The Far Ultraviolet Optical Rotatory Dispersion, Circular Dichroism, and Absorption Spectra of a Myeloma Immunoglobulin, Immunoglobulin G*

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D. L. Ross† and B. Jirgensons
From the Section of Protein Structure, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025

SUMMARY

The optical rotatory properties of a myeloma globulin, immunoglobulin G (IgG), and a normal individual IgG in the far ultraviolet were investigated by rotatory dispersion, circular dichroism, and absorption spectroscopy. The optical rotatory dispersion of the myeloma immunoglobulin displayed two peaks, one at 205 mµ and the other at 210 mµ, and a trough at 228 mµ, a lesser one at 230 mµ, and a deeper one at 198 mµ. The normal individual γ-globulin displayed a peak at 208 mµ and a trough at 228 to 230 mµ. The rotatory dispersion in acid media was suggestive of the formation of right handed α helices in both immunoglobulins.

Circular dichroic analysis of the myeloma IgG showed seven dichroic bands, at 202 mµ (+), 217 mµ (−), 225 mµ (−), 242 mµ (−), 265 mµ (+), 284 mµ (+), and the beginning of a negative band centered below 195 mµ (−192.5 mµ). Application of the Kronig-Kramers transform gave an optical rotatory dispersion curve compatible with the one obtained experimentally.

The peptide bond extinction, calculated from far ultraviolet absorption spectra, displayed a hypochromicity for both immunoglobulins.

The far ultraviolet optical rotatory dispersion analyses of the myeloma IgG cleaved by oxidative sulfitolysis showed the disulfide bonds joining the heavy and light chains to be essential for the maintenance of certain electronic transitions present in the native protein. The major transitions, however, appeared to be unaffected by this treatment. Comparison of the Cotton effects of the sulfitolyzed product in acid with those of the whole molecule in acid (pH 2.4) showed that these S−S bonds are important in restricting conformation change in the presence of this denaturing agent.

The conformation of immunoglobulin, IgG, is unknown. Optical rotatory dispersion analyses by Moffitt plot treatment have given β values near zero, which have been interpreted to indicate little or no α-helical structure in IgG (2–6). Helix-forming solvent, i.e. 2-chloroethanol (3–5, 7), and treatment with sodium dodecyl sulfate (8, 9), however, seem to induce right handed α-helical structures. Several investigators (7, 10) have concluded that some α helix and some β structure are present in native γ-globulin. Cotton effects of normal human IgG in the far ultraviolet have shown a maximum at 210 mµ with [m′] of +5300°, a minimum at 221 to 225 mµ with [m′] of approximately −7000° (9, 11, 12). The position of this maximum or peak of the Cotton effect was close to that found for films of β-poly-l-isoleucine (13), and this also led to the conclusion that some β structure was present.

The optical rotatory dispersion and circular dichroic characteristics of solutions of poly-γ-lysine (14–16), and silk fibroin (17) in the β antiparallel pleated sheet conformation have been reported. These β structures show a Cotton effect with a peak at 205 mµ and a trough at 229–230 mµ. No β structure has been found in IgG by infrared analysis (3, 4), with the exception of films of human γ-globulin-horse antidimensional precipitation (18).

Analysis of the circular dichroism of rabbit IgG, which extends from 250 to 205 mµ, by Sarkar and Doty (14) shows only one shallow, negative band which centers near 217 mµ. The molar ellipticity taken from this plot is approximately −3000° cm² dmol⁻¹. The abbreviations used are: IgG, symbol for immunoglobulin recommended by a conference on human immunoglobulins sponsored by the World Health Organization (1), for the γ-globulin commonly designated 7Sγ, γs, and γα; CD, circular dichroism; ORD, optical rotatory dispersion.
per decimole at 217 mp. Also, K. Nakamura has found a negative CD band at 218 mp and a positive band near 200 mp. The positions of these circular dichroic bands correspond to those found for poly-L-lysine and silk fibroin in the β conformation.

Gould, Gill, and Doty (6) found that the peptide bonds of rabbit γ-globulin displayed a marked hypochromicity in the 190 to 205 mp spectral range. According to the work of others (19, 20) this could be attributed to the peptide bonds’ being in the α-helical conformation. However, this effect for rabbit IgG was attributed to the intramolecular environment of the chromophoric side groups or to the existence of some other periodic structure which provides a repeating arrangement of the peptide bonds. The hypochromicity of the peptide bonds of rabbit IgG does not support the speculation that ψ structure is present in IgG, since Rosenheck and Doty (20) obtained a hyperchromic effect for poly-n-lysine in the p conformation.

The purpose of this report is to present more data on the far ultraviolet optical properties of human immunoglobulins G and to attempt to clarify some of the contradictions. Myeloma IgG was chosen for this study since it represents a more homogeneous population of molecules than IgG found in normal serum. Some studies of IgG obtained from a normal individual donor will also be included for comparison with the myeloma IgG.

EXPERIMENTAL PROCEDURE

Myeloma IgG was extracted by a DEAE-Sephadex batch method (21) from the plasma of a patient diagnosed as having malignant plasmacytoma. This preparation was purified by gel filtration with Sephadex G-200; the IgG obtained gave a typical myeloma IgG immunoelectrophoretic pattern and no serological evidence of other serum proteins. The IgG obtained from a blood donor at this hospital is called normal IgG here. The γ-globulin was removed from the plasma by precipitation with 18% sodium sulfate and purified by chromatography on DEAE-cellulose (22). This preparation was immunoelectrophoretically free of other serum proteins and gave a typical IgG precipitin line.

Oxidative sulfitolysis of the myeloma IgG was done by the method described by Yonesawa et al. (23). Amino acid analyses were done on duplicate hydrolysates of both immunoglobulins with a Beckman/Spinco amino acid analyzer (24). Glicosamine and galactosamine were detected by a modified procedure (25); tryptophan was determined spectrophotometrically (26). The far ultraviolet absorption spectra of the immunoglobulins were determined in matched cylindrical cells with high transmittance in the ultraviolet and 1-mm and 0.5-mm optical path lengths. These spectra were recorded on the Cary 15 spectrophotometer with nitrogen flushing. The slit widths were no greater than 0.08 mm at 205 mp, 0.1 mm at 190 mp, and 0.8 mm at 185 mp. The absence of stray light was checked (20), and “degassing” of the solutions made no difference in the results. The side chain contribution to the mean residue extinction was calculated by the use of the data of Rosenheck and Doty (20), the amino acid content given in Table II, below, the concentration of protein in solution, and the amide content calculated according to the method of Rees (27). All of the half-cystine residues were considered to be in cystine, since less than 0.2 mole of free —SH per mole of this myeloma IgG was found by amperometric titration.

The optical rotatory dispersion measurements reported here were made on the Rudolph RSP-3/4 improved (9, 12, 28) spectropolarimeter. Initial studies of the myeloma IgG on the Rudolph MSP-5/6 spectropolarimeter were identical with those obtained on the recording instrument. The absence of optical artifacts was tested by the use of different concentrations of protein and cells of various light paths. Silica cells (Pyrex cells) were used; the concentrations of protein were generally 0.2 in 0.5 g/100 ml in 5-mm to 2-mm cells in the wave lengths from 400 to 235 mp, 0.1% to 0.04% in 1-mm to 2-mm cells from 250 to 215 mp, 0.04% to 0.02% in 1-mm to 0.5-mm cells from 220 to 208 mp, and 0.02% to 0.01% in 1-mm to 0.5-mm cells from 210 to 190 mp. A 5° symmetrical angle oscillation was used, and the slit widths were from 0.5 to 1.8 mm from 250 to 190 mp. Nitrogen was flushed through the entire optical path to avoid absorption by oxygen at wave lengths below 200 mp, and degassing of solutions made no difference in the optical rotatory dispersion in the far ultraviolet region reported here. All measurements were made at room temperature, 20° ± 2°.

The rotations given represent average values obtained from at least 3 to 6 determinations, and in some cases as many as 15 recordings (201 to 212 mp region). The variation in [α] between the high and low values was approximately 10° to 50° from 400 to 250 mp, ±500° at 230 mp, ±1300° at 210 mp, ±1500° at 205 mp, and ±2000° at 200 mp.

The corrected mean residue rotation, [m°], was calculated with the use of a mean residue weight (MRW) of 109 for both immunoglobulins and the refractive indices of water (29).

The circular dichroism of this myeloma IgG was measured by Dr. Legrand, Jouan Company of Paris, using the Jouan Dichrograph model CD-185. The mean residue ellipticity was obtained by \[ [\theta]_{\text{MRW}} = \Delta E_{10}^{15} \times (\text{MRW}/10) \times 3300 \text{ c.s.} \text{ units} \] where \( \Delta E_{10}^{15} \text{c.s.} \) is the measured difference in the absorbance of the right and left circularly polarized light through a 1-mm light path given by a 1% solution of the myeloma IgG. The factor 3300 is the constant embodying the conversion factors of units and the relationship of absorption to ellipticity, according to Moscowitz (30). The specific viscosity, \( \eta_p \), was calculated by \( \eta_p = R_e \times \frac{0.969 \times 10^{-4} \sqrt{\pi} [\theta]_{\text{MRW}}^1 \Delta \lambda^2 / \lambda^4} {([\theta]_{\text{MRW}}^1)^2} \text{ (c.g.s. units)} \) assuming gaussian curves, and \( \Delta \lambda^2 \) is the wave length of the optically active electronic transition characterized by the circular dichroic band.

RESULTS

Sedimentation Velocity—The sedimentation coefficients were determined in a Beckman/Spinco model E ultracentrifuge at 20.0° and 56,100 rpm. The sedimentation coefficients are given in Table I.

Viscosity—The viscosities of the myeloma and normal IgGs were determined in Cannon-Ubbelholde dilution viscometers at 28.0°. The specific viscosity, \( \eta_p \), was calculated by \( \eta_p = (t_4/t_0) - 1 \), where \( t_4 \) is the flow time, in seconds, of the sample and \( t_0 \) is the flow time of pure solvent.

2 Unpublished data, cited in Reference 17.
$t_0$ is the flow time, in seconds, of the solvent. The data were plotted as $\eta_{sp}/c$ against $c$, where $c$ is the concentration in grams per 100 ml. The intrinsic viscosity, $[\eta]$, in decimals per $g$, was obtained from the intercept of the plot, and is given for each immunoglobulin in Table I.

**Molecular Weight**—The molecular weights of the myeloma and normal immunoglobulins were calculated from the intrinsic viscosities and sedimentation coefficients with the use of the Svedberg-Mandelkern equation (35).

$$
\beta = \frac{(N\eta_0 + \eta_p)}{M^2N(1 - v_p)}
$$

where $N$ is Avogadro’s number, $\eta_0$ is the viscosity of the solvent, $\eta_p$ is the partial specific volume of the protein, and $\rho_p$ is the density of the solvent. $\beta$ is related to the axial ratio of the molecule. The partial specific volume of the myeloma IgG was 0.74 cc per g. The same value was assumed for the normal IgG. The values for $\eta_0$ and $\rho_p$ were taken from Svedberg and Pedersen (36).

Three values for the $\beta$ factor were considered. The axial ratio calculated for $\gamma$-globulin from Kratky's data (31), obtained from low angle x-ray scattering, is 0.8 which, assuming a typical prolate ellipsoid, gives a $\beta$ value of 2.31 x 10$^6$ in Table X of Yang (37). A $\beta$ factor of 2.18 x 10$^6$ can be calculated from the molecular weight determined from sedimentation equilibrium data by Noelken et al. (32) for rabbit $\gamma$-globulin. This value allows the same molecular weight to be obtained from their viscosity and sedimentation velocity data on the same sample of rabbit $\gamma$-globulin. The $\beta$ value often assumed (33, 34) for globular proteins is 2.15 to 2.16 x 10$^6$. The molecular weights chosen for all calculations (32, 38, 39). The molecular weights obtained from sedimentation equilibrium data were extrapolated to zero time (40).

All of the molecular weights thus obtained are higher than those currently appearing in the literature for immunoglobulin (32, 38, 39). The molecular weights chosen for all calculations are 178,000 for the myeloma IgG and 173,000 for the normal IgG. This choice, with the $\beta$ value of 2.18 x 10$^6$, was made because the data used to derive this value of $\beta$ were obtained by experimental methods similar to those used here and also because no assumption regarding the shape of the molecule was involved in obtaining this value.

**Amino Acid Analyses**—The amino acid contents of the immunoglobulins are shown in Table II. The total number of residues per molecule of protein was 1615 for the myeloma IgG and 1573 for the normal IgG. This choice, with the $\beta$ value of 2.18 x 10$^6$, was made because the data used to derive this value of $\beta$ were obtained by experimental methods similar to those used here and also because no assumption regarding the shape of the molecule was involved in obtaining this value.

**Far Ultraviolet Absorption Spectra**—These spectra of the myeloma and normal immunoglobulins in terms of the mean residue extinctions are shown in Fig. 1. The mean residue extinction of the myeloma IgG is decreased in HCl solution at pH 1.5 and also in water when compared with that found in 0.1 M NaF. The mean molar residue extinction coefficients for both immunoglobulins in 0.1 M NaF at 190, 197, and 285 m $\mu$ are listed in Table III, along with the side chain contributions and peptide bond contributions. The values obtained for the peptide bond extinction indicate a hypochromicity which agrees with the data of Gould, Gill, and Doty for rabbit IgG (6).

**Optical Rotatory Dispersion and Circular Dichroism of Native Immunoglobulins**—The dispersion curves are shown in Fig. 2. The two Cotton effects peaks in the dispersion of the myeloma
The mean residue extinctions of normal IgG and myeloma IgG in the far ultraviolet. Curves for both proteins in 0.1 M NaF are shown, as well as those for the myeloma IgG in water at pH 6.5 and in HCl at pH 1.5. The absorptions were determined on 0.007% to 0.04% solutions of the myeloma IgG and on 0.007% and 0.013% solutions of the normal IgG in cells with optical paths of 0.5 mm and 1.0 mm.

**TABLE III**

<table>
<thead>
<tr>
<th>Wave length (μm)</th>
<th>IgG</th>
<th>Mean residue extinction</th>
<th>Side chain contribution</th>
<th>Peptide bond contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>190</td>
<td>Myeloma</td>
<td>9046</td>
<td>3213</td>
<td>5833</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>9085</td>
<td>3475</td>
<td>5010</td>
</tr>
<tr>
<td>197</td>
<td>Myeloma</td>
<td>7904</td>
<td>2415</td>
<td>5489</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>7731</td>
<td>2454</td>
<td>5277</td>
</tr>
<tr>
<td>205</td>
<td>Myeloma</td>
<td>4091</td>
<td>1164</td>
<td>2930</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>4094</td>
<td>1164</td>
<td>2930</td>
</tr>
</tbody>
</table>

The molar extinction coefficients of the peptide bond chromophore determined by Rosenheck and Doty (20) for poly-L-lysine in the α-helical conformation and random coil conformation were, respectively, 4100 and 6900 at 190 μm, 3200 and 6350 at 197 μm, and 2000 and 3200 at 205 μm. Those of the β form were approximately 5800 at 190 μm, 6950 at 197 μm, and 4600 at 205 μm.

*The values were obtained on two different spectropolarimeters and were also found upon measuring the optical rotatory dispersion of two other myeloma IgGs (one prepared in the same manner as the myeloma IgG reported here and the other prepared in the same manner as the normal IgG reported here). Bence-Jones proteins have been reported (44) as having a small anomaly at about 205 μm and a distinct Cotton effect peak at 210 μm. The Cotton effect characteristics are given in Table IV.

The circular dichroism (Fig. 3 and Table V) of the myeloma IgG from 300 to 195 μm showed five distinct bands. The negative band at 217 μm was unsymmetrical, and it has been assumed that another negative band occurs at about 225 μm which is not resolved. ORD plots were calculated by application of the Kronig-Kramers transform (30) to the circular dichroic data. Several calculations were made after arbitrarily placing the CD band which occurs somewhere below 195 μm at 195, 192.5, 190, and 188 μm; the calculations were also made excluding and including the poorly resolved band at 225 μm. All plots confirmed the two positive peaks (210 μm and 205 μm); how-
ever, none of the calculated \([\text{m}']\) values at these wavelengths was as high as had been obtained by spectropolarimetry. The amplitudes at 210 \(\text{m} \mu\) and 205 \(\text{m} \mu\) depend largely upon the \([\theta]\) and half-band width of the negative band below 195 \(\text{m} \mu\). Thus the discrepancies in amplitude are considered to be due to experimental error (±1500° to ±2000°) in ORD at these wavelengths and to the assumptions made concerning the position of the band below 195 \(\text{m} \mu\). An ORD curve in terms of \([\text{m}']\) which was obtained from the CD data is shown in Fig. 4.

The ORD curve of the normal \(\gamma\)-globulin is similar to that obtained by Jirgensons (9, 11) for normal pooled IgG with the exception that here the peak is found at 208 \(\text{m} \mu\) with an amplitude approximately 3000° less, and the minimum for this sample is at 225 \(\text{m} \mu\) instead of 221 \(\text{m} \mu\) and is also of less magnitude. The differences in the rotation values found here from those reported before are explained in part by use of a smaller value for the mean residue weight (109) than was used previously (113.5). The parameters of the Cotton effects of the native immunoglobulins are given in Table IV.

At a position (198 \(\text{m} \mu\)) where a peak is found in the dispersion curves of proteins in the right-handed \(\alpha\)-helical conformation, a trough is found in the case of the myeloma IgG. A trough at this position has also been found for normal IgG (9, 11).

### Table IV

_Cotton effect characteristics of myeloma IgG and normal IgG_

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Myeloma IgG</th>
<th>Normal IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\lambda)</td>
<td>([\text{m}'])</td>
</tr>
<tr>
<td>Native, pH 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximia (peaks)</td>
<td>210</td>
<td>+3,800</td>
</tr>
<tr>
<td>Minima (troughs)</td>
<td>205</td>
<td>+4,000</td>
</tr>
<tr>
<td></td>
<td>230</td>
<td>-1,500</td>
</tr>
<tr>
<td></td>
<td>228</td>
<td>-2,100</td>
</tr>
<tr>
<td>Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 1.5 or 1.75*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximia</td>
<td>199</td>
<td>+7,500</td>
</tr>
<tr>
<td>Minima</td>
<td>220</td>
<td>-1,800</td>
</tr>
<tr>
<td>pH 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximia</td>
<td>205</td>
<td>+3,300</td>
</tr>
<tr>
<td>Minima</td>
<td>226</td>
<td>-2,400</td>
</tr>
<tr>
<td>After oxidative sulfitolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximia</td>
<td>209</td>
<td>+3,400</td>
</tr>
<tr>
<td>Minima</td>
<td>204</td>
<td>+3,400</td>
</tr>
<tr>
<td>pH 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximia</td>
<td>225</td>
<td>-2,500</td>
</tr>
<tr>
<td>Minima</td>
<td>197</td>
<td>-8,700</td>
</tr>
</tbody>
</table>

* Values for myeloma IgG were determined at pH 1.5; those for normal IgG, at pH 1.75.
Fig. 4. ORD curves calculated from CD data for the native myeloma IgG. Broken lines 1, 2, 3, 4, and 5 represent the partial rotations of the CD bands at 192.5, 202, 217, 242, and 225 μm, respectively. The partial rotations of the bands at 265 and 284 μm are too weak below 250 μm to be shown here. The sum of all the partial rotations given in Table V is indicated by the solid line. Note that this curve is in terms of [m'].

linear above 280 μm, tending to curve downward. From 280 to 240 μm, although there was no marked upward or downward trend, there were adjacent points which fell away on either side of the line, which, according to Urnes (49), indicates the presence of Cotton effects. The presence of these Cotton effects, which are confirmed by the CD analysis, indicates that treatment of dispersion data by the Moffitt method is of little value in the case of immunoglobulins, particularly at these wave lengths.

Optical Rotatory Dispersion of Immunoglobulins in Acid Solution—Since the immunoglobulins gave decreased absorptions in acid in the 190 to 205 μm spectral range, their far ultraviolet optical rotatory dispersion characteristics were investigated in acid. All measurements were made after the solutions had been in acid for 24 hours at room temperature (23° ± 2°). Fig. 5 shows the dispersion curves in terms of [α] for the myeloma IgG in HCl at pH 1.5 and in H2SO4 at pH 2.4, and for the normal IgG in HClO4 at pH 1.5.

The Cotton effect peak at 210 μm has disappeared in all three cases. The myeloma IgG at pH 2.4 still displays two peaks: one at 205 μm which was present in the native protein, and a new one at 199 μm, where in the native protein a trough had occurred. At pH 1.5 in HCl only one peak appears at 199 μm with a greater amplitude than the one found at this position at pH 2.4 in H2SO4. The trough at both pH values has shifted toward the ultraviolet, but the amplitude has remained about the same as that of the native protein.

The normal IgG at pH 1.75 shows an increased amplitude for the double trough which now appears at 225 and 250 μm. The peak has shifted to the ultraviolet (201 to 203 μm) and also has an increased amplitude.

The characteristics of the Cotton effects of both proteins in acid solution are given in terms of [m'] in Table IV.

Optical Rotatory Dispersion of Myeloma IgG after Oxidative Sulfitolysis—the disulfide bonds connecting the light and heavy chains of IgG have been broken by several methods, and the molecules that result from subsequent re-formation have been studied. The recombination of undenatured heavy and light chains of antibodies forms an active product even prior to re-formation of the interchain disulfide bonds (46, 47). The sedimentation coefficients and viscosities of the associated heavy and light chains of immunoglobulins are changed but very little (23). To assess any conformational change that may occur after breakage of the disulfide bonds between the heavy and light chains, the myeloma IgG was subjected to oxidative sulfitolysis and this product was analyzed by optical rotatory dispersion.

The Cotton effects of the sulfitolyzed preparation in phosphate
buffer at pH 7.5 are shown in Fig. 6 in terms of [\alpha] along with those of the native myeloma IgG for comparison. The heavy and light chains are known to associate in neutral buffer even in the absence of the disulfide bonds connecting them (43). The sulfitolyzed product does have only one trough at 225 \text{nm}; the Cotton effect of 265 \text{nm} has disappeared and the Cotton effect at 284 \text{nm} is diminished. The native globulin represents an array of IgG with net charges which differ, it is likely that its optical rotatory dispersion represents poorly resolution of such an array of closely similar, but different, optical rotatory dispersion curves. This effect, which has been found with mixtures of specific antibodies and nonspecific immunoglobulins (48) and with mixtures of myeloma \gamma-globulins,\(^4\) points to the importance of homogeneity in the resolution of optical properties.

![Figure 6](http://www.jbc.org/)

**FIG. 6.** Rotatory dispersion of the myeloma IgG after oxidative sulfitolysis (----) in phosphate buffer at pH 7.5 compared with that of the native protein (-----). The concentrations of the protein and the optical paths are the same as those used for the native proteins (Fig. 4). The curve for the sulfitolyzed protein represents points obtained at 5 \text{nm} intervals or less from averaged values of 4 to 10 determinations.

The differences observed between the optical rotatory dispersion curves of normal \gamma-globulin and the myeloma \gamma-globulin are considered to be due to the more homogeneous preparation afforded by a monoclonal gammopathy. Since the normal \gamma-globulin represents an array of IgG with net charges which differ, it is likely that its optical rotatory dispersion represents poorly resolution of such an array of closely similar, but different, optical rotatory dispersion curves. This effect, which has been found with mixtures of specific antibodies and nonspecific immunoglobulins (48) and with mixtures of myeloma \gamma-globulins,\(^4\) points to the importance of homogeneity in the resolution of optical properties.

The optical rotatory dispersion curves of both immunoglobulins in acid solution show alterations similar to those reported for carbonic anhydrases (49, 50). Since Bence-Jones proteins in acid solution (44) gave different optical rotatory dispersion curves from those found for the immunoglobulins, the inference is that the disulfide-bonded heavy chains have a limiting effect upon the conformation change of the light chains in the presence of this denaturing agent. This inference is supported by the work presented on the cleaved myeloma IgG in neutral buffer and in acid.

During the preparation of this manuscript, Dorrington, Zar- lengo, and Tanford (51) published a paper in which the optical rotatory dispersion of a human myeloma IgG was compared with nonspecific rabbit IgG. The rotatory dispersion was measured in the ultraviolet between 220 and 300 \text{nm}, and the reported curves of the homogeneous myeloma globulin and less homogeneous nonspecific rabbit globulin were found to be similar. The ORD pattern was not affected by rupture of interchain disulfide bonds, but it was changed when the proteins were exposed to propionic acid. The curves of the separated H and L chains differed strongly from those of the native globulins and, to a lesser extent, from each other. Most interesting is their finding that the ORD pattern could be restored on combining the H and L chains only in the case of the homogeneous myeloma globulin. The curves of the native human IgG were similar to those presented in this paper. Two troughs were observed between 225 and 230 \text{nm} for the native globulins, and a weak Cotton effect was found near 240 \text{nm}.

**DISCUSSION**

This investigation of a relatively homogeneous preparation of human IgG has revealed that there are more than seven optically active transitions present. The optical rotatory dispersion curves indicated some complexity which was confirmed by circular dichroic analysis. Although only seven transitions are shown by the experimental work, calculation of the rotatory strengths provides evidence for at least one more transition with a positive contribution in the far ultraviolet. Attempts to correlate the observed Cotton effects with conformation seem to be premature because of the complex nature and weakness of the effects.

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The optical rotatory dispersion curves of both immunoglobulins in acid solution show alterations similar to those reported for carbonic anhydrases (49, 50). Since Bence-Jones proteins in acid solution (44) gave different optical rotatory dispersion curves from those found for the immunoglobulins, the inference is that the disulfide-bonded heavy chains have a limiting effect upon the conformation change of the light chains in the presence of this denaturing agent. This inference is supported by the work presented on the cleaved myeloma IgG in neutral buffer and in acid.

During the preparation of this manuscript, Dorrington, Zar- lengo, and Tanford (51) published a paper in which the optical rotatory dispersion of a human myeloma IgG was compared with nonspecific rabbit IgG. The rotatory dispersion was measured in the ultraviolet between 220 and 300 \text{nm}, and the reported curves of the homogeneous myeloma globulin and less homogeneous nonspecific rabbit globulin were found to be similar. The ORD pattern was not affected by rupture of interchain disulfide bonds, but it was changed when the proteins were exposed to propionic acid. The curves of the separated H and L chains differed strongly from those of the native globulins and, to a lesser extent, from each other. Most interesting is their finding that the ORD pattern could be restored on combining the H and L chains only in the case of the homogeneous myeloma globulin. The curves of the native human IgG were similar to those presented in this paper. Two troughs were observed between 225 and 230 \text{nm} for the native globulins, and a weak Cotton effect was found near 240 \text{nm}.

This agrees essentially with our observations on the myeloma globulin. However, we have shown that the Cotton effects at 225 and 265 \text{nm} are disturbed by cleavage of the molecule by oxidative sulfitolysis, which points to the importance of the integrity of the interchain disulfide bonds to that part of the secondary or tertiary structure or both associated with these two electronic transitions. The major structural features of the native myeloma IgG associated

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\(^4\) D. L. Ross, unpublished data.
with the stronger Cotton effects at 217, 202, and 192.5 nm do not appear to be significantly altered by disulfide bond cleavage.

REFERENCES
The Far Ultraviolet Optical Rotatory Dispersion, Circular Dichroism, and Absorption Spectra of a Myeloma Immunoglobulin, Immunoglobulin G

D. L. Ross and B. Jirgensons


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