The Ultraviolet Rotatory Dispersion and Conformation of Bence-Jones Proteins*

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SUMMARY

The optical rotatory dispersion of five individual specimens of Bence-Jones proteins was investigated, including the far ultraviolet spectral zone. The Moffitt constants (b₀) were determined, and either low positive or low negative values were found. The rotatory dispersion curves of all specimens had flat negative minima at 220 to 235 mȝ and positive maxima at 204 to 212 mȝ. Denaturation with acid indicated disorganization of the macromolecules, whereas heating at 50° with decyl sulfate induced a transition indicating the presence of the α-helical conformation in a part of the chains. The finding of Hamaguchi and Migita that the antigenic Type K (or I) proteins in 40% chloroethanol have more negative b₀ values than the Type L (or II) proteins was confirmed. The shallow negative minimum in the curves of the Type K proteins was found at 220 to 224 mȝ, whereas the minimum of the Type L proteins was at 228 to 235 mȝ.

The conformation of Bence-Jones proteins and the related immunoglobulins is unknown. Earlier work on the optical rotatory dispersion of these proteins indicated that they probably are nonhelical, because the Drude constants (λₒ) were found to be very low (1, 2). This has been confirmed recently by others, who found that the Moffitt constants (b₀) of Bence-Jones proteins are near zero (3, 4). However, some exceptions too were found (5). Although it is known that the Bence-Jones proteins exhibit individuality in several properties (6), the exceptional conformations might be only apparent, since both the Drude and Moffitt constants express the conformation less clearly than the Cotton effects in the far ultraviolet spectral zone (7–11). Recent studies showed that the far ultraviolet Cotton effects of a Bence-Jones protein of immunotype K (or I) differed profoundly from those of many other partially α-helical proteins and even from such nonhelical proteins as chymotrypsinogen (8), phosvitin (8), or carbonic anhydrase (12). The Cotton effects of this Bence-Jones protein, however, were similar to those of the serum γ-globulins and myeloma proteins (7, 8).

The purpose of the present communication is to present more data on the far ultraviolet Cotton effects of several other individual specimens of Bence-Jones proteins, chiefly of the immunotype L (or II). The optical rotatory dispersion of the native proteins was compared with the rotatory dispersion of the proteins which were denatured by acid or by heating with decyl sodium sulfate. It is hoped that this information will contribute to the understanding of the conformation of Bence-Jones proteins and immunoglobulins.

EXPERIMENTAL PROCEDURE

Proteins—Five individual samples of the Bence-Jones proteins were used. They were isolated from the urine of patients with the diagnosis of multiple myeloma. The 24-hour collected urine specimens were filtered, and the pH of the urine was adjusted to 4.8 to 5.2 by adding acetic acid. The protein was precipitated by slow addition of saturated ammonium sulfate with stirring, and it was observed that the precipitate began to form at approximately 55% saturation. The precipitate was removed, washed with saturated ammonium sulfate, and dissolved in water. This solution was then dialyzed until free of sulfate, and the aqueous solution of the protein was lyophilized. The dry samples were stored in a freezer. Further purification of the proteins was accomplished by gel filtration on Sephadex G-100 in long columns, 2 × 200 cm, in solutions of 0.05 M sodium phosphate buffer, pH 7.0, containing 0.10 M sodium chloride. The exclusion analysis of the 5-ml fractions of the effluent at 280 mȝ yielded a picture of the homogeneity of the materials. The major components of the effluent were collected, dialyzed, and lyophilized. The elution diagrams of three of the proteins are shown in Fig. 1. It appeared that Sample SH was relatively homogeneous with respect to size, and that Samples OL and PI had several components. The elution patterns of the other two individual samples, NE and CA, were similar to those of SH (13). Some important data characterizing these proteins are compiled in Table I. The chemical characterization of Samples CA and NE is described in another paper (18). The sedimentation diagrams of Samples CA, NE, and SH and the main component of OL indicated homogeneity, whereas the unfractonated protein of Sample PI exhibited two peaks. The sedimentation coefficients were determined on 0.28 to 0.31% aqueous solutions at 20.0°
According to Table I, all of the true Bence-Jones proteins have sedimentation coefficients between 3.4 and 4.0 S, whereas the first eluted component of Sample PI appeared to be a 7 S globulin. Only Component III of PI (Fig. 1) was used for the rotatory dispersion work. The absorption of the proteins PI-III and SH was measured with the aid of the Cary model 12 recording spectrophotometer in the far ultraviolet region below 240 nm in 0.01% aqueous solutions in 1-mm cells. Both samples yielded the high peptide backbone absorbance peak with a maximum at 192 to 194 nm, and a weaker maximum at 220 to 230 nm appearing in the form of a shoulder. These curves were very similar to those reported for serum γ-globulin by Gould, Gill, and Doty (14). The concentration of the aqueous solutions of the proteins was determined by absorbance measurements at 280 nm with the use of a value of 1.40 for 0.10% protein in a 1.0-cm optical path.

All chemicals were reagent grade. Decyl sodium sulfate was a gift from du Pont’s Organic Chemicals Division and, according to the specifications, was a pure product. 2-Chloroethanol was restilled before use.

**Optical Rotation Measurements** The measurements were made with two improved spectropolarimeters as described in previous papers (7, 8). Both instruments had the narrow angle, fused silica, double prism monochromators. Additional improvements were made in the electronics of the recording Rudolph model RSP-3 unit to increase the sensitivity of the detector and decrease the electrical noise. The absence of stray light was tested by working with various amounts of protein in the beam. The concentration of the proteins was 0.1 to 0.3% in the 250- to 350-nm range, and it was 0.01 to 0.02% at 190 to 240 nm. The optical path of the solutions was 0.10, 0.20, or 0.50 cm. The slit width was 0.2 to 0.5 mm at 250 to 350 nm, and it was 1.0 to 1.6 mm at the lower wave lengths down to 190 nm. The absorbance values at 190 to 200 nm of the solutions used for spectropolarimetry were between 0.8 and 1.8. In spite of all improvements, the reproducibility at 190 to 200 nm was not satisfactory (in terms of the specific rotation it was approximately ±3000°). In the region of the positive maximum at 207 to 212 nm, and at higher wave lengths, the reproducibility was better. All curves reported represent average values from between three and five series of measurements with both instruments.

The specific rotation [α] was calculated in the ordinary way, i.e. [α] = 100 α/εL, in which α is the rotatory power in angular degrees; ε, the optical path in decimeters; and c, concentration in grams per 100 ml. The Moffitt constants, bo, were computed from measurements in the 240- to 350-nm spectral zone by plotting [α'](λ2 − λ2) against λ4/(λ2 − λ2), and finding the bo value from the slope. Values of 212, 216, and 220 were used for the parameter λ0 (7, 8, 15). The values for the corrected specific rotation [α'], often denoted also by the symbol [m'], were found from [α'] = 8 M [α](100/n² + 2), in which M is the mean residual molecular weight of the amino acid residues and n is the refractive index of the solvent. A value of 115 was used for n.

**RESULTS**

In Fig. 2 are shown the Moffitt plots of Bence-Jones protein Sample SH. It is obvious that satisfactory straight lines were obtained in the 350- to 240-nm wave length zone with values of either 216 or 220 for the parameter λ0. However, the fit was less good for λ0 = 212. The bo values became less negative as the value for the parameter λ0 was raised (7, 15). The most negative value for bo of −46° was found for Sample SH by using a λ0 of 212. However, positive values for bo were found for Samples OL and PI-III. For NE, the bo was zero when λ0 was taken as 216; it was +13 when λ0 was taken as 220, and −15 with a λ0 of 212. These data are compiled in Table II.

The far ultraviolet rotatory dispersion of Samples PI-III and SH is shown in Figs. 3 and 4, respectively. The curves of the other specimens, CA, NE, and OL, were similar to those of PI-III and SH. In Fig. 3, the dashed curve shows the rotatory dispersion of serum albumin, which is typical of proteins that have a high content of α helix. In Fig 4, the dashed curve represents phosvitin, which is a completely disordered protein (8). Clearly, the rotatory dispersion of the Bence-Jones proteins differs profoundly from that of the helical and the disordered proteins. The Bence-Jones protein curves have a very shallow trough at 220 to 235 nm and a positive maximum at 210 nm, as do the serum γ-globulins (7, 8). The positive peak of the Bence-Jones proteins was somewhat broader than the maximum of the γ-globulins (immunoglobulins), and a shoulder appeared in the broad peak of the Bence-Jones proteins at 203 to 205 nm. Although this shoulder was poorly reproducible, the existence of the positive maximum itself is unquestionable. This was confirmed not only with both our advanced instruments but also with the
Fig. 2. The Moffitt plots of Bence-Jones protein Sample SH with assumed values of 212, 216, and 220, respectively, for the parameter \( \lambda_0 \). The values for the corrected specific rotatory power \([R']\) were computed from measurements in the 240- to 350-\(\mu\) spectral zone on a 0.287% aqueous solution of the protein in a cell with an optical path of 0.50 cm.

**TABLE II**

Moffitt constants and Cotton effect data of native Bence-Jones proteins

<table>
<thead>
<tr>
<th>Sample</th>
<th>( b_0 )</th>
<th>( \lambda_0 )</th>
<th>Minimum ( [R']_{\text{min}} )</th>
<th>Maximum ( [R']_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>-44</td>
<td>212</td>
<td>228 ( -1500^\circ )</td>
<td>210 ( +3700^\circ )</td>
</tr>
<tr>
<td></td>
<td>-21</td>
<td>216</td>
<td>229 ( -2200^\circ )</td>
<td>210 ( +5000^\circ )</td>
</tr>
<tr>
<td></td>
<td>-8</td>
<td>220</td>
<td>229 ( -2200^\circ )</td>
<td>210 ( +5000^\circ )</td>
</tr>
<tr>
<td>NE</td>
<td>-15</td>
<td>212</td>
<td>229 ( -1600^\circ )</td>
<td>210 ( +4500^\circ )</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>216</td>
<td>229 ( -1600^\circ )</td>
<td>210 ( +4500^\circ )</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>220</td>
<td>229 ( -1600^\circ )</td>
<td>210 ( +4500^\circ )</td>
</tr>
<tr>
<td>OL</td>
<td>+19</td>
<td>216</td>
<td>230 ( -1700^\circ )</td>
<td>209 ( +3800^\circ )</td>
</tr>
<tr>
<td>SH</td>
<td>-46</td>
<td>212</td>
<td>230 ( -1700^\circ )</td>
<td>209 ( +3800^\circ )</td>
</tr>
<tr>
<td></td>
<td>-25</td>
<td>216</td>
<td>230 ( -1700^\circ )</td>
<td>209 ( +3800^\circ )</td>
</tr>
<tr>
<td></td>
<td>-10</td>
<td>220</td>
<td>230 ( -1700^\circ )</td>
<td>209 ( +3800^\circ )</td>
</tr>
<tr>
<td>PI-III</td>
<td>+11</td>
<td>212</td>
<td>224 ( -1400^\circ )</td>
<td>209 ( +4700^\circ )</td>
</tr>
<tr>
<td></td>
<td>+14</td>
<td>216</td>
<td>224 ( -1400^\circ )</td>
<td>209 ( +4700^\circ )</td>
</tr>
<tr>
<td></td>
<td>+17</td>
<td>220</td>
<td>224 ( -1400^\circ )</td>
<td>209 ( +4700^\circ )</td>
</tr>
</tbody>
</table>

Jasco model ORD/UV-5 spectropolarimeter. In these experiments, 0.019% aqueous solutions of Samples SH and CA were used in a 1-mm cell. The minimum at 195 to 200 \(\mu\) was difficult to investigate, because of high absorbance. The Cotton effect data, i.e. the positions and amplitudes of the peaks, are compiled in Table II.

Denaturation of the Bence-Jones proteins was studied with HCl and decyl sodium sulfate as denaturing agents. After addition of the acid, the protein was left at room temperature at least 24 hours. The final pH of these solutions was between 1.8 and 1.9. Denaturation with decyl sulfate was accomplished by heating 0.10 to 0.12% protein with 0.05 M decyl sodium sulfate at 50°.

These measurements were made at a Conference on Optical Rotatory Dispersion and Circular Dichroism, Instrument Workshop, Bonn, Germany, September 25-October 1, 1965, by one of us (D. L. R.).

Fig. 3. The rotatory dispersion of the immunotype K (or I) Bence-Jones protein PI-III in the far ultraviolet region. On the abscissa are plotted the wave lengths; on the ordinate, the corrected specific rotatory power. Measurements were made with 0.01 to 0.028% aqueous solutions, pH 6.1, in cells with optical path lengths of 0.10 or 0.20 cm. (At the longer wave length zone above 220 \(\mu\), the measurements were made in a thicker layer of 0.50 cm.) The dashed curve represents serum albumin; the vertical bars show variations of the rotatory power observed on the same solution with different instruments.
for 2 hours. The rotatory power was measured after the mix-
tures had been completely cooled down to room temperature of
23-25°. The pH of these solutions was 7.1 to 7.7. The results
with Sample PI-III are shown in Fig. 5. The acid-denatured
Bence-Jones protein, according to these findings, has a quite
different conformation from that denatured by heating with decyl
sulfate. Similar observations were made on Specimens CA and
SH upon treatment with detergent or denaturation with acid.

The Moffitt constants ($b_o$) of the proteins treated with decyl
sulfate at 50° or with acid also were determined. It was found
that in this respect, too, the resulting conformational transitions
were different; i.e. upon the treatment with the detergent, the
$b_o$ value became more negative, whereas upon acid treatment it
changed very little. For example, by using the value of 216
for the parameter $\lambda_o$, the $b_o$ of Sample CA changed from $-16$
to $-80$ in the treatment with detergent, whereas in the acid
treatment it changed from $-16$ to $-22$, which is within the
limits of experimental error.

The effect of 2-chloroethanol on the Bence-Jones proteins
also was investigated. This was of particular interest, since
Hamaguchi and Migita have reported (3) that the $b_o$
values of the Type L immunoproteins in 40% (by volume) chloroethanol
 differed strongly from the $b_o$ of the Type K proteins in the same
 solvent. This was tested with our Samples CA, NE, and
SH, which are of Type L, and compared with PI-III, which is of
Type K. In agreement with the Japanese workers, we found

that Type K proteins indeed have more negative $b_o$ values than
Type L samples. The $b_o$ of the Type K sample, PI-III, in 40%
chloroethanol was $-132$, whereas the $b_o$ values of CA, NE, and
SH were $-42$, $-74$, and $-68$, respectively ($\lambda_o = 216$). We
also found that the trough in the dispersion curve at 230 to 233
$\mu$m was deepened in all cases when 40% chloroethanol was used
instead of water. However, it was impossible to make an ade-
quate investigation of the 190 to 220 $\mu$m spectral zone, because
of excessive absorption of light in this solvent.

**DISCUSSION**

Up to now, the Drude constants ($\lambda_o$) and the Moffitt constants
($b_o$) have been used as criteria for the conformation of Bence-
Jones proteins (1-4, 17, 18). Two important conclusions were
drawn from these studies: (a) that the polypeptide chains in
these proteins do not have a significant amount of the $\alpha$-helical
conformation, and (b) that the unknown conformation is similar
to that of the immunoglobulins. However, very large variations
in the conformation between individual samples of these proteins
have been noted by van Eijk, Monfoort, and Westenbrink (5).
These authors concluded that some individual specimens of the
Bence-Jones proteins possibly possess a significant amount of
$\alpha$ helix. The present study, if confined to the Moffitt constants
as criteria for conformation, confirms this individuality to some
extent. Negative \( b_0 \) values were found for two of the specimens, as shown in Table II.

In the case of the ordinary proteins, which can be considered as having a relatively high \( \alpha \) helix content and also regions of disordered chains, the Moffitt constant \( b_0 \) is determined largely by the juxtaposition of two Cotton effects of opposite sign (9). In the instance of Bence-Jones protein these Cotton effects are not observed. The negative trough at 220 to 240 \( \mu m \) is very shallow, and the positive maximum at 198 to 200 \( \mu m \) is absent. Instead, a maximum was observed at 204 to 212 \( \mu m \). This maximum can be considered as an expression of the cross-\( \beta \) conformation (7, 19, 20). Furthermore, there are indications that other Cotton effects are present in the farther ultraviolet region. Obviously, the parameter \( b_0 \) in these instances, too, is a composite factor and cannot disclose the conformation in any detail. Moreover, it is known that the values found for \( b_0 \) depend not only on the value of the parameter \( \lambda_\alpha \) but also on experimental conditions, such as the choice of wave length range (7, 22). For these reasons, any exceptional \( b_0 \) values, if used as criteria for conformation, should be supported by other evidence, such as data on Cotton effects in optical rotation or circular dichroism measurements.

Troitskii (23) has tried recently to apply an extended Moffitt equation developed by Wada, Tsuboi, and Konishi (24) to various proteins, chiefly immunoglobulins. This approach should make it possible to disclose the presence of orders other than \( \alpha \) helix, and it is assumed that this other conformation might be the \( \beta \) form. According to the calculations of Troitskii (23), the immunoglobulins and Bence-Jones proteins have about 10 to 20\% of \( \alpha \) helix and 20 to 44\% of the cross-\( \beta \) (or some similar) conformation. The shifting of \( b_0 \) to more negative values on treatment with detergents, according to Troitskii, can be explained as destruction of the \( \beta \) structure. These interesting conclusions deserve further investigation. The presently available data on the far ultraviolet Cotton effects of the Bence-Jones proteins and immunoglobulins (7, 8) seem not to be reconcilable even with the lower value of 10\% \( \alpha \) helix. (Similar calculations of Callaghan and Martin for the immunoglobulins (25), in which 30\% \( \alpha \) helix was estimated, are obviously in error, as pointed out by Troitskii (23).) Examination of polymer films has shown that the \( \beta \) conformation can be correlated with the appearance of a positive maximum in the rotatory dispersion curves at 207 \( \mu m \) (20). The Bence-Jones protein curves have similar maxima.

Simmons and Blout (26) in 1960 suggested using the amplitude of the negative Cotton effect at 233 \( \mu m \) as a measure of \( \alpha \) helix content. Although there seems to be some correlation between these parameters, recent studies have thrown doubt on the unequivocal validity of this interpretation, especially if the troughs are shallow. Troughs in the rotatory dispersion curves in the 230- to 250-\( \mu m \) zone appear to depend on several "short range" structures, e.g. the disulfide linkages (29). For this reason, nothing much can be concluded from the finding that the amplitudes of the negative troughs of the Bence-Jones proteins are between \( -1400^\circ \) and \( -2200^\circ \) (Table I). However, it may be mentioned that other authors have reported similar values for immunoglobulins and similar proteins (30-32). We believe that one is on a somewhat firmer ground in the 190- to 220-\( \mu m \) spectral zone, although current interpretations of data in this zone have also been criticized recently (33). In spite of these objections, it seems that the positive maxima observed for Bence-Jones proteins and immunoglobulins at 204 to 212 \( \mu m \) are indicative of some order other than \( \alpha \) helix, notably the \( \beta \) structure. This is supported by the fact that denaturation of the Bence-Jones proteins with acid resulted in disappearance of this maximum (Fig. 5). The rotatory dispersion curves of the acid-denatured Bence-Jones proteins resemble those of disorganized proteins, such as phosvitin.

Differentiation of the immunotype K from L proteins by means of the Cotton effects is difficult. A slight difference was found in the position of the negative trough. All four Type L proteins have it at 228 to 235 \( \mu m \), whereas the Type K sample has it at 224 \( \mu m \). The position of the minimum of another Bence-Jones protein of Type K, which was studied earlier, was found even at 220 \( \mu m \) (8). Finding differences in the curves below 200 \( \mu m \) is hampered by prevailing experimental difficulties. With our samples we confirmed the observation of Hamaguchi and Migita (3) that the differentiation is possible by using solutions in 40\% chloroform. Since small but significant differences have been established between the amino acid composition of the Type K and L Bence-Jones proteins (13, 34, 35), a slight difference in conformation is not surprising.

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