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Equine Antihapten Antibody. VI. Subunits of Polyalanylated γG(T)-Immunoglobulin*

Robert J. Genco,† Fred Karush,‡ and Harriet S. Tenenhouse

ABSTRACT: After extensive alanylation both γG(T) antihapten and γG(T) diphtheria antitoxin were converted into their monomeric subunits. The proteins were mildly reduced and alkylated and underwent spontaneous dissociation into the subunits in neutral aqueous solution. The heavy and light chains were separated by gel filtration with Sephadex G-200 in buffered saline. Their identification was established by immunoelectrophoresis with rabbit antisera specific for equine heavy and light chains. The heavy chain derived from the polyalanylated antihapten antibody exhibited a reduction of at least 100-fold in its association constant for the homologous hapten compared with the parent antibody. The optical rotatory dispersion patterns of the polyalanylated γG(T) and subunits revealed no extensive conformational alteration resulting from their dissociation. The calculated curve for an equimolar mixture of heavy and light chains differed significantly from that observed for the parent protein only in the smaller Cotton effect at 224 mμ of the mixture. The interpretation of this finding, coupled with the earlier measurements of the optical rotatory dispersion pattern of the Fab fragment of rabbit γG (Steiner, L. A., and Lowey, S. (1966), *J. Biol. Chem. 241*, 281), is that the light-chain–heavy-chain interaction affects the conformation of the binding region of the antibody. Furthermore, in view of the optical rotatory dispersion behavior of reconstituted myeloma γG (Dorrington, K. J., Zarlengo, M. H., and Tanford, C. (1967), *Proc. Natl. Acad. Sci. U. S. 58*, 996), it is inferred that this structure is dependent upon the specific interaction of complementary heavy and light chains. The loss of binding activity following dissociation may then be attributed to a change in the conformation of the heavy chain associated with its combining region.

The polyalanylation of immunoglobulins has served as a useful chemical modification of these proteins primarily because of the enhanced aqueous solubility acquired by the subunits of the immunoglobulins (Fuchs and Sela, 1965; Freedman and Sela, 1966; Haber and Richards, 1966; Dorrington *et al.*, 1967). The availability of soluble light and heavy chains, particularly if prepared without exposure to denaturing solutes, would allow further clarification of their respective roles in determining the combining region of the antibody molecules. To exploit this possibility it was necessary to establish that polyalanylation of the antibody molecule did not alter its binding sites. The absence of extensive alteration of these sites was inferred from the results of an earlier study (Karush and Sela, 1967) in which it was shown that alanine enrichment of 200–600 residues/molecule of antihapten antibody did not reduce the average association constant for hapten binding by more than twofold.

In the course of this earlier study it was observed that mild reduction and alkylation of polyalanylated γG(T)[1] resulted in the spontaneous dissociation of the proteins into subunits in neutral aqueous solution, that is, in the absence of any additional dissociating solute. This observation was the basis for the further investigation whose results are described in this report. Extensively alanylated γG(T) anti-Lac antibody and γG(T) diph-

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† Present address: Department of Oral Biology, School of Dentistry, State University of New York at Buffalo, Buffalo, N. Y.

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: Lac, *p*-azophenyl β-lactoside; Hy, hemocyanin; PSS, 0.15 M NaCl–0.02 M phosphate buffer (pH 7.4); *S*-CmC, *S*-carboxymethylcysteine; Lac dye, *p*-(*p*-dimethylaminobenzeneazo)phenyl β-lactoside; γG(T), T component of equine antiserum (Weir *et al.*, 1966).

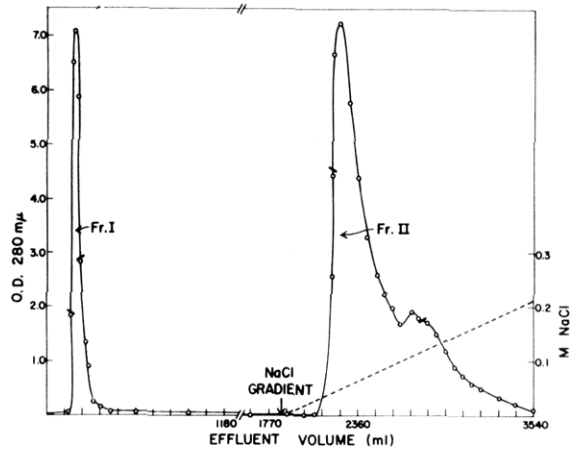

FIGURE 1: Purification of γG and γG(T) from the 7S fraction of diphtheria antitoxin by DEAE-cellulose chromatography. The starting solvent was 0.01 M sodium phosphate (pH 7.95) and 0.002 M EDTA followed by the addition of NaCl as indicated. The pools designated fraction I and fraction II were taken as γG and γG(T), respectively.

theria antitoxin were used for the preparation of their monomeric subunits by mild reduction and alkylation. The separated subunits have been studied with respect to their antigenic characteristics, their conformational properties as revealed by optical rotatory dispersion, and the binding of hapten.

Materials and Methods

Equine Anti-Lac Antibody. This antibody was purified from antiserum obtained by immunization of a 500-kg horse with hemocyanin (Hy) (*Limulus polyphemus*) to which had been coupled the Lac haptenic group. The description of the preparation of the antigen and the immunization procedure have been given earlier (Klinman *et al.*, 1966). The anti-Lac-containing serum was nonprecipitating when tested with a series of Lac-coupled proteins, including human serum albumin carrying 15–20 Lac groups/molecule.

Because of the nonprecipitability of the antiserum, the anti-Lac antibody was purified by coprecipitation of the anti-Lac and antihemocyanin antibodies with Lac-Hy. The anti-Lac antibody was then extracted from the precipitate in 0.5 M lactose. The detailed procedure has been described previously (Klinman *et al.*, 1966). The purified antibody was free of detectable antigen (Lac-Hy) as judged by optical density at 365 mμ and analysis by double diffusion in gel with antihemocyanin antiserum. Immunoelectrophoresis revealed no serum protein other than immunoglobulins. Antihemocyanin antibody was detectable in the anti-Lac preparation but it constituted less than 5% of the total antibody.

The fractionation of the anti-Lac antibody into γG and γG(T) was achieved by column chromatography with DEAE-cellulose as previously described (Klinman and Karush, 1967). The γG(T) fraction was found to contain not more than 8% of γG when precipitin analysis of this fraction was carried out with rabbit antiserum specific for γG(T).

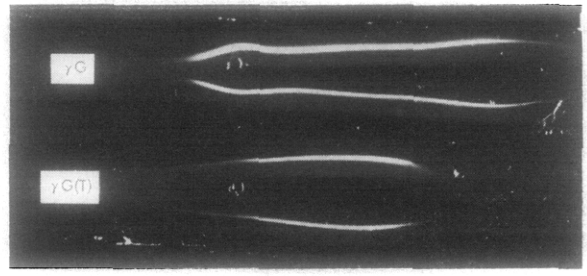

FIGURE 2: Immunoelectrophoretic analysis of γG and γG(T) with rabbit antiserum to equine heavy chains.

Preparation of γG(T) from Diphtheria Antitoxin. A commercial preparation of diphtheria antitoxin (Wyeth Laboratories, Inc., Marietta, Pa.) was fractionated by gel filtration through Sephadex G-150 in PSS (0.15 M NaCl–0.02 M phosphate buffer, pH 7.4) containing 0.002 M EDTA. The 7S fraction, containing most of the original γG and γG(T) proteins, was subjected to further fractionation by chromatography with DEAE-cellulose by a procedure similar to that employed for the anti-Lac antibody. The elution pattern observed is shown in Figure 1. The γG and γG(T) fractions were examined by immunoelectrophoresis with rabbit antiserum to equine serum (Figure 2).

Preparation of Polyalanylated Proteins. The alanylation of the immunoglobulins was done by the procedure described by Fuchs and Sela (1965) as modified by Karush and Sela (1967). The anhydride of *N*-carboxy-DL-alanine was used at a weight ratio to protein of 5:1. The alanylated products were stored in the lyophilized state or in the frozen state at −20°.

Mild Reduction and Alkylation. The reduction of protein cystines in the alanylated samples was carried out with two thiol reagents. In the case of PA-γG(T) anti-Lac antibody, 0.14 M redistilled 2-mercaptoethanol was used as previously described (Karush and Sela, 1967). With polyalanylated γG(T) antitoxin, reduction was effected with dithiothreitol (Cleland, 1964) over a concentration range of 4–16 × 10^{-3} M. Other reaction conditions were the same in both cases. Recrystallized iodoacetate was used for reaction with the protein sulfhydryl groups.

Alanine Enrichment and S-CMC Content. The number of alanine residues acquired by the alanylated protein was determined by amino acid analysis. The sample was hydrolyzed under reduced pressure in constant-boiling HCl (6 N) for 24 hr at 110° (Spackman *et al.*, 1958) and analyzed with an automatic amino acid analyzer. The analysis for *S*-CMC content was done in the same way with correction for decomposition. This correction was made by extrapolating the measured values at 24, 48, and 72 hr to the value corresponding to zero hydrolysis time.

Chain Separation by Gel Filtration. The fractionation into heavy- and light-chain populations of polyalanylated proteins following mild reduction and alkylation was achieved by gel filtration through a column (2.5 × 50 cm) of Sephadex G-200. The filtration was carried out at room temperature in PSS with 0.002 M EDTA. The pools containing the protein fractions were concentrated by ultrafiltration.

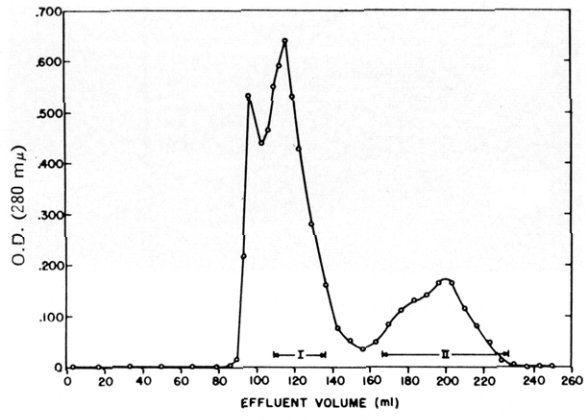

FIGURE 3: Gel filtration in PSS of mildly reduced and alkylated polyalanylated γG(T) anti-Lac antibody which had been reduced with 0.14 M mercaptoethanol. Pools I and II were used for the analysis and identification of the respective peaks. Mildly reduced and alkylated polyalanylated γG(T) diphtheria antitoxin gave a similar pattern on gel filtration.

Sedimentation Analysis. Measurements of sedimentation velocity were made in the Spinco Model E analytical ultracentrifuge at 20–22° with the schlieren optical system. The runs were made at a speed of 59,780 rpm and at protein concentrations ranging from 3 to 10 mg/ml.

Antibody Binding of Hapten. The specific binding of the Lac dye was measured by equilibrium dialysis following a procedure similar to that previously described (Karush and Sela, 1967).

Optical Rotatory Dispersion. Optical rotation was measured from 250 to 210 mμ in a Durum-Jasco spectropolarimeter with a protein concentration of about 1 mg/ml. The solvent was 0.04 M potassium phosphate buffer (pH 7.4) and its rotation was subtracted from that of the protein solution. Protein concentrations were determined by optical density measurement at 280 mμ using the following values of $E^{1\%}_{1\,cm}$ (Rockey, 1967): 14.8 for γG(T), 15.2 for the heavy chains of γG(T), and 13.6 for the light chains of γG(T). The concentrations calculated on this basis do not include the additional mass of the DL-alanyl peptides. Since they do not contribute to the optical rotation, nor the absorption at 280 mμ, a mean residue weight, M, of 108 was used corresponding to nonalanylated rabbit γG (Crumpton and Wilkinson, 1963). The same value of M was assumed for the separated chains. The measured rotation was converted into mean residue rotation, $[R']_\lambda$, using the following relation between $[R']_\lambda$ and the specific rotation, $[\alpha]_\lambda$

$$[R']_\lambda = \frac{3M[\alpha]_\lambda}{100(n^2 + 2)}$$

The value of the refractive index, n, was taken as 1.37 for the wavelength range used (Fasman, 1963).

Results

Dissociation of Mildly Reduced and Alkylated Polyalanylated γG(T). The observation in the analytical ultracentrifuge of the spontaneous dissociation of mildly reduced and alkylated polyalanylated γG(T) anti-Lac antibody in neutral solvent (Karush and Sela, 1967) was the basis for the isolation of the subunits in presumably undenatured form. Gel filtration with Sephadex G-200 in PSS gave the pattern shown in Figure 3. The minor peak which is associated with the leading edge of the first major peak was due to the undissociated impurity of mildly reduced and alkylated polyalanylated γG. Its presence was due to the original 8% contamination of the γG(T) anti-Lac preparation with γG anti-Lac antibody. After correction for the γG component, the recovery of protein nitrogen in the first major peak was 73% and in the second 27%. A similar gel filtration pattern was obtained with the mildly reduced and alkylated polyalanylated γG(T) derivative of the diphtheria antitoxin.

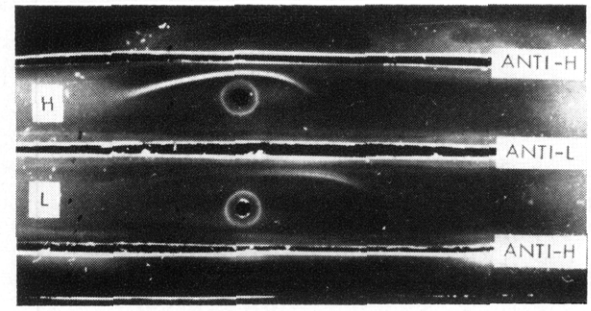

FIGURE 4: Immunoelectrophoretic analysis of fractions I (heavy chain, H) and II (light chain, L) of Figure 3 with rabbit antisera specific for heavy chains (anti-H) and light chains (anti-L).

Further characterization of the separated fractions was provided by examination of the two effluent pools indicated by I and II in Figure 3. Immunoelectrophoretic analysis with rabbit antisera specific for equine heavy chains and light chains demonstrated, as shown in Figure 4, that separation had been achieved between the heavy chain (fraction I) and the light chain (fraction II). The monomeric state of these separated subunits was established by analytical sedimentation of the fractions in the same solvent employed for gel filtration. These gave symmetrical sedimentation peaks (Figure 5) with $s_{20,w}$ values of 3.36 and 2.03 S for the heavy and light chains, respectively. These values correspond to the monomeric form of the subunits. The fractions obtained by gel filtration of the antitoxin derivative exhibited parallel behavior in immunoelectrophoresis and in analytical ultracentrifugation. The values of $s_{20,w}$ for the polyalanylated heavy and light chains of the antitoxin were 3.7 and 2.1 S, respectively. The reduction in this case was also carried out with 0.14 M mercaptoethanol.

The Reductive Cleavage of Polyalanylated γG(T). For the most effective utilization of the subunit preparations described above it is desirable to employ reduction conditions which cleave only interchain disulfides. Analysis for the *S*-CMC content of the above preparations of anti-Lac light and heavy chains, resulting from reduction with 0.14 M mercaptoethanol, gave a value of 5.9 mol of *S*-CMC/50,000 g of heavy chain and 1.7/20,000 g of light chain. These figures indicated that split-

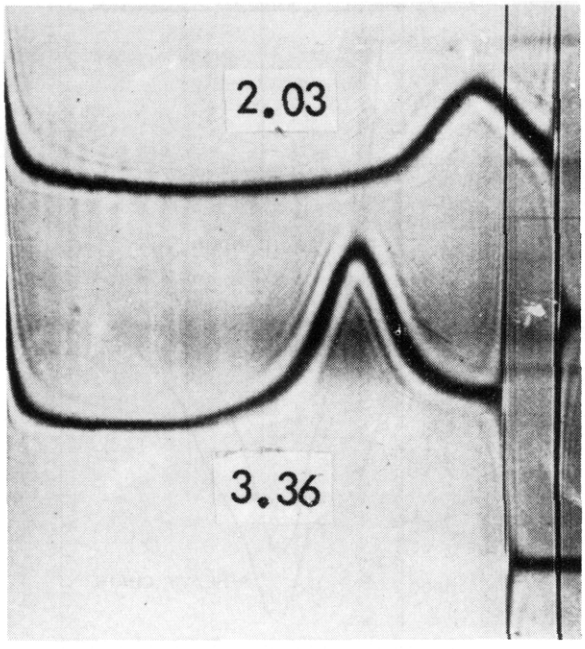

FIGURE 5: Analytical ultracentrifugation in PSS of the heavy-chain (I) and light-chain (II) fractions of Figure 3. The values of $s_{20,w}$ are shown for the heavy chain (lower) and the light chain (upper).

ting of some intrachain disulfides had occurred. In order to establish more appropriate reduction conditions a study was made of the number of disulfides cleaved as a function of the concentration of dithiothreitol with polyalanylated γG(T) diphtheria antitoxin.

The results of this study, shown in Figure 6, demonstrate that a plateau was reached in the number of *S*-CMC groups found and that the plateau value was obtained within the concentration range of dithiothreitol of 1–2 × 10^{-2} M. For purposes of comparison PA-γG(T) anti-Lac antibody was reduced under the same conditions with 1 × 10^{-2} M dithiothreitol. The number of resulting *S*-CMC groups, as seen in Figure 6, was the same as the plateau value, thus indicating that the antitoxin and anti-Lac antibody are equally susceptible to reduction. It may be presumed therefore that the curve of Figure 6 is probably generally applicable to equine γG(T)-immunoglobulin. The sedimentation pattern observed with the above preparation of reduced polyalanylated γG(T) anti-Lac antibody was similar to that resulting from reduction with 0.14 M mercaptoethanol (Karush and Sela, 1967).

For the calculation of the number of disulfides cleaved a control experiment was carried out involving the alkylation of γG(T) diphtheria antitoxin in neutral buffer without prior reduction. Since no *S*-CMC was detected in the subsequent amino acid analysis of the sample, the observed values of *S*-CMC content can be directly related to the number of disulfides split. Thus it appears that the plateau value corresponds to the cleavage of six disulfides per molecule. Since the number of interchain disulfides of γG(T) probably does not exceed four (Weir and Porter, 1966), reduction to the plateau value involves the cleavage of intrachain disulfides as well.

In view of this intrachain scission an attempt was

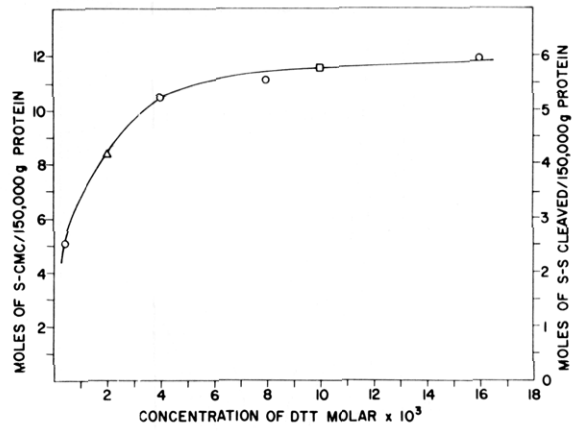

FIGURE 6: The content of *S*-CMC of samples of polyalanylated γG(T) reduced with various concentrations of dithiothreitol. (○) Diphtheria antitoxin and *S*-CMC by amino acid analysis; (△) portion of reduced sample assayed for sulfhydryl content by the Ellman method (Ellman, 1959); (□) anti-Lac antibody and *S*-CMC by amino acid analysis.

made to prepare subunits from polyalanylated γG(T) anti-Lac antibody using a concentration of dithiothreitol, namely, 2 × 10^{-3} M, which would be expected to result in the cleavage of only four disulfides. The resulting reaction mixture, after alkylation, was fractionated by gel filtration through Sephadex G-200 in PSS with 2 × 10^{-3} M EDTA. Two fractions were obtained and were analyzed by immunoelectrophoresis with rabbit antisera to equine heavy and light chains. The major portion of the first peak was identified as heavy chain and the second peak gave evidence only of light chain. However, the low yield of light chain in the second peak (16.5% protein nitrogen) and the detection of light chain in the first peak indicated that not all of the interchain bonds between heavy and light chains had been split. Evidently some of the intrachain disulfides are more labile than these interchain bonds. In view of this difficulty the samples of heavy and light chains from γG(T) antitoxin used for the measurement of optical rotatory dispersion were prepared with 1 × 10^{-2} M dithiothreitol under the same conditions which gave the results shown in Figure 6. Their alanine enrichment is shown in Table I.

TABLE I: Alanine Enrichment of the Subunits of the Polyalanylated γG(T) Proteins.

Sample	Additional Alanine (residues/mole)[a]
Heavy chain of antitoxin	166
Light chain of antitoxin	72
Heavy chain of anti-Lac antibody	141
Light chain of anti-Lac antibody	53

[a] Calculated on the basis of molecular weights of 50,000 and 20,000 for the heavy and light chains, respectively.

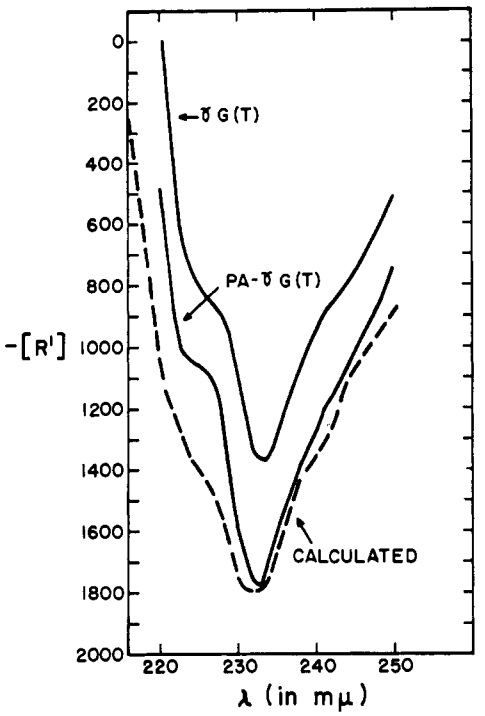

FIGURE 7: Optical rotatory dispersion curves for γG(T) diphtheria antitoxin, the polyalanylated γG(T) derivative, and a calculated curve for an equimolar mixture of polyalanylated heavy and light chains. The calculated curve was based on the dispersion curves of the isolated polyalanylated subunits (Figure 8) using molecular weights of 50,000 and 22,000 for the equivalent nonalanylated heavy and light chains, respectively.

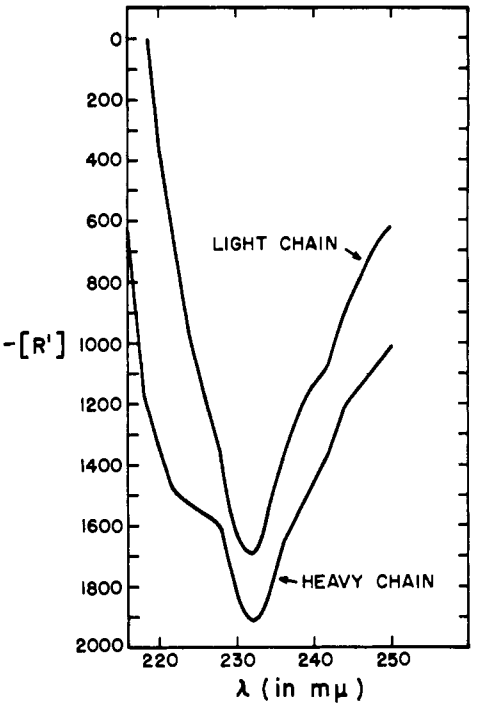

FIGURE 8: Optical rotatory dispersion curves for the polyalanylated heavy and light chains obtained by mild reduction and alkylation of polyalanylated γG(T) diphtheria antitoxin.

Binding of Hapten. Measurement of the binding of Lac dye by the heavy-chain fraction derived from polyalanylated γG(T) anti-Lac antibody showed a large decrease in association constant. Whereas the original γG(T) antibody had an association constant of 1.8×10^7 l./mole and the parent polyalanylated γG(T) antibody an estimated association constant of 1×10^7 l./mole, the value for the heavy chain was at least 100-fold less than the latter figure.

Optical Rotatory Dispersion. The dispersion curves of γG(T) antitoxin (Figure 7) and γG(T) anti-Lac antibody (Figure 9) exhibit several features in common. Both possess deep troughs at 233 mμ and show small Cotton effects at about 224 and 241 mμ. Polyalanylation of the γG(T) antitoxin did not alter the dispersion curve qualitatively but served to lower the entire curve and to render the Cotton effect at 224 mμ more distinct (Figure 7).

The dispersion curves for the light and heavy chains of polyalanylated γG(T) antitoxin (Figure 8) retain the characteristic feature of a deep trough at about 231 mμ but differ with respect to their behavior at lower wavelengths. The heavy chain exhibits an accentuated Cotton effect at about 224 mμ whereas the light chain shows none. A calculated curve for the reconstituted molecule based on the dispersion curves of the isolated polyalanylated subunits is shown in Figure 7. For the purpose of the calculation molecular weights of 50,000 and 22,000 were used for the heavy chain and light chain, respectively, excluding the mass of the additional alanine. Comparison of the calculated curve with that observed with the parent polyalanylated γG(T) molecule shows close agreement except for the striking difference in the region below 230 mμ.

The examination of the derivatives of γG(T) anti-Lac antibody was limited because of lack of materials. A comparison is shown in Figure 9 between γG(T) anti-Lac antibody and the heavy chain obtained from the polyalanylated product. The main difference is a more pronounced Cotton effect in the 224-mμ region as is also apparent from a similar comparison in the case of the antitoxin (Figures 7 and 8). In contrast to the antitoxin, however, there is a much smaller downward displacement of the dispersion curve, as reflected, for example, in the much greater levorotation at 233 mμ of the heavy chain of the antitoxin (1920°) than that of the antibody heavy chain (1520°).

Discussion

The pattern of optical rotatory dispersion in the range from 220 to 250 mμ observed here for equine γG(T)-immunoglobulin exhibits considerable similarity to that found with rabbit γG-immunoglobulin (Steiner and Lowey, 1966) and human γG-immunoglobulin (Dorrington *et al.*, 1967). The common features include a trough at 230–233 mμ, a minimum or shoulder at about 225 mμ, and a shoulder at about 240 mμ. The quantitative relations among these features, however, do vary both with respect to antibodies of different specificity within a species as well as between species. The limited comparison available in this study, between anti-

Lac γG(T) and antitoxin γG(T), shows very close agreement between the dispersion curves.

Particularly useful information is provided by the dispersion curves of the alanylated and separated heavy and light chains of the antitoxin (Figure 8). Since their preparation did not involve the use of denaturing solutes, *e.g.*, propionic acid, their dispersion curves aid in ascertaining the existence of structural features of the original molecules which depend upon light-chain–heavy-chain interaction. It may be noted first that the values of $[R']$ at the minimum (−1920 and −1680° for the heavy and light chains, respectively; see Table II) differ

TABLE II: Values of the Reduced Mean Residue Rotation at the 224-mμ Shoulder and 233-mμ Trough for Equine γG(T) and Its Polyalanylated Derivatives.

Sample	$-[R']_{224\ m\mu}$	$-[R']_{233\ m\mu}$
Diphtheria antitoxin γG(T)	800	1370
Anti-Lac γG(T)	700	1440
Polyalanylated γG(T) antitoxin (experimental)	1080	1760
Polyalanylated γG(T) antitoxin (calculated)	1460	1800
Heavy chain of polyalanylated γG(T) antitoxin	1540	1920
Light chain of polyalanylated γG(T) antitoxin		1680
Heavy chain of polyalanylated γG(T) anti-Lac	1050	1520

very little from the value of −1760° for the alanylated parent molecule. This result is quite different from that reported for alanylated heavy and light chains of rabbit γG-immunoglobulin (Dorrington *et al.*, 1967). The heavy chains of rabbit γG, for example, showed a levorotation at the 230-mμ minimum about twice as large as that of the parent alanylated molecule. It appears quite likely that exposure to 1 M propionic acid, the condition used to dissociate the alanylated chains, resulted in a conformational change which was not fully reversed by adjustment to neutral pH.

The absence of extensive conformational change arising from the dissociation of heavy and light chains of polyalanylated γG(T) is clearly evident from the similarity of the calculated curve for the equimolar mixture of light and heavy chains to the observed curve of the parent molecule (Figure 7). In particular the value of −1800° for $[R']$ at the 233-mμ minimum is virtually identical with the observed value of −1760°. Further support for the conclusion that dissociation is accompanied, at most, by limited conformational alteration, is provided by the ability of the dissociated chains to precipitate antibody from antiserum induced with γG(T).

The main difference between the calculated and experimental curves of Figure 7 is found in the wavelength

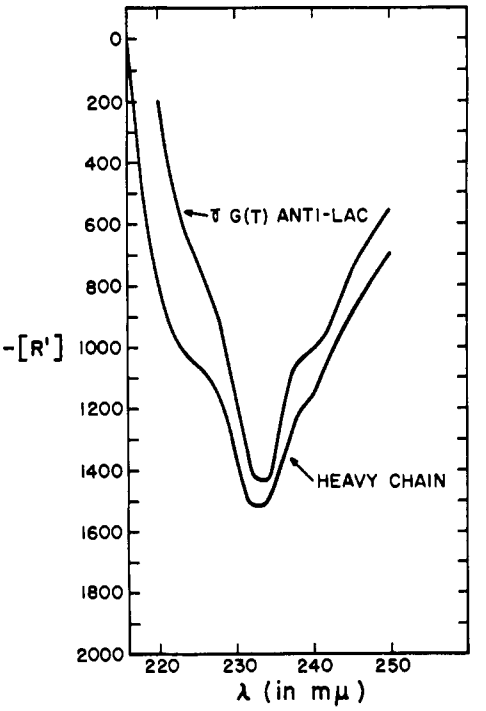

FIGURE 9: Optical rotatory dispersion curves for γG(T) anti-Lac antibody and for the polyalanylated heavy chain obtained by mild reduction and alkylation of the polyalanylated γG(T) antibody.

region below 233 mμ. In particular there is a much smaller Cotton effect at 224 mμ in the calculated curve than there is in the experimental one. This difference is of special interest since it is associated in rabbit γG with the Fab fragment and exhibits variation with immunological activity (Steiner and Lowey, 1966). It appears, therefore, that dissociation has brought about structural alteration in the N-terminal half of the heavy chain and/or the light chain and that the structure of the region of the molecule thus affected is intimately related to the antibody combining site. This view finds support in the optical rotatory dispersion study of the stabilization of antibody provided by specifically bound hapten (Cathou and Haber, 1967). It was concluded that the contact amino acid residues of the combining region are widely distributed along the peptide chains and are dependent for their functional juxtaposition upon the integrity of the tertiary structure.

Although a rigorous argument cannot be made with present information, it is likely that this structural alteration occurs in the N-terminal half of the heavy chain rather than in the light chain because the separated heavy chain exhibits a Cotton effect at 224 mμ whereas the light chain does not (Figure 8). If this view is valid, then we may conclude that the conformation of the N-terminal half of the heavy chain is, in part, imposed by the light chain. In any case we may infer that the light-chain–heavy-chain interaction does affect the conformation of the Fab part of the molecule. The possibility that the cleavage of the disulfide bond between the heavy and light chains may independently account for this conformational effect is rendered unlikely by the recovery of the original dispersion curve when reduced and alkyl-

ated human myeloma γG chains were recombined (Dorrington *et al.*, 1967).

The existence of such an interaction-dependent structure is, in all likelihood, related to the specificity of the light-chain–heavy-chain combination. It has been suggested that there are two noncovalent interaction sites (Dorrington *et al.*, 1967). One of these is relatively nonspecific and is responsible for random recombination of heavy and light chains as occurs, for example, with normal γG-immunoglobulin. The second is specific for complementary chains and provides recognition of chains synthesized in the same clone. The evidence for the existence of a recognition site comes from the preferential recombination of complementary chains as shown, for example, with a myeloma γG (Grey and Mannik, 1965; Mannik, 1967). Further support is provided by the finding that only in the recombination of the heavy and light chains of a myeloma γG was the original optical rotatory dispersion curve restored (Dorrington *et al.*, 1967). In view of the latter finding we would tentatively conclude that the interaction-dependent structure associated with the 224-mμ Cotton effect requires the specific interaction between complementary chains for its manifestation.

The greatly reduced binding of Lac dye by the polyalanylated heavy chain from γG(T) anti-Lac antibody carries with it several implications arising from the monomeric form of the heavy chain and the avoidance of a denaturing solute. The loss or reduction of specific binding which is generally observed with isolated heavy chains from antibody (Fleischman, 1966) cannot be primarily attributed to aggregation of the chains although aggregation does often occur and may be a contributing factor. Furthermore, the structural alteration of the heavy chain resulting from its exposure to a denaturing solute, the usual condition employed, cannot be the sole cause of loss of activity. Again, such a change may be a contributory factor since the heavy chain isolated by the propionic acid procedure does exhibit a greatly altered dispersion curve compared with the parent molecule (Dorrington *et al.*, 1967) and compared with the heavy chain described in this study.

An alternative interpretation of the loss of activity and the associated role of the light chain is provided by the interaction-dependent structure. Although the direct participation of the light chain in the combining site of the antibody cannot be excluded, the present results provide suggestive evidence that the light chain serves to stabilize that aspect of the conformation of the heavy chain associated with its combining region. This stabilization is presumed to involve the specific complementary interaction between light and heavy chains. Thus, not only is binding reduced by removal of the light chain but, as emphasized by Dorrington *et al.* (1967), random recombination of heavy and light chains would not fully restore the original conformation. More specifically, that aspect of structure associated with the Cotton effect at 224 mμ would not be recovered. Further studies with a variety of γG(T) antibodies are necessary to evaluate the validity of the proposed role of the light chain.

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