Optical Rotatory Dispersion of Nonhelical Proteins*

B. Jirgensons

From The University of Texas M.D. Anderson Hospital and Tumor Institute, Department of Biochemistry, Section of Protein Structure, Houston, Texas 77025

SUMMARY

The optical rotatory dispersion of a group of proteins having the Moffitt constant b0 near zero was studied in the far ultraviolet spectral zone. Sodium poly-α-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 μm is near zero.

Type II is exemplified by oxidized serum albumin. These curves have two minima, one at 230 to 233 μm and another at 210 to 215 μm. The major conformation of oxidized serum albumin seems to be a disordered chain, with a small amount of α 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 thyroglobulin. These proteins have curves with a minimum at 218 to 225 μm, and they are weakly dextrorotatory at 195 to 200 μm. It was concluded that these proteins are devoid of complete α-helical strands; instead, they are characterized by some other kind of ordered structures.

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

Addition of the disordered polyglutamate or phosvitin to the solutions of the highly α-helical myoglobin or serum albumin did not produce a displacement of the negative minimum at 233 μm, 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 α helix formation. The type III curve of chymotrypsinogen was thereby transformed into the ordinary curve of the partially α-helical proteins; i.e. it then possessed a trough at 233 μm and a peak at 198 μm. The detergent had a similar effect on the rotatory dispersion of trypsin, Bence-Jones protein, and γ-globulin, but not on the highly charged phosvitin.

Recent studies on the optical rotatory dispersion of proteins and polynamino acids have shown that there is a significant difference between the highly α-helical and disordered chain conformations (1-4). 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. compact macromolecules which seem to be devoid of the α helix. Pepsin, trypsin, chymotrypsinogen, the luteneizing hormone (6), serum γ-globulin (6, 7), elastase, soybean trypsin inhibitor (7), and several others belong to this group. The Moffitt constant b0 of these proteins is close to zero, and the negative minimum of the Cotton effect is not at 233 μm but at 215 to 225 μm (6, 7). Since the disordered poly-α-L-glutamate has a minimum at 205 μm (1), it seemed worthwhile to consider the possibility that the abnormal proteins are characterized simply by a very low α helix content, and that the rest of the molecular structure is disordered. If this is true, mixtures of highly α-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 α 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 μm, which are essential in the calculation of the α helix content.

EXPERIMENTAL PROCEDURE AND RESULTS

Proteins—Chymotrypsinogen A was a chromatographically homogeneous protein (crystallized five times) provided by...
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 eluted 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 contained only traces of extraneous material. Di-sulfide 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 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 metmyoglobin 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,-l-glutamate, which was also obtained from Mann. The specimen carried the designation M2428 and, according to Mann, its average molecular weight was 61,000.

The reduced specific viscosity, \( \eta_{sp} / c \), of the aqueous solutions of the polyglutamate at 28.0°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 all were used in aqueous solutions. Trypsin was dissolved in either water or 0.001 N HCl. Oxidized serum albumin was dissolved in diluted 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 mμ; 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°. The protein concentration was 0.02 to 0.2% at the wave length limits of 220 to 350 mμ, and it was 0.007 to 0.02% in the 190 to 220 mμ zone. The Moffitt constants b₀ were determined from measurements in the 250 to 350 mμ spectral region by using the value of 216 for the parameter λ₀ (7, 20, 21). The corrected specific rotation [m] values were computed from the relationship 

$$[m] = \frac{[\alpha]_3 \lambda}{100(n^2 + 2)}$$

where [α] is the specific rotation, and n 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 mμ, and in the range of ±1500° to ±2000° at the 190 to 200 mμ zone. By using the manual unit, 5 to 10 readings were made at each wave length, usually beginning at 210 mμ and proceeding downward in 2- or 3-mμ increments to 195 or 190 mμ. The lowest wave length at which the instrument responded with a reasonable precision was 185 or 188 mμ.) The results obtained with the recording instrument in the 200 to 240 mμ 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-L-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 mμ, a peak at 210 mμ, and another trough at about 198 mμ. The curves are shown in Fig. 2. Curve 1 (Fig. 2) 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 mμ, whereas polyglutamate has the minimum at 205 mμ; 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 mμ and a high positive maximum at 195 mμ (1-7). Trypsin, pepsin, the luteinizing hormone (6), elastase (7), and several other proteins exhibit this type of curve. The entire curve of the normal partially α-helical proteins is shown in Fig. 4. The ultraviolet rotatory dispersion of aqueous phosvitin (Curve 1), chymotrypsinogen (Curve 2), oxidized serum albumin (Curve 3), and human serum γ-globulin (Curve 4). All points on the curves represent average values from three to five series of measurements. The concentration of the proteins was 0.02 to 0.15% in the 220 to 240 mμ spectral zone, and it was 0.007 to 0.02% at 190 to 220 mμ. The solution layer thickness was 0.20 or 0.50 cm at 220 to 240 mμ, and 0.10 or 0.20 cm at 190 to 220 mμ. The pH of the various phosvitin preparations was 5.1 to 7.1. The pH of the chymotrypsinogen solutions was 3.0 to 5.2, the more acid solutions containing HCl. Oxidized serum albumin was dissolved in approximately 0.02 M NaOH, and the final pH of the solutions was 9.8 to 10.6. Serum γ-globulin was dissolved in 0.02 M sodium phosphate buffers, pH 7.0 to 9.6. The dashed segment of the curve in the positive part of the chart, with an arrow pointing to 198 mμ, indicates the position of the positive peak as observed with partially α-helical proteins. The continuous segment in the negative part of the chart, with the arrow pointing to 233 mμ, indicates the position of the negative trough in the curves of partially α-helical proteins.

[Fig. 2. The ultraviolet rotatory dispersion of aqueous phosvitin (Curve 1), chymotrypsinogen (Curve 2), oxidized serum albumin (Curve 3), and human serum γ-globulin (Curve 4).]
on mixtures of the α-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 3 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 α-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 μm, whereas the amplitude is diminished. In the 200 to 220 μm zone, the excessive disordered form leads to the formation of another

`TABLE I

Moffitt constants \(b_0\) and Cotton effect values for various proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>(b_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-α-L-glutamic acid,</td>
<td>-562</td>
</tr>
<tr>
<td>pH 4.3</td>
<td></td>
</tr>
<tr>
<td>Poly-α-L-glutamate,</td>
<td>-292</td>
</tr>
<tr>
<td>aqueous, pH 8.1</td>
<td></td>
</tr>
<tr>
<td>Myoglobin, aqueous,</td>
<td>-202</td>
</tr>
<tr>
<td>pH 7.2</td>
<td></td>
</tr>
<tr>
<td>Serum albumin, aqueous,</td>
<td>-286</td>
</tr>
<tr>
<td>pH 5.1</td>
<td></td>
</tr>
<tr>
<td>Phosvitin, aqueous, pH 7.1</td>
<td>-15</td>
</tr>
<tr>
<td>Phosvitin, in 0.10 M NaCl, pH 6.8</td>
<td>0</td>
</tr>
<tr>
<td>Histone H1, aqueous, pH 3.7</td>
<td>+13</td>
</tr>
<tr>
<td>Oxidized albumin, pH 10.6</td>
<td>+32</td>
</tr>
<tr>
<td>Chymotrypsinogen, aqueous, pH 5.2</td>
<td>+6</td>
</tr>
<tr>
<td>Chymotrypsinogen, decyl sodium sulfate treatment</td>
<td>-65</td>
</tr>
<tr>
<td>Trypsin, 0.001 N HCl, pH 3.0</td>
<td>-19</td>
</tr>
<tr>
<td>Trypsin, decyl sodium sulfate treatment</td>
<td>-57</td>
</tr>
<tr>
<td>Thyrotropin, aqueous γ-Globulin, normal, pH 8.0</td>
<td>+36</td>
</tr>
<tr>
<td>γ-Globulin, reconstituted, pH 8.6</td>
<td>-10</td>
</tr>
<tr>
<td>γ-Globulin, decyl sodium sulfate treatment</td>
<td>-42</td>
</tr>
<tr>
<td>Bence-Jones protein, aqueous, pH 7.0</td>
<td>+54</td>
</tr>
<tr>
<td>Bence-Jones protein, decyl sodium sulfate treatment, pH 7.1</td>
<td>-58</td>
</tr>
<tr>
<td>Myeloma globulin, pH 8.6</td>
<td>+54</td>
</tr>
</tbody>
</table>

---

The rotary dispersion of trypsin and thyrotropin was similar to that of chymotrypsinogen. The rotary dispersion curve of trypsin treated with the chymotrypsin 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 rotary dispersion of trypsin.

Optical Rotatory Dispersion of Systems Composed of α-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 μm) of a α-helical polypeptide or protein. Since measurement
minimum at about 210 m\(\mu\). Such curves were observed with some proteins, e.g. with oxidized serum albumin (Curve 3, Fig. 2).

Since the conformation of the highly \(\alpha\)-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 m\(\mu\) 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 m\(\mu\).

The high positive peak of the myoglobin at 199 m\(\mu\) 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 observed 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 chymotrypsinogen, trypsin, and thyroglobulin are not caused by an extremely low \(\alpha\) 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 \(\alpha\)-helical conformation.

**Effect of Decyl Sodium Sulfate on Conformation of Some Non-\(\alpha\)-helical Proteins**—On treatment of chymotrypsinogen with decyl sodium sulfate, the \(b_0\) became more negative, indicating the formation of the \(\alpha\) helix (23). In Fig. 4, Curve 2 illustrates the effect of this detergent on the far ultraviolet rotatory dispersion of chymotrypsinogen, while Curve 1 represents untreated chymotrypsinogen 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 m\(\mu\) and a positive maximum at 198 m\(\mu\), which are characteristic for a protein having part of its polypeptide chain in the \(\alpha\)-helical conformation. A similar transition was observed with trypsin, but not with phosvitin. Treatment of the Bence-Jones protein or serum \(\gamma\)-globulin with the detergent (0.05% protein or serum \(\gamma\)-globulin with the detergent) resulted in a transition similar to that observed with chymotrypsinogen and trypsin (Table I).

**Far Ultraviolet Rotatory Dispersion of Histone Fl**—Curve 3 in Fig. 4 shows the rotatory dispersion of the histone in aqueous solution. This curve is similar to the curves of the disordered polyglutamate (Curve 4, Fig. 3) and to the curve of phosvitin (Curve 1, Fig. 2). The conformation of the positively charged histone in aqueous solutions thus is similar to the conformation of the negatively charged phosvitin and polyglutamate (the latter at pH 7.0 to 8.1). More data on the optical rotatory dispersion of this and the other histone fractions can be found elsewhere (19, 24).

**Rotatory Power of Fully Disordered Proteins at Wave Lengths of 233 and 198 m\(\mu\)**—Another purpose of this study was to provide data for fully disordered proteins at the Cotton effect extrema, since these data are necessary as corrections for calculation of the \(\alpha\) helix content. The optical activities of various denatured proteins, when measured with the wave length of 233 m\(\mu\), have been found different for different cases (3, 7). However, a complete disorganization in these cases has not been established. The two examples mentioned in this report, e.g. aqueous phosvitin and histone Fl, behaved very much like the disordered polyglutamate and thus can be tentatively considered as fully disordered proteins. Comparison of their rotatory power at 233 m\(\mu\), however, showed a significant difference, i.e. a [m\(\chi\)]\(_{233}\) of -1,900° for phosvitin (Curve 1, Fig. 2) and a much more negative value of -3,700° for the histone (Curve 3, Fig. 4). This discrepancy is difficult to explain. However, it was gratifying to find that the rotatory power of both phosvitin and histone Fl, behaved very much like the disordered polyglutamate and thus can be tentatively considered as fully disordered proteins. Comparison of their rotatory power at 233 m\(\mu\), however, showed a significant difference, i.e. a [m\(\chi\)]\(_{233}\) of -1,900° for phosvitin (Curve 1, Fig. 2) and a much more negative value of -3,700° for the histone (Curve 3, Fig. 4). This discrepancy is difficult to explain. However, it was gratifying to find that the rotatory power of both phosvitin and histone Fl, behaved very much like the disordered polyglutamate and thus can be tentatively considered as fully disordered proteins.

**DISCUSSION**

According to Krauskopf, High, and Sieker (25), x-ray diffraction has shown that there are no straight \(\alpha\)-helical rods in the macromolecules of chymotrypsinogen. Since chymotrypsinogen did not show the trough at 233 m\(\mu\) and the peak at 198 m\(\mu\) 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 crystalize, 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 phoebvinn 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 rotary 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 rotary 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 mp, 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 mp (30, 33). All these effects of the amino acid side chains indicate that conclusions about the polypeptide backbone conformation from rotary dispersion should be made with great caution. This is particularly true for the 220 to 290 mp spectral zone.

It was shown that the far ultraviolet rotary 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 mp, whereas the latter cross the zero line at 220 to 225 mp and have a shoulder in the positive part of the chart at 212 to 216 mp (1-7).

REFERENCES

Optical Rotatory Dispersion of Nonhelical Proteins
B. Jirgensons


Access the most updated version of this article at [http://www.jbc.org/content/241/1/147](http://www.jbc.org/content/241/1/147)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/241/1/147.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/241/1/147.full.html#ref-list-1](http://www.jbc.org/content/241/1/147.full.html#ref-list-1)