 2,420 per single group. This may be compared with the values of 2,300 for tyrosine[10,11] and 2,430 for bovine serum albumin[11] at the same wave length in 0.15 M KCl. The CT data are in agreement with those previously obtained by Wilcox and Chervenka. Wilcox[12] reported that 2 out of 4 tyrosyl groups titrate abnormally in chymotrypsinogen. Chervenka[13] noticed that perturbations of the tyrosyl spectra accompany the activation of chymotrypsinogen to α-chymotrypsin. He found, however, the tyrosyl ionization behavior to be the same in the enzyme and its precursor.

The spectrophotometric titration data for CT and DIP-CT in GU at 16° is shown in Fig. 5.

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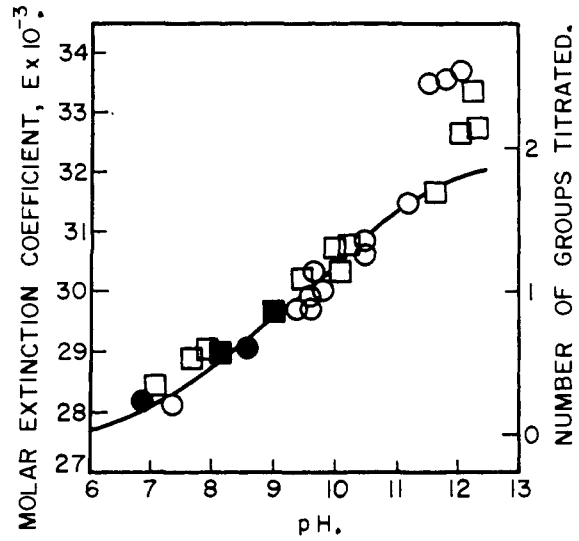

Fig. 4.—Spectrophotometric titration curve of CT and DIP-CT in 0.14 M KCl at 16° at a wave length of 295 mμ. The open circles and squares are for the forward titration of CT and DIP-CT respectively, the filled circles and squares are for the corresponding back titration. The coördinates defining the solid curve were obtained from a line computed by the method of least squares (see text). This line applies to the reversible portion of the titration curve only.

The solid line was obtained from a computed line of a log $A^-/(AH - A^-)$ *versus* pH plot. In this plot the line computed by the method of least squares has the same slope and intercept for both CT and DIP-CT. The apparent pK for the reversibly titrating tyrosyl groups in both enzymes is 10.6. Whereas only 2 out of 4 tyrosyl groups of DIP-CT and CT titrate reversibly in 0.70 M and 0.14 M KCl, all 4 behave normally in GU as is indicated by the single stage titration curve of Fig. 5. There was no difference in the spectrophotometric titration curves in GU between solutions aged for 1 and 7 hr., respectively. Therefore, the denaturation of CT and DIP-CT in GU at 16° has been assumed to be complete within 60 minutes.

The change in molar extinction coefficient E at 290 mμ in GU is 11,000 for 4 tyrosyl groups or 2,750 per single group. This may be compared with the value of 2,630 for the tyrosyl groups of ribonuclease in GU.[6]

Discussion

The spectrophotometric titration of tyrosyl residues in CT and DIP-CT in 0.14 M and 0.70 M KCl and in GU indicate that the number of reversibly titrating tyrosyl residues and the apparent pK's of these groups are the same for the two enzyme preparations in both aqueous solution and in GU. This agrees with previous studies[3,9] which demonstrated that ΔD_{295} is zero for the difference spectrum of DIP-CT *versus* CT at the same pH in the pH region 7 to 12, both in aqueous solution and in 8 M urea.

In spite of the excellent agreement of the spectrophotometric titration data of CT and DIP-CT both in aqueous solutions and in GU, the results must be viewed with caution. Structural differences between CT and DIP-CT could cause the

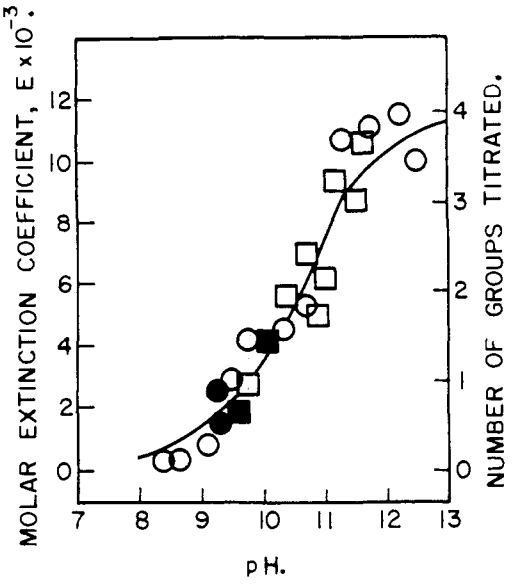

Fig. 5.—Spectrophotometric titration curve of CT and DIP-CT in GU at 16° at a wave length of 295 mμ. The open circles and squares are for the forward titration of CT and DIP-CT, respectively, the filled circles and squares are for the corresponding back titration. The coördinates defining the solid curve were obtained from a line computed by the method of least squares (see text).

apparent pK's of the reversibly titrating tyrosyl groups to be the same, although the intrinsic pK's are different. Differences in the aggregation behavior of CT and DIP-CT are probably not important considerations. The experiments reported were carried out with a protein concentration of 0.40 mg./ml. or less and an ionic strength greater than 0.1 M. Investigations of the aggregation behavior of CT and DIP-CT in the pH range 7 to 9.0 indicated that at an enzyme concentration of 1 mg./ml. and an ionic strength greater than 0.1 aggregation of these proteins is negligible.[14] However, correction for the electrostatic interaction between the protons of the tyrosyl groups and the net charge of the protein molecule may reveal differences in the intrinsic pK's of these groups between the two enzymes. Settlement of this point awaits a careful evaluation of the net charge of both CT and DIP-CT as a function of pH.

Assuming that all groups titrate normally in GU, the Z values for CT can be calculated from the known amino acid composition[15] of this enzyme. pK' intrinsic of the reversibly titrating tyrosyl groups in GU can then be found from the following equation which applies to each type of ionizable group[16]

$$p\mathrm{H} - \log\left(\frac{X}{1-X}\right) = pK'_{\mathrm{intr.}} - 0.868\,wZ \quad (1)$$

where X is the fraction of the groups of the given kind which have dissociated protons at the given

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(16) See, *e.g.*, C. Tanford in T. Shedlovsky, ed., "Electrochemistry in Biology and Medicine," John Wiley and Sons, Inc., New York N. Y., 1955, p. 248.

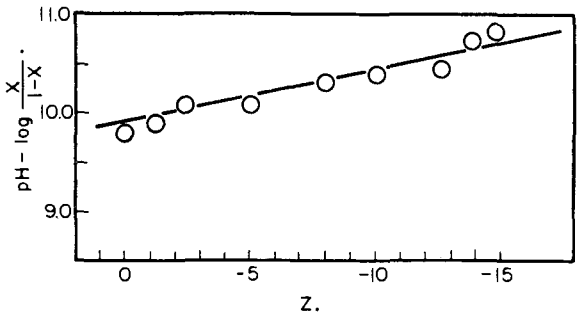

Fig. 6.—Plot of data for tyrosyl groups in GU according to equation 1. The values for log $X/1 - X$ were computed from the solid line of Fig. 5 (see text).

pH, and $pK'_{\mathrm{intr.}}$ is the negative logarithm of the intrinsic dissociation constant for the corresponding groups in the solvent used. The term 0.868 wZ arises from the electrostatic interaction between the proton and the protein molecule which bears the net charge Z. The quantity w is regarded as an experimental parameter and would be expected to be constant at a given temperature and ionic strength over a pH range in which the size, shape and permeability of the molecules do not vary.

The data for the tyrosyl groups of CT and DIP-CT in GU were plotted according to equation 1 and are shown in Fig. 6. The experimental points lie on a straight line from which w and $pK'_{\mathrm{intr.}}$ were determined from the slope and intercept at $Z = 0$. A value for $pK'_{\mathrm{intr.}}$ of 9.9 was obtained. This value may be compared with the values of 10.0 for the tyrosyl groups in ribonuclease[6] and 10.03 for phenol[5] in GU.

The w value of 0.057 can not easily be compared to other values. Although a theoretical w can be obtained from the equations of Linderstrøm-Lang,[17] the necessary other experimental parameters are not available for CT. Nevertheless, the agreement of $pK'_{\mathrm{intr.}}$ found in this experiment with the values obtained for other proteins and phenol in GU suggests that the internal interactions which render the two tyrosyl groups of CT and DIP-CT abnormal in aqueous solution are removed in GU. The internal interactions may be due to hydrogen bonding between tyrosyl residues and a hydrogen bond acceptor, specific local electrostatic effects or hydrophobic bonding. All these interactions probably require the specific configuration of the native enzyme which is disrupted by GU.

While the tryptophyl residues in CT and DIP-CT are not equivalent,[3,9] the data presented here suggest that the tyrosyl residues are equivalent. Whether the reversibly titrating tyrosyl residues in aqueous solution are normal and whether minor differences between these tyrosyl groups in the two enzymes exist will have to be decided by further investigations.

Experimental

Material.- Salt free, three times recrystallized α-chymotrypsin (Lot number CDI 700–706) was obtained from

(17) K. Linderstrøm-Lang, *Compt. rend. Lab. Carlsberg Ser. Chim.*, **15**, No. 7 (1924).

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[5,9] 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.

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[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

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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

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