Optical Rotatory Dispersion of Nonhelical Proteins*

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SUMMARY

The optical rotatory dispersion of a group of proteins having the Moffitt constant $b_0$ near zero was studied in the far ultraviolet spectral zone. Sodium poly-$\alpha$-L-glutamate, myoglobin, and serum albumin were used as reference substances. It was found that, in addition to the normal type of rotatory dispersion, as displayed, for example, by myoglobin, four other types of curves could be demonstrated. They are as follows.

Type I is represented by phosvitin and the lysine- and proline-rich histone, which are flexible disordered macromolecules. The rotatory dispersion of these proteins is similar to that of disordered polyglutamate. The rotatory power of phosvitin and histone at 198 $\mu$m is near zero.

Type II is exemplified by oxidized serum albumin. These curves have two minima, one at 230 to 233 $\mu$m and another at 210 to 215 $\mu$m. The major conformation of oxidized serum albumin seems to be a disordered chain, with a small amount of $\alpha$ helix. Computed curves from data of helical and disordered polyglutamate yielded this type of curve when the helix content was about 25% or less.

Type III is represented by chymotrypsinogen, trypsin, and thyrotropin. These proteins have curves with a minimum at 218 to 225 $\mu$m, and they are weakly dextrorotatory at 195 to 200 $\mu$m. It was concluded that these proteins are devoid of complete $\alpha$-helical strands; instead, they are characterized by some other kind of ordered structures.

Type IV curves are observed with serum $\gamma$-globulin, the myeloma protein, and Bence-Jones protein. These curves have a shallow minimum at 220 to 225 $\mu$m, a positive maximum at 210 $\mu$m, and a minimum at 198 to 200 $\mu$m.

Addition of the disordered polyglutamate or phosvitin to the solutions of the highly $\alpha$-helical myoglobin or serum albumin did not produce a displacement of the negative minimum at 233 $\mu$m, although the amplitude was diminished. If the ratio of the disordered polymer relative to myoglobin was high, type II curves were obtained.

Treatment of chymotrypsinogen with decyl sodium sulfate caused a conformational transition resulting in some $\alpha$ helix formation. The type III curve of chymotrypsinogen was thereby transformed into the ordinary curve of the partially $\alpha$-helical proteins; i.e. it then possessed a trough at 233 $\mu$m and a peak at 198 $\mu$m. The detergent had a similar effect on the rotatory dispersion of trypsin, Bence-Jones protein, and $\gamma$-globulin, but not on the highly charged phosvitin.

Recent studies on the optical rotatory dispersion of proteins and polynucleic acids have shown that there is a significant difference between the highly $\alpha$-helical and disordered chain conformations (I-4). The same has been ascertained by the study of circular dichroism (4, 5). However, there may be more than two types of conformations in globular proteins, i.e. $\alpha$ helix and random chain, as it has been often tacitly assumed. As shown in two recent publications (6, 7), there are "abnormal" globular proteins, i.e. compact macromolecules which seem to be devoid of the $\alpha$ helix. Pepsin, trypsin, chymotrypsinogen, the luteinizing hormone (6), serum $\gamma$-globulin (6, 7), elastase, soybean trypsin inhibitor (7), and several others belong to this group. The Moffitt constant $b_0$ of these proteins is close to zero, and the negative minimum of the Cotton effect is not at 233 $\mu$m but at 215 to 225 $\mu$m (6, 7). Since the disordered poly-$\alpha$-L-glutamate has a minimum at 205 $\mu$m (1), it seemed worthwhile to consider the possibility that the abnormal proteins are characterized simply by a very low $\alpha$ helix content, and that the rest of the molecular structure is disordered. If this is true, mixtures of highly $\alpha$-helical and disordered macromolecules could be expected to yield curves resembling those of the abnormal proteins. The purpose of this paper is to report experiments directed at testing this possibility, and to provide more data for various nonhelical proteins. Myoglobin, serum albumin, and poly $\alpha$-L-glutamate were used as reference substances. Moreover, this report supplements a previous paper (7) on the Cotton effects as new criteria of conformation. In the present paper, data are provided on the optical activity of fully disorganized proteins at the important wave lengths of 198 and 233 $\mu$m, which are essential in the calculation of the $\alpha$ helix content.

EXPERIMENTAL PROCEDURE AND RESULTS

Proteins—Chymotrypsinogen A was a chromatographically homogeneous protein (crystallized five times) provided by

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Worthington, Sample CGC 762. The protein appeared homogeneous in sedimentation tests in the analytical ultracentrifuge. Upon gel filtration on Sephadex G-100, in a long column (189 x 1.8 cm) with a buffer of pH 7.0 composed of 0.05 M sodium phosphate and 0.10 M NaCl, the elution diagram exhibited only one sharp peak. The optical rotatory properties of the Worthington material were identical with the properties of the starting material. Several trypsin preparations were used, primarily a sterile preparation (crystallized three times) from Worthington. The preparations were enzymatically active even in dilutions of 0.001%; e.g., the three times crystallized enzyme was found to have 10,400 units per mg with N-benzoyl-n-arginine ethyl ester as substrate. The three times crystallized enzyme was found to have 10,400 units per mg with N-benzoyl-n-arginine ethyl ester as substrate.

The optical rotatory properties of the Worthington trypsin were compared with the properties of a trypsin treated with the chymotrypsin inhibitor I-(1-lysylamido-2-phenyl)ethyl chloromethyl ketone (8) according to Kostka and Carpenter (9). Oxidized human serum albumin was prepared some time ago in this laboratory by Dr. T. Ikenaka and was described in a previous paper (10). Phosvitin was obtained from Nutritional Biochemicals. One of the samples was studied here several years ago (11) and appeared similar to the preparations described by Mecham and Olcott (12). Phosvitin contains 9.7% phosphorus, which is in the form of ionizable monoesterified phosphate, and is bound to the hydroxyl groups of serine residues (12). The recently provided samples appeared similar to the older sample in optical activity, viscosity, and sedimentation. One symmetrical peak was observed on sedimentation in the analytical ultracentrifuge. Gel filtration on a Sephadex G-100 column (189 x 1.8 cm) with a buffer composed of 0.05 M sodium phosphate and 0.10 M NaCl, pH 7.0, revealed one major peak and two additional components in small amounts. The latter appeared as small peaks on both sides of the major peak. No significant differences could be found in the optical activity between the major fraction and the unfractionated phosvitin. The aqueous solutions of phosvitin were very viscous, and the reduced specific viscosity increased on dilution, as shown in Fig. 1. After salt was added, the anomaly disappeared and the normal linear dependence of the viscosity on concentration was observed (Fig. 1). These observations indicate that phosvitin is a flexible polyelectrolyte (13). A small specimen (3 mg) of thyrotropin (thyroid-stimulating pituitary hormone) was provided by Dr. John G. Pierce (University of California, Los Angeles). The properties of this substance are described in a paper of Carsten and Pierce (14). Because of the small amount available, no homogeneity tests could be made. The hormone seems to be composed of several components which differ somewhat in their molecular properties but which are all physiologically active (14). Thyrotropin is a glycoprotein similar to the luteinizing hormone; i.e., it has a high content of serine, threonine, proline, and cystine (14). Human serum γ-globulin was isolated in this laboratory by methods described in previous papers (6, 11); one sample was provided by the Protein Foundation (Jamaica Plain, Massachusetts). The preparations contained only traces of extraneous material. Disulfide bond cleavage of the globulin and reconstitution of the macromolecules have been studied in this laboratory (15), and the reconstituted γ-globulin was compared with the native material. The γ-globulin was purified by gel filtration on Sephadex G-100, and only the major component, which was a 7S globulin, was used. A pathological myeloma γ-globulin (designated Py) of a case in which this 7S protein appeared in the urine also was investigated (16). In purity, amino acid composition, and optical activity it was similar to the normal serum γ-globulin (10). A Bence-Jones protein was isolated from the urine of another patient suffering from multiple myeloma. This protein had a molecular weight of 28,000, and the molecule was composed of two polypeptide chains of unequal size (17). It belonged to the antigenic B-type. Sedimentation tests and chromatography indicated that this protein was practically homogeneous. Calf thymus histone Fraction F1 (Sample CTH) was kindly provided by Dr. Lubomir S. Hnilica of our Department. This histone fraction contains 27.1% lysine and 9.4% proline, among other amino acids, and is described in more detail elsewhere (18, 19).

The aqueous solutions of this histone were viscous and the reduced specific viscosity increased on dilution, as in the case of phosvitin. When salt was added the anomaly disappeared. The histone showed one peak in the analytical ultracentrifuge, but some inhomogeneity has been revealed on electrophoresis in starch gel (18).

A four times crystallized human serum albumin (Nutritional Biochemicals) and a crystallized specimen of whale myoglobin, i.e., metmyoglobin (Mann), were used as reference materials. The optical rotatory properties of the crystallized myoglobin from Mann have been compared by others with specially purified preparations and no significant differences have been found (4). Another reference substance was sodium poly-a-lysinate, which was also obtained from Mann. The specimen carried the designation M242S and, according to Mann, its average molecular weight was 61,000. The reduced specific viscosity, nsp, of the aqueous solutions of the polyglutamate at 20.0°C was found to be 1.23 dl per g at a concentration of 0.19%, and it rose to 2.79 dl per g at a lower concentration of 0.024%.

Decyl sodium sulfate was a gift from du Pont; according to the manufacturer's data, it was a pure product. All other chemicals were reagent grade.

Serum albumin, myoglobin, sodium polyglutamate, chymotrypsinogen, histone F1, phosvitin, thyrotropin, and Bence-Jones protein were all used in aqueous solutions. Trypsin was dissolved in either water or 0.001 N HCl. Oxidized serum albumin was dissolved in dilute NaOH, and the final pH of these solutions was 9.8 to 10.6. The normal and pathological γ-globulins were dissolved in 0.02 M phosphate buffers of pH 7.0 to 9.6. The pH of the phosvitin and polyglutamate solutions was varied by adding 0.1 N HCl or NaOH.

The concentrations of the proteins and polyglutamate were determined chiefly by micro-Kjeldahl nitrogen analyses. In the case of oxidized albumin and thyrotropin, the protein was weighed, and the measured weight was corrected, by assuming 10% moisture. The concentration of γ-globulin, myeloma globulin, and Bence-Jones protein was determined by absorbance measurement at 280 μm; the absorbance value of 1.40 was used for solution containing 1 mg per ml and a 1.0-cm optical path.

Optical Rotatory Dispersion Measurements—The measurements were made with the two modified Rudolph spectropolarimeters described in a previous paper (7). All measurements were made in cylindrical optical quartz cells (Pyrocell) with an optical path of 0.10, 0.20, or 0.50 cm, at a room temperature of 22–25°C. The protein concentration was 0.02 to 0.2% at the wave length limits of 230 to 350 μm, and it was 0.007 to 0.02% in the 190 to 220 μm zone. The Moffitt constants b₀ were determined from measurements in the 250 to 350 μm spectral region by using the value of 216 for the parameter λₑ (7, 20, 21). The corrected specific rotation [m] values were computed from the relationship

\[ [\alpha] = 216/([\alpha] + b) \]

where [α] is the specific rotation, and b is the refractive index of the solvent. The refractive index values of water (22°) were used also for the diluted buffer solutions. The mean residue molecular weight M was found from amino acid composition. The reproducibility of the b₀ values was approximately ±8°, while the reproducibility of the [m] values was approximately ±500° at the negative trough of 233 μm, and in the range of ±1500° to ±2000° at the 190 to 200 μm zone. By using the manual unit, 5 to 10 readings were made at each wave length, usually beginning at 210 μm and proceeding downward in 2- or 3-μm increments to 195 or 190 μm. (The lowest wave length at which the instrument responded with a reasonable precision was 187 or 188 μm.) The results obtained with the recording instrument in the 200 to 240 μm zone were checked with the more accurate manual unit.

Four Types of Rotatory Dispersion Curves in Far Ultraviolet Zone—Four general types of the far ultraviolet rotatory dispersion curves were found for the proteins having the Moffitt constant b₀ near zero: (a) curves resembling those of the disordered poly-α-γ-glutamate; (b) curves such as that exhibited by chymotrypsinogen; (c) curves with two flat minima; and (d) the γ-globulin curves having a flat trough at about 220 μm, a peak at 210 μm, and another trough at about 195 μm. The curves are shown in Fig. 2. Curve 1 shows the rotatory dispersion of aqueous phosvitin, which, as indicated before, is a flexible polyelectrolyte, like the disordered polyglutamate. The rotatory dispersion of phosvitin is similar to the rotatory dispersion of the disordered polyglutamate (Curve 4, Fig. 3). A few differences, however, are noteworthy: first, the deep minimum in the phosvitin curve was found at 208 μm, whereas polyglutamate has the minimum at 205 μm; second, the optical activity of the aqueous phosvitin was strongly affected by electrolytes, whereas polyglutamate was relatively insensitive to them. In the presence of 0.10 M NaCl, the amplitude of the negative Cotton effect of phosvitin decreased. Curve 2 (Fig. 2) represents chymotrypsinogen A. This curve differs strongly from the other three curves, as well as from the curves of the normal partially α-helical proteins, which have a minimum at 233 μm and a high positive maximum at 198 μm (1–7). Trypsin, pepsin, the luteinizing hormone (8), elastase (7), and several other proteins exhibit this type of curve. In the case of chymotrypsinogen, the optical activity was insensitive to a variation of salt concentration and to pH variation, tested between pH 3.0 and 7.5. Curve 3 (Fig. 2) shows the rotatory dispersion of oxidized serum albumin, which appears to be a largely disorganized protein (10). This curve has a flat trough at 230 to 233 μm and another flat minimum at 210 to 215 μm. Curve 4 in Fig. 2 exhibits the rotatory dispersion of human serum γ-globulin. This curve differs from all others in its positive maximum at 210 μm and its negative minimum at 198 to 200 μm. Of all the many proteins thus far tested, only the myeloma globulins and Bence-Jones proteins were similar to the γ-globulin. A specimen of normal serum γ-globulin was
Fig. 3. Observed and calculated curves for helical poly-$\alpha$-glutamic acid and disordered poly-$\alpha$-L-glutamate. Curve 1, the observed curve of 0.02% polyglutamic acid, pH 4.3. At shorter wavelengths the curve forms a high positive peak at 198 m$\mu$. Curve 4 represents data obtained with aqueous 0.02% sodium polyglutamate, pH 8.1. At shorter wavelengths the curve ascends into the positive part of the chart and forms a maximum at 191 m$\mu$. Curve 2 was calculated for a mixture composed of 1 part of $\alpha$-helical and 2 parts of disordered polyglutamate; and Curve 5 was computed for a mixture of 1 part of helical and 4 parts of disordered polymer.

cleaved by oxidative sulfitolysis and reconstituted to the $\gamma$S macromolecule by reduction (15) displayed slightly different rotatory behavior; i.e. the rotatory power of the reconstituted globulin at 195 to 205 m$\mu$ was near zero. Also, the $b_0$ value of the reconstituted globulin was slightly negative, whereas that of the native material was positive. This indicates that the conformation has changed somewhat on cleavage and reconstitution. The numerical values are included in Table I, and compared with the values of the other proteins and reference substances. The reference values are in a reasonable agreement with the data obtained by others (1-4).

The rotatory dispersion of trypsin and thyrotropin was similar to that of chymotrypsigen. The rotatory dispersion curve of trypsin treated with the chymotrypsogen inhibitor (8, 9) was practically the same as that of the untreated enzyme. Changes of pH within the limit of 3.0 to 5.2 had no pronounced effect on the rotatory dispersion of trypsin.

Optical Rotatory Dispersion of Systems Composed of $\alpha$-Helical and Disordered Macromolecules—The purpose of this part of the work was to test whether a large amount of disordered polypeptide could change the position of the negative minimum (at 233 m$\mu$) of a $\alpha$-helical polypeptide or protein. Since measurement on mixtures of the $\alpha$-helical polyglutamic acid and disordered polyglutamate cannot solve this problem, because the conformation of both components changes with the change of pH, the dispersion curves for such mixtures were calculated from the measured data of the components. In Fig. 3, Curve 2 was calculated for a mixture of 1 part of helical polyglutamic acid (pH 4.3) and 2 parts of disordered polyglutamate (pH 8.1), and Curve 5 is the calculated curve for a mixture in which the disordered form is in an excess of 4:1. Curve 1 (Fig. 3) represents the $\alpha$-helical polyglutamic acid, and Curve 4 shows the behavior of the disordered polyglutamate. As expected, an excess of the disordered form does not cause a displacement of the minimum at 233 m$\mu$, whereas the amplitude is diminished. In the 200 to 220 m$\mu$ zone, the excessive disordered form leads to the formation of another minimum at 208 m$\mu$. Table I lists the $b_0$ and Cotton effect values for various proteins.

### Table I

<table>
<thead>
<tr>
<th>Protein</th>
<th>$b_0$, m$\mu$</th>
<th>Minimum at</th>
<th>$\alpha'$ at $\lambda_{\text{max}}$</th>
<th>Maximum at</th>
<th>$\alpha'$ at $\lambda_{\text{max}}$</th>
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</thead>
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<tr>
<td>Poly-$\alpha$-L-glutamic acid, pH 4.3</td>
<td>-562$^*$</td>
<td>233</td>
<td>-16,200$^*$</td>
<td>198</td>
<td>+72,000$^*$</td>
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<td>Poly-$\alpha$-L-glutamate, aqueous, pH 8.1</td>
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<td>205</td>
<td>-17,400</td>
<td>191</td>
<td>+23,000</td>
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<td>Myoglobin, aqueous, pH 7.2</td>
<td>-292</td>
<td>233</td>
<td>-9,800</td>
<td>199</td>
<td>+42,000</td>
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<tr>
<td>Serum albumin, aqueous, pH 5.1</td>
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<td>233</td>
<td>-9,100</td>
<td>198</td>
<td>+37,000</td>
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<td>Phosvitin, aqueous, pH 7.1</td>
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<td>208</td>
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<td>191</td>
<td>+17,000</td>
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<td>Phosvitin, in 0.10 M NaCl, pH 6.8</td>
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<td>-4,900</td>
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<td>208</td>
<td>-10,600</td>
<td>190</td>
<td>+12,000</td>
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<td>Oxidized albumin, pH 10.6</td>
<td>+32</td>
<td>232</td>
<td>-2,800</td>
<td>210</td>
<td>-1,100</td>
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<td>221</td>
<td>-5,200</td>
<td>190</td>
<td>+14,000</td>
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<td>Chymotrypsogen, decyl sodium sulfate treatment</td>
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<td>233</td>
<td>-5,300</td>
<td>198</td>
<td>+10,500</td>
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<td>Trypsin, 0.001 N HCl, pH 3.0</td>
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<td>224</td>
<td>-3,100</td>
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<td>+3,500</td>
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<tr>
<td>Trypsin, decyl sodium sulfate treatment</td>
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<td>232</td>
<td>-4,800</td>
<td>201</td>
<td>+16,000</td>
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<tr>
<td>Thyrotropin, aqueous</td>
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<td>-2,900</td>
<td></td>
<td></td>
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<td>-4,800</td>
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<td>Bence-Jones protein, aqueous, pH 7.0</td>
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<td>+3,000</td>
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<td>-5,400</td>
<td>198</td>
<td>+7,700</td>
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<td>+54</td>
<td>221</td>
<td>-2,700</td>
<td>210</td>
<td>+3,700</td>
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</table>
minimum at about 210 μ. Such curves were observed with some proteins, e.g. with oxidized serum albumin (Curves 3, Fig. 2).

Since the conformation of the highly α-helical myoglobin or serum albumin is known to be insensitive to small changes of pH, tests on mixtures composed of these highly helical and disordered macromolecules were made also. The disordered polyglutamate was added to a solution of myoglobin; the pH was maintained within the limits of 6.5 and 8.0 to keep the disordered macromolecules in that state. It was found that addition of the disordered polyglutamate did not cause a shift of the trough from 233 μ to lower wave lengths. The amplitude of the Cotton effect, however, was strongly diminished. In the presence of a larger excess of the polyglutamate (0.012% myoglobin + 0.020% polyglutamate) a new trough was formed at 210 to 215 μ. The high positive peak of the myoglobin at 199 μ was affected by the disordered polyglutamate in the same way as the trough; i.e. the position was not altered but the amplitude was diminished. Essentially the same results were obtained by using serum albumin instead of myoglobin, or phosvitin instead of polyglutamate.

These observations indicate that the Cotton effect positions in the dispersion curves of chymotryptsinogen, trypsin, and thyrotrypsin are not caused by an extremely low α-helix content and disorder, but that they are determined by some other factors. Also, these results support the contention that the macromolecules of oxidized serum albumin are disordered to a large extent and have small portions of the chains in the α-helical conformation.

Effect of Decyl Sodium Sulfate on Conformation of Some Non-helical Proteins—On treatment of chymotryptsinogen with decyl sodium sulfate, the b₀ became more negative, indicating the formation of the α-helix (23). In Fig. 4, Curve 2 illustrates the effect of this detergent on the far ultraviolet rotatory dispersion of chymotryptsinogen, while Curve 1 represents untreated chymotryptsinogen under comparable conditions. This pair of curves shows quite clearly the difference between the "abnormal" (Curve 1) and normal curve (Curve 2). The latter has a minimum at 233 μ and a positive maximum at 198 μ, which are characteristic for a protein having part of its polypeptide chain in the α-helical conformation. A similar transition was observed with trypsin, but not with phosvitin. Treatment of the Bence-Jones protein or serum γ-globulin with the detergent (0.05% protein or serum γ-globulin with the detergent (0.02% in the very far ultraviolet region.

The helix content calculation for the proteins which have a peak in their curves at 198 μ then is simplified to dividing the [m']₁₉₈ by the corrected rotatory power of the reference substance, which usually is polyglutamic acid. Since the [m']₁₉₈ of the helical polyglutamic acid was found to be +72,000° and that of the disordered polyglutamate was found to be -3,000°, the percentage of helix content would be 100 [m']₁₉₈/[m']₁₉₈ = 73,000°. For example, the helix content for chymotryptsinogen treated with decyl sodium sulfate would be 10,500/[m']₁₉₈ = 0.14, or 14%. Extreme caution, however, should be exercised in interpreting these results, because (a) the experimental errors are large; (b) there are discrepancies in the [m']₁₉₈ value of the polyglutamic acid reference (7); and (c) the amplitude may depend on factors other than the polypeptide backbone conformation.

DISCUSSION

According to Kurnt, High, and Sieker (25), x-ray diffraction has shown that there are no straight α-helical rods in the macromolecules of chymotryptsinogen. Since chymotryptsinogen did not show the trough at 233 μ and the peak at 198 μ in its
rotary dispersion curves, the rotary dispersion data are in accord with the x-ray diffraction results. Trypsin and thyrotropin had curves similar to those of chymotrypsinogen and thus are probably nonhelical. Moreover, the evidence provided in this paper supports the contention that these nonhelical proteins cannot be considered as being disordered and having a low \( \alpha \) helix content. The compact gross conformation and ability to crystallize, especially in the cases of trypsin and chymotrypsinogen, are other features suggesting some order in the secondary and tertiary structure. Other examples of such nonhelical proteins have been described before by this author (6, 7) and by others (26). However, oxidized serum albumin can be considered as being largely disordered and having a low \( \alpha \) helix content. While phoebatin and histone appear to be completely disordered macromolecules, chymotrypsinogen, trypsin, and thyrotropin probably have some ordered structures other than the \( \alpha \) helix. Because of insufficient resolution, the regions of high electron density in the molecular model of chymotrypsinogen, as obtained from x-ray diffraction studies (26), may be interpreted in several ways. There could be some order differing from that of the \( \alpha \) helix or there could be incompletely developed or distorted helices. Unfortunately, no detailed conclusions about conformation can be made from the rotatory dispersion curves, because the recent work of others (26-30) has shown that the aromatic side groups of the polypeptide chain, as well as disulfide bonds (31) and other short range structures (32), affect the rotatory dispersion and circular dichroism in the ultraviolet spectral zone. This may, at least in part, also explain the discrepancies between values of the helix content obtained by various methods. The fact that the negative minimum in the chymotrypsinogen curve is not at 233 but at 221 m\( \mu \), however, cannot be explained as a result of the aromatic side chain interactions, because in polytyrosine the negative trough is displaced to the opposite direction, i.e. from 233 to 238 m\( \mu \) (30, 33). All these effects of the amino acid side chains indicate that conclusions about the polypeptide backbone conformation from rotatory dispersion should be made with great caution. This is particularly true for the 220 to 290 m\( \mu \) spectral zone.

It was shown that the far ultraviolet rotatory dispersion of serum \( \gamma \)-globulin and Bence-Jones protein differs profoundly from the rotatory dispersion of all other proteins. The conformation of Bence-Jones proteins has been studied recently by others (34, 35).

The calculated curves for the mixtures of helical and disordered polyglutamate (Fig. 3) showed that a curve of the chymotrypsinogen type does not result from a mixture in which the disordered form is in excess. One has to conclude that factors other than \( \alpha \) helix and disorder are involved even in the conformation of the ordinary proteins with a moderate \( \alpha \) helix content, because the computed curves for about 20 to 35\% helix differ from the real protein curves. The former have a plateau or flat minimum at 210 to 215 m\( \mu \), whereas the latter cross the zero line at 220 to 225 m\( \mu \) and have a shoulder in the positive part of the chart at 212 to 216 m\( \mu \) (1-7).

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