Structural Features of γ-Immunoglobulin, Antibody, and Their Fragments. Circular Dichroism Studies*

Renata E. Cathou, Anthony Kulczycki, Jr., and Edgar Haber

ABSTRACT: The far-ultraviolet circular dichroism of rabbit antidinitrophenyl antibody is the sum of separate contributions from the Fab and Fc fragments.

Analysis of the circular dichroism of Fab revealed the presence of at least seven bands, located at 217 (−), 232 (+), 242 (−), 265 (+), 275 (+), 285 (+), and 291 mμ (+), all of which were found to be sensitive to the conformational state of the protein. In particular, the latter four optically active transitions, which had not been previously observed in optical rotatory dispersion studies, disappeared when the protein was exposed to 4 M guanidine hydrochloride for 5 hr; these bands are due to tyrosine, tryptophan, and/or cystine in asymmetric environments. The negative band at 217 mμ is probably due to β structure. The positive band at 232 mμ has been tentatively assigned to un-ionized tyrosine residues on the basis of molar ellipticity and alteration on raising the pH to 12. The ellipticity of the negative band at 242 mμ and the effect on it of exposing Fab to guanidine hydrochloride suggest that random coil may be the source of this band, although cystines may also make contributions. The circular dichroism of nonspecific rabbit γ-immunoglobulin Fab showed bands at the same wavelengths as those of antidinitrophenyl Fab but the molar ellipticities of all bands were smaller. The circular dichroism of antidinitrophenyl Fab in the presence of a threefold molar excess of ε-dinitrophenyllysine was the same as that of the protein in the absence of ligand indicating that no conformational changes detectable by circular dichroism had occurred on formation of the antibody–hapten complex.

Optical rotatory dispersion studies of rabbit IgG immunoglobulins have shown that purified antibody directed against a simple antigenic determinant, the ε-dinitrophenyllysyl group (anti-DNP), has a distinctive spectrum in the ultraviolet region which differs from that of nonspecific IgG (Steiner and Lowey, 1966; Cathou and Haber, 1967).

Circular dichroism, although originating in the same basic phenomenon as optical rotatory dispersion, is manifest as discrete bands, generally at the same wavelengths as those of the optically active transitions and with similar band widths. Since these bands are usually set on a background of zero optical activity, it is easier to resolve a circular dichroism spectrum into component bands than a complex optical rotatory dispersion spectrum into Cotton effects. Instrumentation has now progressed to the point where measurement of circular dichroism is not only very feasible, but also capable of great sensitivity.

We present here a detailed investigation of the circular dichroism of high-affinity rabbit anti-DNP and of its papain fragments, particularly the hapten-binding Fab fragments. Some of the circular dichroism bands of the Fab fragment have been tentatively identified.

We have found that all of the bands are sensitive to the conformational state of the protein. At least four bands in the 260–300-mμ region, which were not clearly observed in previous optical rotatory dispersion spectra, and which are due to the aromatic amino acids, and probably also disulfide bridges, disappear when the Fab fragment is exposed to 4 M guanidine hydrochloride. Because these bands are in an experimentally accessible wavelength region and are far removed from the peptide-bond transitions, they can be used as sensitive conformational probes.

We have compared the spectrum of anti-DNP with that of nonspecific IgG[1] and have found that although both exhibit the same circular dichroism bands, the molar ellipticities of these bands differ, suggesting that the two species of molecules either contain differing proportions of the structures giving rise to the circular dichroism bands or that the environments of these structures differ.

Materials and Methods

Materials. Rabbit anti-DNP, its papain fragments, and guanidine hydrochloride were prepared as previously described (Cathou and Haber, 1967); the antibody had $K_0 \cong 10^9$ moles/l. with ε-DNP-lysine

* From the Cardiac Unit, Medical Services, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts. *Received July 25, 1968.* Supported by Grant AI-04967, National Institute of Allergy and Infectious Diseases, and Grant HE-06664, National Heart Institute, U. S. Public Health Service.

[1] The abbreviations used are as listed in *Biochemistry 5*, 1445 (1966). The nomenclature used for immunoglobulins and the papain fragments Fab and Fc are those adopted by the World Health Organization and summarized in *Bull. World Health Organ. 30*, 447 (1964).

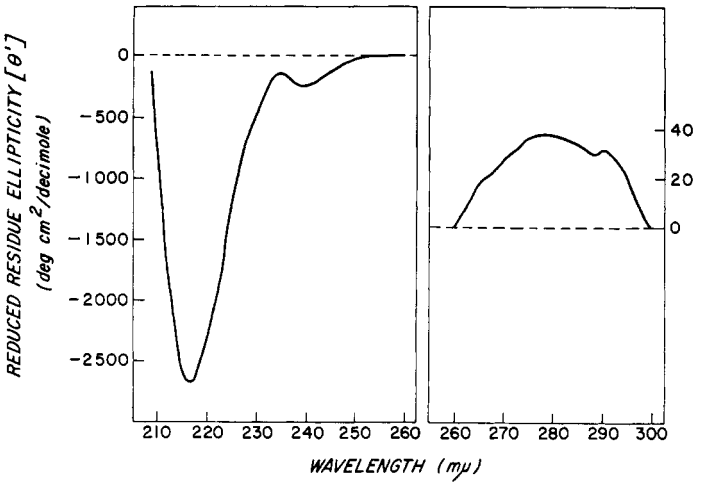

FIGURE 1: Circular dichroism spectrum of rabbit anti-DNP in 0.01 M potassium phosphate–0.15 M NaCl (pH 7.4).

as measured by equilibrium dialysis. All solutions were prepared in 0.01 M potassium phosphate buffer–0.15 M NaCl (pH 7.4). Nonspecific rabbit IgG (fraction II) was obtained from Pentex and found to contain no detectable impurities by cellulose acetate strip electrophoresis. The Fab fragment of IgG was prepared according to the same procedure as that used for the hydrolysis of anti-DNP.

Concentration Determinations. Concentrations of anti-DNP, IgG, and papain fragments were determined spectrophotometrically at 280 mμ using the following extinction coefficients for 1% solutions and a path length of 1 cm: anti-DNP, 16.5; anti-DNP Fab, 18.1; Fc, 13.8; nonspecific IgG, 15.0; and nonspecific IgG Fab, 16.0 (Steiner and Lowey, 1966). Concentrations of ε-DNP-lysine were determined spectrophotometrically using the molar extinction coefficient ϵ_{363} 17,530 (Eisen and Siskind, 1964).

Circular Dichroism. Circular dichroism measurements were made using a Cary Model 6001 recording spectropolarimeter with circular dichroism attachment at 27°, in cells with path lengths ranging from 5 cm to 1 mm. Samples generally contained 0.1–1.0 mg/ml of protein. The absorbance of solutions was always less than 1.5. Ellipticity is expressed as reduced residue ellipticity $[\theta']$ which is defined as

$$[\theta'] = \frac{3}{n^2 + 2}\frac{M\theta}{10cl} \quad (1)$$

where n is the refractive index of the solvent, M is the mean residue weight, θ is the observed ellipticity in degrees, c is the concentration of optically active solute in grams per cubic centimeters, and l is the path length in centimeters. A mean residue weight of 108 was used for nonspecific IgG, anti-DNP, and their papain fragments (Crumpton and Wilkinson, 1963).

Solvent affects the rotational strength of an optically active transition (Cassim and Taylor, 1965); the Lorentz field correction, $3/(n^2 + 2)$, is generally used to compensate for this effect. However, at present it is very difficult to assess the effective refractive index of the environment surrounding any given optically active group in a protein especially in the interior. Therefore, the refractive index of the bulk solvent water was used in the Lorentz field correction even though this is probably not rigorously correct. For the purpose of comparison of our experimentally determined ellipticities with those given in the literature, the latter have been converted to reduced molar ellipticities when necessary.

Results

The circular dichroism of anti-DNP in the range of 210–300 mμ is given in Figure 1. Although the positions of all bands were constant from preparation to preparation, the molar ellipticity varied somewhat; similar results were previously found in the optical rotatory dispersion of anti-DNP (Cathou and Haber, 1967; Steiner and Lowey, 1966); therefore results of a representative preparation are shown. There are negative bands at 217 and 240 mμ and a positive shoulder at 235 mμ. Above 260 mμ the ellipticity is positive with several overlapping bands which can be partially resolved at 275–280 and 290 mμ. The circular dichroism of nonspecific rabbit IgG (not shown) displays the same general features; however, the negative band at 217 mμ is smaller, the positive shoulder at 235 mμ is much less prominent, and the negative band at 240 mμ is deeper. A comparison of the circular dichroism of IgG and specific antibody will be considered in greater detail below in a discussion of the Fab fragments.

The circular dichroism spectra of the papain fragments, Fab and Fc, of anti-DNP are shown in Figures 2 and 3, respectively. Papain hydrolysis yields two populations of Fab fragments derived from two species of IgG with differing charge (Porter, 1959). Fab I (terminology of Porter, 1959) (Figure 2) shows negative bands at 217 and 242 mμ, and a positive band at 232 mμ. Above 250 mμ the ellipticity is positive. At least four positive bands can be seen at 265, 275, 285, and 291 mμ. The circular dichroism spectrum of anti-DNP Fab II is similar to that of Fab I except that the negative bands at 217 and 242 mμ are about 30% larger. The ellipticity

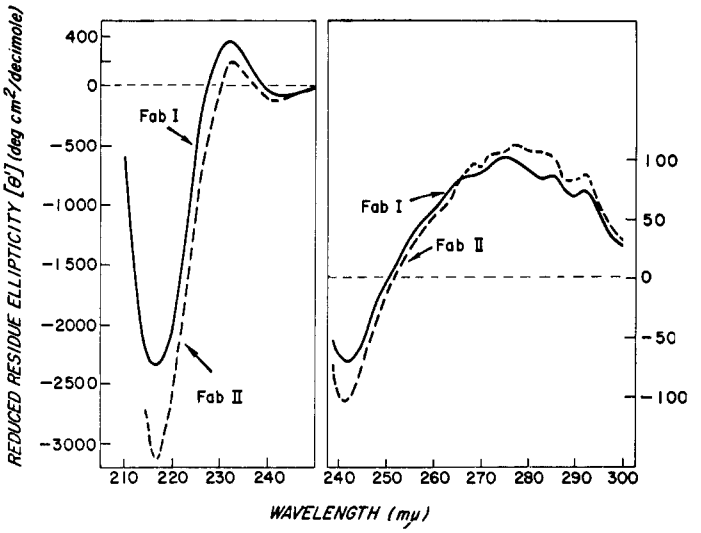

FIGURE 2: Circular dichroism spectra of the Fab fragments of rabbit anti-DNP in 0.01 M potassium phosphate–0.15 M NaCl (pH 7.4). (——) Fab I and (– – – –) Fab II.

of the bands in the range 280–295 mμ exhibited by Fab II is about 15% greater than that shown by Fab I. The Fc fragment (Figure 3), which is the same in nonspecific IgG and specific antibody (this is not surprising since the amino acid sequence is constant (Hill *et al.*, 1966)), exhibits a negative band at 217–218 mμ, a suggestion of poorly resolved negative bands at 225–228 and 240 mμ, a shoulder at 235 mμ, and a negative region from 250 to 285 mμ. The spectra of the papain fragments shown in Figures 2 and 3 and the spectrum of the whole molecule shown in Figure 1 cannot be quantitatively compared, as different preparations were used. However, the spectra of the subunits are additive, as shown by the fact that the circular dichroism spectrum of a papain digest of anti-DNP is superimposable on that of the original undigested material.

Figure 4 shows the circular dichroism of the Fab II fragments of nonspecific IgG and anti-DNP. IgG Fab exhibits a negative band at 217 mμ which is about one-third as large as that shown by anti-DNP; in addition, the positive band at 232 mμ is reduced to a shoulder and the ellipticity of the bands in the 260–300-mμ region is lower.

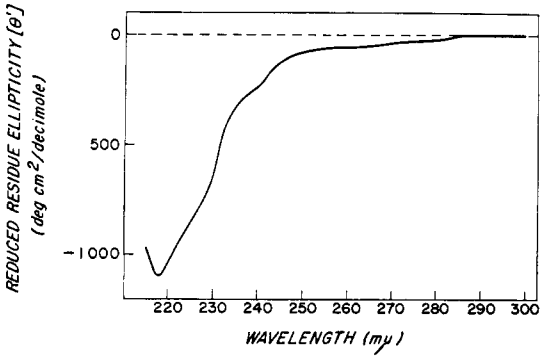

FIGURE 3: Circular dichroism spectrum of the F_c fragment of rabbit anti-DNP in 0.01 M potassium phosphate–0.15 M NaCl (pH 7.4).

The circular dichroism spectrum of anti-DNP Fab (I and II) in the presence of a threefold molar excess of ε-DNP-lysine is similar to that found in the absence of hapten, within experimental error, confirming earlier optical rotatory dispersion studies (Cathou and Haber, 1967; Steiner and Lowey, 1966).

The effect of increasing the pH from 9.8 to 11.9 on the circular dichroism spectrum of anti-DNP Fab in the range 230–260 mμ is shown in Figure 5. The positive band at 232 mμ decreases in size and eventually disappears while a new positive band centered at 248 mμ appears. The size of the band at 248 mμ at pH 11.93 is about the same as that of the band at 232 mμ at pH 9.8. At pH 11.93 the band at 232 mμ has almost disappeared and is seen only as a distortion in the lower wavelength limb of the band at 248 mμ. As the band at 248 mμ becomes evident, the negative band centered at 240 mμ disappears. The positive bands of the Fab spectrum in the 270–300-mμ region are smaller at pH 11.9 than at pH 7.4 by a factor of about 40% for Fab I and of about 65% for Fab II. When the pH of a solution of Fab is brought back to 7.4 from 11.9, the original spectrum reappears, although the positive bands at 232 mμ and in the 260–300-mμ region are slightly smaller than in the untitrated material.

At pH 11.5 in 5 M guanidine hydrochloride the ellipticity of the positive band at 248 mμ is increased to about twice the value found in the absence of guanidine hydrochloride.

The effects on the circular dichroism spectrum of exposure of Fab I to both 4 and 6 M guanidine hydrochloride at pH 8 are shown in Figure 6. The protein solution was kept in 4 M guanidine hydrochloride for 16 days to allow the denaturation reaction to come to equilibrium. Under these conditions the positive band at 232 mμ was almost eliminated and became a shoulder. The position of the negative band at 242 mμ was shifted to about 235–237 mμ and the ellipticity became more negative in this region. The positive bands in the region of 260–300 mμ completely disappeared; these bands

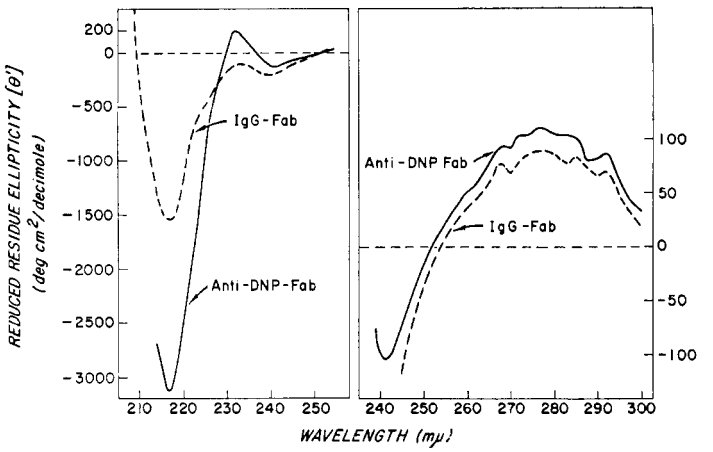

FIGURE 4: Circular dichroism spectra of the Fab II fragments of rabbit anti-DNP and nonspecific IgG. (——) Anti-DNP Fab II and (– – – – –) nonspecific IgG Fab II.

are very sensitive to environment and disappeared after 5 hr in 4 M guanidine hydrochloride. Very surprisingly, the negative band at 217 mμ was hardly affected; the ellipticity in 4 M guanidine hydrochloride after 16 days decreased only by 15–20%. When the protein that had been in 4 M guanidine hydrochloride was then exposed to 6 M guanidine hydrochloride, the positive shoulder at 232 mμ was further decreased by about 15% and the ellipticity of the negative band at 217 mμ dropped to about 50% of the value found in the absence of guanidine hydrochloride. The effects of guanidine denaturation will be discussed in greater detail in a subsequent publication.

Discussion

Since the circular dichroism spectra of anti-DNP and an unresolved papain digest of anti-DNP are identical, it can be safely assumed that no gross conformational changes have occurred during digestion and that therefore the spectrum of anti-DNP is the additive result of the spectra of the component papain fragments Fab and Fc. The same result had been previously obtained in an optical rotatory dispersion study of IgG (Steiner and Lowey, 1966). Thus, the positive shoulder seen at 235 mμ in the spectrum of anti-DNP is due to the positive band at 232 mμ of Fab and is not merely a region of no optical activity between two negative bands. In order to gain information on the three-dimensional structure of the antibody molecule, we have, therefore, concentrated on a study of the structure of the papain fragments, and in particular of the Fab fragment, which from the viewpoint of antigen binding is the more interesting.

The simplest analysis of the circular dichroism spectrum in the range of 210–250 mμ of the Fab fragment of anti-DNP yields three bands: a negative band at 217–218 mμ, a positive band at 232 mμ, and a negative band at 241–242 mμ. Because the 242-mμ band is somewhat skewed, there may be another negative band at about 247 mμ. Above 250 mμ at least four positive bands can be discerned, located at 265, 275, 285, and 291 mμ.

The negative band at 217–218 mμ is probably due to β structure. The appearance of a negative band at this wavelength due to the n–π* transition of polypeptides in the β conformation has been predicted from calculations of the ultraviolet optical properties (Pysh, 1966) and demonstrated for poly-L-lysine (Townend *et al.*, 1966), *B. mori* silk fibroin (Iizuka and Yang, 1966, 1968), and acetoacetate decarboxylase (Lederer, 1968). The existence of intrachain cross β structure in the immunoglobulin molecule has also been inferred from a study of the infrared spectra of oriented films of antibody–antigen complexes (Imahori, 1963). Earlier circular dichroism studies (Sarkar and Doty, 1966) of nonspecific IgG revealed the presence of a negative band at 217–218 mμ. Of the amino acids most likely to participate in the formation of β structure *i.e.*, serine, threonine, valine, asparagine, and isoleucine (Blout, 1962; Blake *et al.*, 1967), the first three are present in large quantities in the Fab fragments (Crumpton and Wilkinson, 1963).

Depending upon whether the anti-DNP Fab β structure is in a predominantly hydrophilic or hydrophobic environment, there could be 20–45% β structure present

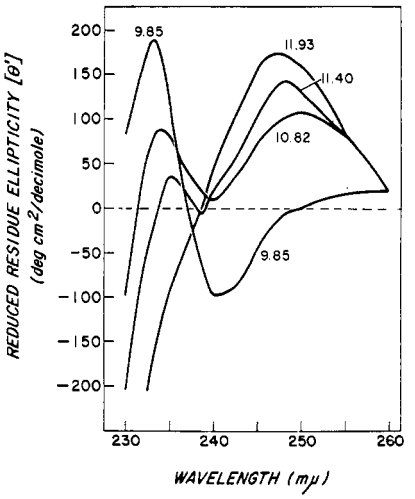

FIGURE 5: Effect of pH on the circular dichroism spectrum of rabbit anti-DNP Fab II in 0.01 M potassium phosphate–0.15 M NaCl.

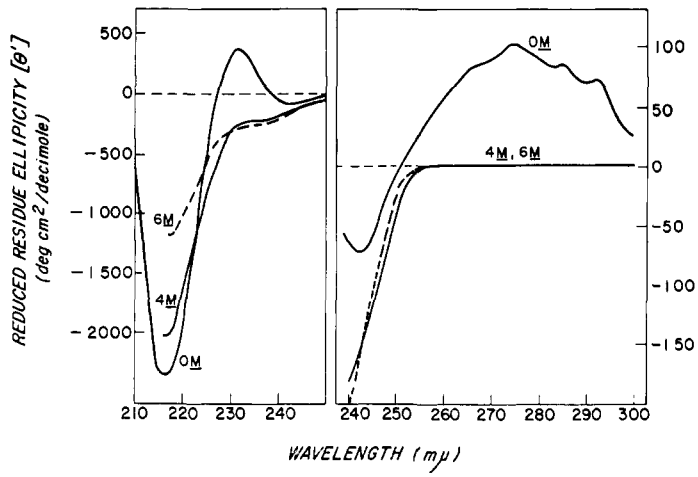

FIGURE 6: Effect of guanidine hydrochloride on the circular dichroism spectrum of rabbit anti-DNP Fab I in 0.01 M potassium phosphate–0.15 M NaCl (pH 8). (——) No guanidine hydrochloride and 4 M guanidine hydrochloride and (– – – –) 6 M guanidine hydrochloride. See text for experimental details.

(Sarkar and Doty, 1966). Since Fab II consistently has a more intense 217-mμ band than Fab I, it would be expected that Fab II either contains more β structure or that the environment of the latter is more hydrophilic (Sarkar and Doty, 1966). Using these criteria, Fab of nonspecific IgG contains 10–20% β structure. These values must, of course, be considered very approximate since (a) the molar ellipticities of a polypeptide completely in the β form in different environments are not known with certainty, and (b) the β structure in Fab is no doubt in a mixture of both hydrophobic and hydrophilic environments. The low proportion of β structure found in nonspecific IgG Fab and the possibility that it may be imbedded in the hydrophobic interior may possibly explain why this structure has not been identified in infrared studies of IgG (Winkler and Doty, 1961; Imahori and Momoi, 1962).

It is possible that the negative 217-mμ band is not due to β structure, but rather to aromatic amino acids or histidine. To date, however, the transitions of tyrosine, tryptophan, phenylalanine, and histidine in this wavelength region have all been shown to exhibit positive bands (Beychok, 1967). Although it is unlikely that these amino acids are the source of the 217-mμ band, the resolution of this point must await further information on the behavior of the circular dichroism of aromatic amino acids in proteins.

When anti-DNP Fab is exposed to 6 M guanidine hydrochloride, there is still negative ellipticity at 217 mμ. The circular dichroism of a random coil exhibits a positive band at this wavelength (Holzworth and Doty, 1965). Therefore, unless there are negative ellipticity contributions from sources other than β structure at 217 mμ, these preliminary results suggest, but certainly do not prove, that there may still be some ordered structure present when Fab is exposed to 6 M guanidine hydrochloride. This would be contrary to the results of Tanford and his coworkers (Tanford *et al.*, 1967a,b) who concluded that proteins, even though they may contain disulfide bridges, exist essentially as random coils in 6 M guanidine hydrochloride.

The absence of any negative band at 222 mμ in the circular dichroism spectrum of the Fab fragment of either anti-DNP or IgG precludes the presence of any appreciable amount of α helix.

The 232-mμ positive band exhibited by Fab (both anti-DNP and nonspecific IgG) at neutral pH may be due to tyrosine. The free amino acid exhibits a positive band at about 230 mμ when un-ionized and at 245 mμ when ionized (Beychok and Fasman, 1964). *N*-Acetyl-L-tyrosinamide, which may be considered a model of tyrosine in peptide linkage, exhibits a positive band at 227 mμ which shifts to 242 mμ upon ionization (Simmons and Glazer, 1967). The oligopeptide oxytocin shows a positive band at 222–225 mμ which shifts to 245 mμ (Beychok and Breslow, 1968). A similar shift in circular dichroism bands from 229 to 245 mμ has recently been demonstrated for *B. mori* silk fibroin (Iizuka and Yang, 1968). When the reduced molar ellipticity of the anti-DNP Fab 232-mμ band is calculated on the basis of tyrosine, assuming mol wt 45,000 and 18 residues (Crumpton and Wilkinson, 1963), $[\theta'] = 8800$, a value which is very similar to that of the tyrosine in oxytocin (Beychok and Breslow, 1968). Although the molar ellipticity of the 248-mμ band at pH 12 is somewhat lower than that expected, *i.e.*, $[\theta'] = 6000$, when the protein is exposed to 5 M guanidine hydrochloride at pH 11.5, $[\theta'] \cong 12{,}000$, a normal value for ionized tyrosine (Beychok and Breslow, 1968). Some of the tyrosine residues are therefore probably inaccessible to solvent in the native structure. Indeed, if one looks at the lower wavelength limb of the positive band at 248 mμ of Fab fragment that had not been exposed to guanidine hydrochloride at pH 12 one can still see a shoulder at about 235 mμ. This behavior is compatible with that observed by Gould *et al.* (1964) when studying the ionization of the tyrosine residues of IgG.

Other possible sources of the Fab 232-mμ band are tryptophan and cystine (Beychok, 1966; Green and Melamed, 1966). However, neither of these amino acids show the requisite large band shifts on raising the pH from 7.4 to 12 (Beychok, 1966; R. Cathou and A. Kul-

czycki, unpublished results). Furthermore, since disulfides usually exhibit bands only above 240 mμ (Beychok, 1966) it is unlikely that the band at 232 mμ is due to cystine. However, the possibility exists that the new band seen at 248 mμ at basic pH is due to this amino acid. This would require that the environment of cystine becomes more asymmetric at pH 12, giving rise to a new or more intense band, or that the negative band at 242 mμ, which may have contributions from cystine (see below), changes sign and becomes positive. Preliminary experiments with a model cystine-containing peptide, glutathione (R. Cathou, unpublished results), showed no significant shifts in either the position or sign of the 250-mμ band on raising the pH from 8 to 12.

Cystine may be responsible for the Fab negative bands seen at 242 mμ. However, the reduced molar ellipticity per cysteine residue, when calculated on the basis of 13 cysteines (Crumpton and Wilkinson, 1963), $[\theta'] = -2600$, is higher than that generally observed for other cystine-containing compounds (Beychok and Breslow, 1968; Beychok, 1965). If one now uses the value of the ellipticity of the 242-mμ band of Fab in 4 M guanidine hydrochloride (the position has now shifted to 237–238 mμ), the ellipticity per cysteine, $[\theta'] = -7000$. To date, the only disulfides which have been found to possess such great optical activity are cyclic disulfides with a single screw sense (Carmack and Neubert, 1967). Thus, if cystines are responsible for this band, they must have primarily the same screw sense and must retain their configuration in 4 M guanidine hydrochloride. It is more probable that this band is due to random coil. Such a band is seen in the circular dichroism spectra of both poly-α-L-glutamate and poly-α-L-lysine in the random conformation (Velluz and Legrand, 1965) and has been predicted theoretically (Carver *et al.*, 1966). An increase in ellipticity of this band on exposure of Fab to 4 M guanidine hydrochloride is compatible with this hypothesis. The slightly skewed nature of this band suggests that there may be additional contributions at about 247 mμ which may be due to cystine (Beychok, 1966).

The fine structure observed in the circular dichroism spectrum of the Fab fragment between 250 and 300 mμ had not been previously observed in optical rotatory dispersion studies of anti-DNP probably because the small overlapping Cotton effects were superimposed on the rapidly increasing negative rotation of the larger Cotton effect with a trough at 224 mμ. A number of proteins have now been found to possess optical activity in this wavelength region (Beychok, 1966; Beychok *et al.*, 1966; Green and Melamed, 1966; Lederer, 1968; Simmons and Glazer, 1967). The Fab fragment of anti-DNP exhibits at least four bands at 265, 275, 285, and 291 mμ which are difficult to resolve. Tyrosine, tryptophan, and cystine residues with a dihedral angle greater than 90° could all make contributions (Beychok, 1967) and it is thus difficult to attempt to specifically identify these bands. When the Fab fragment is exposed to 4 M guanidine hydrochloride for 5 hr, these bands disappear, indicating that the amino acids responsible were originally in asymmetric environments. This wavelength region (250–300 mμ) is thus very useful as a sensitive conformational probe, especially since it is far removed from the peptide transitions and is easily accessible experimentally. We have used this probe to study the effects of hapten binding on antibody conformation in guanidine solutions and have found that significant stabilization occurs, in agreement with earlier optical rotatory dispersion studies (Cathou and Haber, 1967).

Nonspecific IgG Fab exhibits the same spectrum in the 250–300-mμ region as that of anti-DNP Fab although the ellipticity of all bands in the former is lower, suggesting that in the nonspecific protein there are fewer chromophoric side chains in asymmetric environments.

If tyrosines are responsible for any of the bands above 260 mμ one might expect a shift or change in size of the bands upon ionization (Simmons and Glazer, 1967). In fact, the ellipticity of these bands does decrease upon raising the pH. However, it is difficult in this case to distinguish between a decrease in ellipticity due primarily to ionization or to loss of asymmetric environment.

The anti-DNP Fab–ε-DNP-lysine complex has the same circular dichroism spectrum within experimental error from 215 to 300 mμ as the Fab fragment in the absence of ligand. There is thus no conformational change detectable by circular dichroism on binding of hapten.

The circular dichroism spectrum of the Fc fragment is more difficult to analyze than that of the Fab. The negative band at 217–218 mμ is probably due to β structure. The remaining shoulders in the spectrum are poorly resolved and there does not appear to be any detectable fine structure above 250 mμ. The difficulty stems from the low ellipticity compared to absorption which results in a less favorable signal-to-noise ratio than in the case of Fab. This is unfortunate because the Fc fragment is crystallizable and it would be valuable to be able to compare the structural information obtainable by X-ray crystallography and circular dichroism for such a protein.

During the preparation of this manuscript a report of the circular dichroism of a human myeloma IgG and human nonspecific IgG appeared (Ross and Jirgensons, 1968). The curve presented for the myeloma protein is in agreement with the results presented in this paper. These authors concluded that optically active transitions existed at 202, 217, 225, 242, 265, and 284 mμ. Bands were seen at all of these wavelengths except at 225 mμ; the latter band was postulated to exist because of asymmetry of the 217-mμ band. No mention was made of a positive band at about 230–235 mμ, although a shoulder is present in the circular dichroism spectrum of the myeloma protein. This omission was probably made because the ellipticity at the positive extremum of this shoulder was essentially zero and thus appeared as a region of no optical activity between two negative bands. However, as we have shown, the circular dichroism of the whole immunoglobulin molecule is the additive result of the circular dichroism of the Fab and Fc fragments. The positive shoulder at about 235 mμ in anti-DNP (which does not return to zero for either anti-DNP or nonspecific rabbit IgG) is due to the positive band at 232 mμ in Fab and has negative ellipticity because of the negative contributions of Fc. The asymmetry of the higher wavelength limb of the 217-mμ band of human myeloma protein noted by these authors could

be due to a negative band at about 225 mμ similar to that seen in the circular dichroism of rabbit Fc.

Ross and Jirgensons also found, in the optical rotatory dispersion spectrum of sulfitolyzed myeloma protein, that the Cotton effect at 265 mμ had disappeared and the Cotton effect at 284 mμ was greatly diminished. This suggests that integrity of disulfide bonds is necessary for the optically active transitions seen at these wavelengths in the circular dichroism of anti-DNP Fab. These bands may be due to disulfide bonds themselves or to aromatic amino acids in asymmetric environments maintained by the presence of disulfide bonds.

The main structural features of anti-DNP Fab to emerge from these studies are the probable presence of β structure, random coil, and aromatic amino acids in asymmetric environments. The two main populations of Fab, I and II, differ primarily in the strength of the 217-mμ band which has been assigned to β structure. Nonspecific IgG Fab exhibits much weaker 217-mμ and 232-mμ bands and a stronger 240-mμ band, and thus appears to contain less β structure, more random coil, and fewer tyrosines in asymmetric environments. Since all the observed bands are sensitive to the conformational state of the protein, they make excellent conformational probes.

Acknowledgment

The authors gratefully thank Dr. Hermann Kalckar and Dr. Donald Wallach for the use of their Cary 6001 spectropolarimeter.

References

Beychok, S. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 999.
Beychok, S. (1966), *Science 154*, 1288.
Beychok, S. (1967), *in* Poly-α-Amino Acids, Fasman, G. D., Ed., New York, N. Y., Marcell Dekker, p 293.
Beychok, S., Armstrong, J. McD., Lindblow, C., and Edsall, J. T. (1966), *J. Biol. Chem. 241*, 5150.
Beychok, S., and Breslow, E. (1968), *J. Biol. Chem. 243*, 151.
Beychok, S., and Fasman, G. D. (1964), *Biochemistry 3*, 1675.
Blake, C. C. F., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarma, V. R. (1967), *Proc. Roy. Soc. (London) B167*, 365.
Blout, E. R. (1962), *in* Polyamino Acids, Polypeptides and Proteins, Stahmann, M., Ed., Madison, Wis., University of Wisconsin, p 275.
Carmack, M., and Neubert, L. A. (1967), *J. Am. Chem. Soc. 89*, 7134.
Carver, P., Schechter, E., and Blout, E. R. (1966), *J. Am. Chem. Soc. 88*, 2550.
Cassim, J. Y., and Taylor, E. W. (1965), *Biophys. J. 5*, 553.
Cathou, R., and Haber, E. (1967), *Biochemistry 6*, 513.
Crumpton, M. J., and Wilkinson, J. M. (1963), *Biochem. J. 88*, 228.
Eisen, H. N., and Siskind, G. W. (1964), *Biochemistry 3*, 996.
Gould, H. J., Gill, T. J., III, and Doty, P. (1964), *J. Biol. Chem. 239*, 2842.
Green, N. M., and Melamed, M. D. (1966), *Biochem. J. 100*, 614.
Hill, R. L., Delaney, R., Lebovitz, H. E., and Fellows, R. E., Jr. (1966), *Proc. Roy. Soc. (London) B166*, 159.
Holzworth, G., and Doty, P. (1965), *J. Am. Chem. Soc. 87*, 218.
Iizuka, E., and Yang, J. T. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 1175.
Iizuka, E., and Yang, J. T. (1968), *Biochemistry 7*, 2218.
Imahori, K. (1963), *Biopolymers 1*, 563.
Imahori, K., and Momoi, H. (1962), *Arch. Biochem. Biophys. 97*, 236.
Lederer, F. (1968), *Biochemistry 7*, 2168.
Porter, R. R. (1959), *Biochem. J. 73*, 119.
Pysh, E. S. (1966), *Proc. Natl. Acad. Sci. U. S. 56*, 825.
Ross, D. L., and Jirgensons, B. (1968), *J. Biol. Chem. 243*, 2829.
Sarker, P. K., and Doty, P. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 981.
Simmons, N. S., and Glazer, A. N. (1967), *J. Am. Chem. Soc. 89*, 5040.
Steiner, L., and Lowey, S. (1966), *J. Biol. Chem. 241*, 231.
Tanford, C., Kawahara, K., and Lapanje, S. (1967a), *J. Am. Chem. Soc. 89*, 729.
Tanford, C., Kawahara, K., Lapanje, S., Hooker, T. M., Jr., Zarlengo, M. H., Salahuddin, A., Aune, K. C., and Takagi, T. (1967b), *J. Am. Chem. Soc. 89*, 5023.
Townend, R., Kumosinski, T. F., Timasheff, S. N., Fasman, G. D., and Davidson, B. (1966), *Biochem. Biophys. Res. Commun. 23*, 163.
Velluz, L., and Legrand, M. (1965), *Angew. Chem. Intern. Ed. Engl. 4*, 838.
Winkler, M., and Doty, P. (1961), *Biochim. Biophys. Acta 54*, 448.