*CONFORMATIONAL CHANGE AND COMPLEMENTARITY IN THE COMBINATION OF H AND L CHAINS OF IMMUNOGLOBULIN-G**

By Keith J. Dorrington,† Mario H. Zarlengo, and Charles Tanford

DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY MEDICAL CENTER, DURHAM, NORTH CAROLINA

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The basic four-chain model for the immunoglobulins originally proposed by Porter[1] has been confirmed by subsequent studies.[2] Mild reduction of IgG[3] at neutral pH, followed by gel filtration in suitable dissociating solvents, results in separation of the heavy (H) and light (L) polypeptide chains.[4] It has been demonstrated repeatedly that separated chains can be recombined, and that the product has hydrodynamic properties essentially similar to the original IgG molecule.[5] Hybrid recombination between heavy and light chains from a chemically and functionally heterogeneous population of IgG molecules (even from different species) can be easily achieved.[5] However, Mannik[6] has recently shown, using human IgG myeloma proteins, that H chains preferentially recombine with the L chains with which they were previously associated, even in the presence of an excess of L chains from another source.

When IgG molecules with specific antibody activity have been studied, recombination of heavy and light chains have yielded a product capable of combining with the specific antigen.[7-11] Higher recovery of activity followed recombination of specific H chain with specific L chain than with nonspecific L chains.[8, 9, 11-16]

These findings strongly suggest that full recovery of the binding site conformation requires correct pairing of constituent polypeptide chains. The optical rotatory dispersion studies described below give physical confirmation of this specificity requirement.

Materials and Methods.—Immunoglobulin G was isolated from pooled rabbit plasma as previously described.[17] Human myeloma IgG was isolated from the plasma of a patient with multiple myelomatosis; this plasma was kindly donated to us by Dr. C. E. Buckley. The crude protein was precipitated by 40% saturated ammonium sulfate, and purified by *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) chromatography employing gradient elution from 0.01 *M* phosphate, pH 8.0, to 0.5 *M* phosphate, pH 6.25. The bulk of the myeloma protein appeared as an unabsorbed peak which was dialyzed against 0.1 *N* NaCl, concentrated, filtered through a Millipore membrane, and stored at 4°. Human nonspecific IgG (fraction II) was obtained from Pentex Corp. and further purified by DEAE-cellulose chromatography. All the IgG preparations sedimented in the ultracentrifuge as a single peak ($s^0_{20,w}$ = 6.7*S*), although rabbit IgG showed approximately 10% of a 9.0*S* component.

Poly-DL-alanylated derivatives of rabbit and myeloma IgG were prepared according to the method of Fuchs and Sela,[18] using 4 gm N-carboxy-DL-alanine anhydride (Pilot Chemicals) per gram of IgG.

The characteristics of both the intact and polyalanylated proteins are given in Table 1. Molecular weights were determined by sedimentation equilibrium, using the meniscus depletion method of Yphantis.[19] Alanine content based on molecular weight differences was in good agreement with data based on amino acid analysis. Details of the physical properties of the polyalanylated proteins and their polypeptide chains will be published elsewhere.

Samples of rabbit, human, and myeloma IgG and their polyalanylated derivatives were reduced with 0.2 *M* 2-mercaptoethanol and alkylated according to Fleischman *et al.*[4] Separation of heavy and light chains was achieved by gel filtration of reduced and alkylated IgG on Biogel P-150 in 1.0 *M* propionic acid. The isolated chains were dialyzed against 0.01 *M* sodium phos-

TABLE 1

PREPARATIONS OF IgG*

	Mol. wt.†	No. of Residues		M_0
		Protein	DL-ala	
Rabbit IgG	145,000	1350	—	108
PA-Rabbit IgG‡	187,000	1350	580	139
Myeloma IgG	143,000	1330	—	108
PA-Myeloma IgG‡	189,000	1330	640	143
Human IgG	Not determined	Not determined		108

* The only parameter from this table which is used in the determination of ORD data is the mean residue weight, M_0, which is defined as the weight of total protein (including poly-DL-alanine, where appropriate) per residue of protein amino acid. The poly-DL-alanyl chains do not contribute to the observed rotation.
† Determined by sedimentation equilibrium.
‡ PA = poly-DL-alanylated.

phate/0.1 M NaCl, pH 7.0, except nonalanylated rabbit heavy chain, which was dialyzed against 0.01 M sodium acetate, pH 5.5.

Recombination of polypeptide chains was carried out as described by Metzgar and Mannik[10] except that the buffer used was 0.01 M sodium phosphate/0.1 M NaCl, pH 7.0.

Optical rotatory dispersion (ORD) curves were measured with a Cary 60 spectropolarimeter at 25°C, using cells with a 1-mm light path. Protein concentrations of 1 to 2 gm/liter were used. Results are expressed in terms of mean residue rotation, $[m']_\lambda$, which is related to the specific rotation, $[\alpha]_\lambda$, by the relation

$$[m']_\lambda = \frac{3\,M_0}{100\,(n^2+2)}\,[\alpha]_\lambda, \qquad (1)$$

where n is the refractive index of the solvent and M_0 the mean residue weight of the protein, given for the whole proteins in Table 1. M_0 values for H and L chains of the unmodified proteins were taken as 108. M_0 values for polyalanylated chains were calculated with the assumption that two thirds of the added alanyl residues are associated with the H chain.[18]

Results.—This study has two objectives: First we wished to determine whether the separation of H and L chains from each other results in a significant conformational change. Secondly, the answer being positive, we wished to determine whether the original conformation was restored upon recombination of the chains. Measurements or ORD between 220 and 300 mμ were used as a probe for conformational change.

These objectives require that ORD measurements of the separated chains and of native and reconstituted IgG molecules be carried out under identical conditions. Because the native conformation of intact IgG itself is unstable at acid pH, near neutral pH was required. Unfortunately, separated H chains have very low solubility at neutral pH, and, for this reason, poly-DL-alanylated IgG was used for some of the experiments. This modification of the protein is known to increase chain solubility,[18] without affecting other properties of the molecule. The results below will demonstrate that polyalanylation has no significant effect on conformation.

ORD of native IgG: The ORD curve of native IgG between 220 and 300 mμ is unique. The principal features are (1) the mean residue rotation is remarkably small throughout this wavelength range; (2) there is a double trough between 220 and 235 mμ, and in the results reported here, minima always appear at 225 and 230 mμ; and (3) there is a small Cotton effect centered near 240 mμ. These features appear to be characteristic of native IgG from all sources for which data are available.[20, 21]

ORD curves for native nonspecific rabbit IgG, for human nonspecific IgG, and for the native myeloma protein are shown in Figures 1, 5, and 6. They all

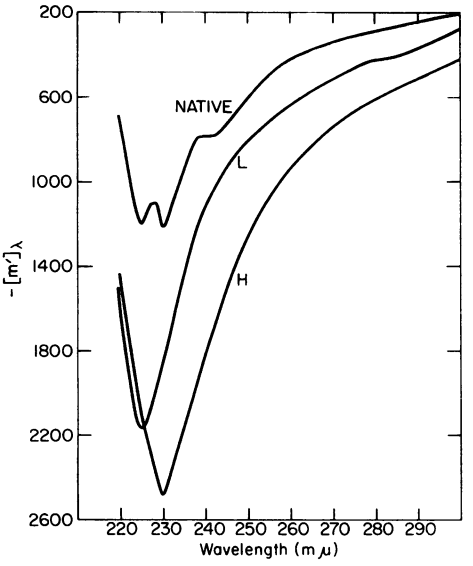

FIG. 1.—ORD curves of native rabbit IgG and of separated H and L chains derived from it.

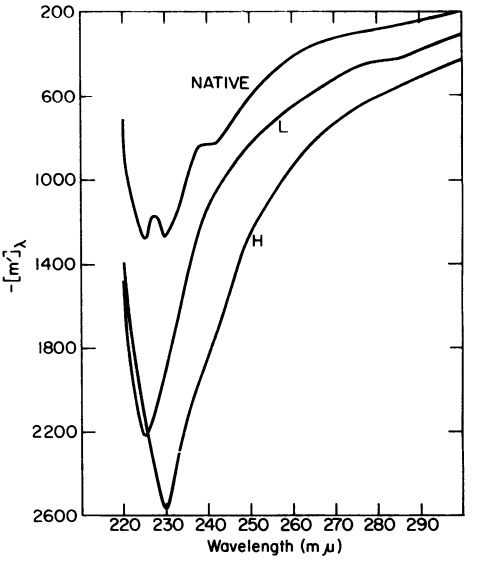

FIG. 2.—ORD curves of native poly-DL-alanylated rabbit IgG and of separated poly-alanylated H and L chains derived from it.

show these specific features. The ORD curve for the myeloma protein shows minor differences from the other two curves, e.g., the troughs at 225 mμ and 230 mμ are unequal in depth. Similar variations between ORD curves of different IgG preparations from the same species have been observed by Steiner and Lowey.[20]

Figures 2 and 3 show ORD curves for polyalanylated rabbit IgG and polyalanylated myeloma IgG. Comparison with Figures 1 and 5 shows that polyalanylation has no effect on the characteristic ORD spectrum. The curve for each derivative is, within experimental error, identical with the curve for the parent protein. The minor differences between rabbit IgG and myeloma IgG are also present in the polyalanylated derivatives.

Effect of reduction, alkylation, and exposure to propionic acid: We have found that the mild reduction and alkylation procedure which is used to break the interchain disulfide bonds of IgG has of itself little or no effect on the characteristic ORD spectrum. On the other hand, we have found that this spectrum is destroyed on exposure of IgG to 1.0 *M* propionic acid, regardless of whether interchain disulfide bonds have been broken or remain intact.[22] These results are comparable with published data on the affinity between H and L chains. The affinity resides primarily in strong noncovalent interactions between H and L chains. It is not significantly affected by reduction of interchain disulfide bonds,[5] but it is destroyed in 1.0 *M* propionic acid,[4] in which solvent the H and L chains are readily separated from each other by gel filtration if interchain disulfide bonds have been broken.

ORD curves of separated H and L chains at neutral pH: H and L chains were separated from each other, after reduction and alkylation of the parent protein by gel filtration in 1.0 *M* propionic acid. The propionic acid was removed, and the separated chains were returned to neutral or near-neutral pH. As was pointed

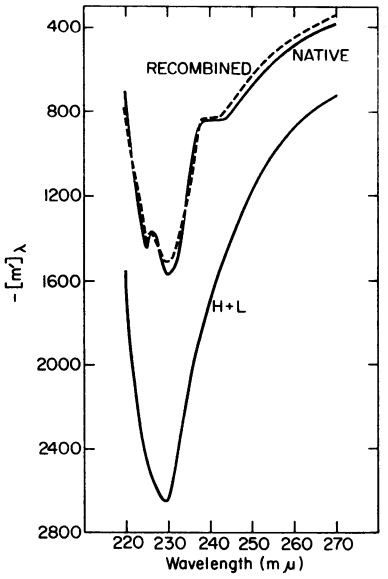

FIG. 3.—ORD curves of native poly-DL-alanylated human myeloma IgG, of an equimolar mixture of separated H and L chains derived from it, and of the protein obtained on recombination of these chains. The curve for the equimolar mixture of separated chains is a calculated one, based on actual data for the isolated chains, similar to those of Figs. 1 and 2.

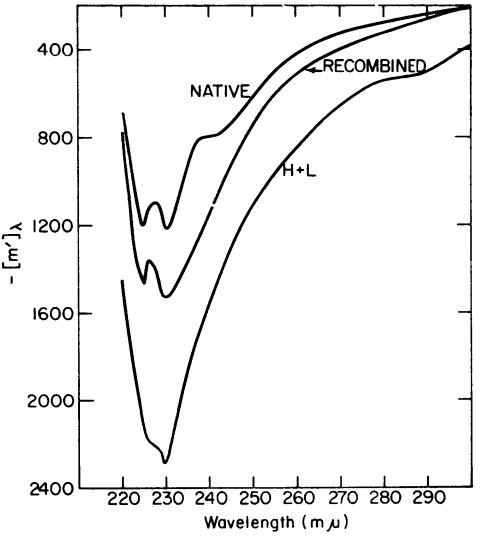

FIG. 4.—ORD curves of native rabbit IgG, of an equimolar mixture of separated H and L chains (calculated from the data of Fig. 1), and of the protein obtained on recombination of the chains.

out above, the H chains of unmodified proteins are quite insoluble near neutral pH, but it was found that rabbit IgG chains had sufficient solubility at pH 5.5, which is within the pH range of stability of native IgG, and measurements at that pH were therefore made.

The chains were studied by sedimentation and ORD. It was found that they were nativelike in their properties, i.e., relatively compact, with ORD curves quite different from those which are observed for disordered polypeptide chains.[23] The ORD spectra do not, however, display the characteristic features of native IgG. Figure 1, for example, shows the data for rabbit chains. It is seen that the mean residue rotation is about twice as large as for native IgG, and that the Cotton effect at 240 mμ is absent. The L chain has a single trough at 225 mμ, and the H chain has one at 230 mμ, but their sum does not correspond to the double trough of the native molecules, as is seen in Figure 4, where the calculated ORD curve of an equimolar mixture of the two chains is shown.

Data for the H and L chains of polyalanylated rabbit IgG are shown in Figure 2. The results are essentially identical to those of Figure 1. Polyalanylation evidently has as little effect on the properties of the separated chains as on those of the native protein.

Essentially similar results were obtained for human myeloma protein. Only polyalanylated chains were studied in this case. Data for the separated chains are not given, but the curve for an equimolar mixture is shown in Figure 3.

ORD curves of recombined chains: The calculated ORD spectrum for a simple equimolar mixture of the H and L chains of polyalanylated human myeloma IgG is shown in Figure 3. As was mentioned previously, the spectrum is quite unlike that of the native parent IgG. On the other hand, the ORD curve obtained experimentally following actual mixing of the chains and consequent recombination (also shown in Fig. 3) is virtually identical with that of the original native protein. The conformational change which accompanies the separation of the chains is clearly reversed when the chains are recombined.[24] Figure 5 shows similar data for human myeloma IgG which was not polyalanylated. Complete recovery of the native ORD curve is obtained when previously separated H and L chains are recombined.

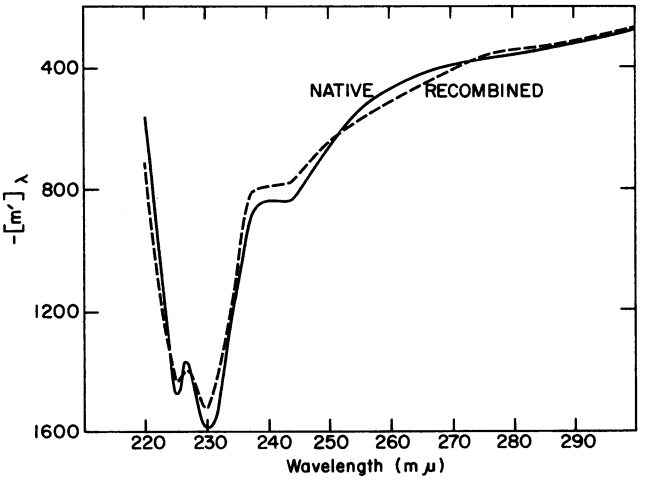

FIG. 5.—ORD curves of native myeloma protein and of the protein reconstituted from separated H and L chains. (The chains were separated by gel filtration in 1.0 *M* propionic acid, but their ORD curves could not be measured at neutral pH because of insufficient solubility.)

A different result is obtained when the H and L chains from nonspecific IgG are recombined. The data for human IgG are shown in Figure 6 and those for rabbit IgG in Figure 4. They show that a substantial change in conformation occurs when H and L chains are recombined. The unique curves for the native protein are, however, not re-created. In particular; the characteristic Cotton effect at 240 mμ is missing. The original native conformation has evidently been only partly restored.

Discussion.—The foregoing results have shown that an ORD curve with characteristic unique features is associated with the native conformation of IgG, of either rabbit or human origin. This conformation is not retained in the separated H and L chains derived from these proteins. Individual isolated chains apparently assume distinct ordered conformations at neutral pH. Their properties do not resemble those of disordered polypeptide chains.[23, 25] However, they clearly lack

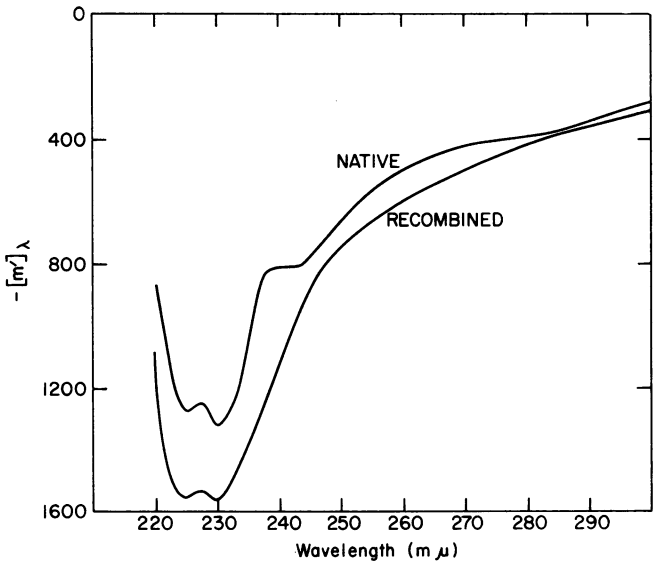

FIG. 6.—Data similar to those of Fig. 5, but for nonspecific human IgG.

those conformational features which give rise to the native ORD spectrum. This appearance of a new structure in the separated chains could explain the numerous failures to demonstrate anything beyond low levels of antigen-binding activity in separated chains derived from specific antibodies.[26, 27] It is reasonable to suppose that the specific combining ability of an IgG antibody molecule is exhibited fully only when the native conformation is intact.

A change in the ORD spectrum occurs when H and L chains are recombined, but the characteristic features of the native protein were recovered only in the experiments using myeloma IgG. They were lacking in recombined nonspecific IgG. These results suggest that complementary pairs of H and L chains are required to generate the native IgG conformation fully. Complementarity is presumably assured for all IgG molecules at the time of biosynthesis, since each antibody-producing cell predominantly makes IgG of a single specificity.[28] Complementary recombination of previously separated H and L chains occurs with myeloma proteins, because they come closest to representing a homogeneous population of IgG molecules, each myeloma protein being the normal product of an abnormally active, neoplastic clone of cells.

The original native conformation is, however, not recovered after *in vitro* recombination of the H and L chains from nonspecific IgG. This protein consists of a heterogeneous mixture of the products of many cells of diverse specificities, and the separated chains are similarly heterogeneous. The probability of correct pairing of H and L chains upon recombination must be vanishingly small. Recombination of an H chain with its complementary L chain appears to be favored over combination with noncomplementary chains,[6] but this advantage is overcome by the enormous excess of noncomplementary chains when a completely unselected IgG sample is used as starting material.

Other evidence to support the foregoing interpretation has been obtained in this laboratory.[22] If nonspecific IgG is exposed to propionic acid with interchain disulfide bonds intact, the native conformation is disrupted to the same extent as when these bonds are broken, but physical separation of the H and L chains is prevented. This reaction has proved to be completely reversible. Clearly, regeneration of the native IgG conformation can be achieved with any population of molecules, regardless of homogeneity, provided that each H chain remains associated in the final product with its complementary L chain.

These conclusions provide a conformation-related counterpart to the recombination experiments cited in the introduction. They indicate that the characteristic ORD pattern observed for native IgG is associated with the specific (i.e., variable) part of the IgG structure.[29]

It is remarkable that the ORD spectra which are obtained when complementary chains combine are closely similar for all IgG molecules, regardless of specificity. For example, the 240-mμ Cotton effect is always observed. We are not able at this time to assign this effect to a known asymmetric transition; neither peptide groups nor aromatic side chains produce Cotton effects at this wavelength.[30, 31] However, it clearly must reflect some very similar structural feature which results from the interaction of complementary H and L chains of all kinds. The results thus suggest that H and L chains can interact with each other in three ways:

(1) There is a region of noncovalent interaction which is common to all H and L chains. It allows H and L chains to associate without regard for specificity.

(2) There is a second site of interaction which is specific to individual H and L chains and which serves as a recognition site for complementary pairs.

(3) There is a third region which is responsible for the 240-mμ Cotton effect, which again must be identical or very similar in all H-L pairs. But this interaction is closely related to the complementary site and cannot be expressed unless complementary pairs are formed.

Summary.—(1) It is shown that native IgG from rabbit or human sources has a characteristic ORD spectrum between 220 and 300 mμ. (2) The ORD pattern is not affected by polyalanylation or by rupture of interchain disulfide bonds. It is destroyed when the protein is exposed to propionic acid. (3) Separate H and L chains do not possess the characteristic ORD pattern. (4) The ORD pattern is restored when complementary H and L chains, such as are obtained from myeloma protein, are recombined. (5) The ORD pattern is not restored when H and L chains from nonspecific serum IgG are recombined, unless physical separation of complementary chain pairs is prevented.

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