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Gross Conformation of Free Polypeptide Chains from Rabbit Immunoglobulin G. I. Heavy Chain*

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ABSTRACT: Heavy chain, isolated by gel chromatography in propionic acid, was renatured by dialysis against 0.01 M sodium acetate buffer (pH 5.5). Under these conditions aggregation is slow, but increasing ionic strength and pH causes the formation of high molecular weight material, which must be removed by gel chromatography prior to measurement. The molecular weight of free heavy chain is 107,000–117,000 at pH 5.5–8.5; it is thus a dimer at near-neutral pH. (At pH 2.5 or in 6 M Gdn·HCl (guanidine hydrochloride) at pH 7, monomers are formed.) From an $s^0_{20,w}$ value of 5.7 S, f/f_{min} is calculated to be about 1.4, which exceeds the value for typical globular proteins, but is similar to f/f_{min} for native IgG (immunoglobulin G). The optical rotatory dispersion spectrum shows features reminiscent of native IgG, namely a small Cotton effect at 240 mμ, a minimum at 231 mμ ($[m']$ = −1750) and a shoulder at 225 mμ, but the mean residue rotation is more negative than that of the parent protein. Papain cleaves the molecule into two main parts, each with a native molecular weight of about 50,000, dissociating to half this value in 6 M Gdn·HCl. Small amounts of fragments with molecular weights in 6 M Gdn·HCl of about 12,000 and 1000 were also present, which indicates some further cleavage of the chains. Consideration of these results suggests that free heavy chain is a dimer, held together by interactions in both the Fc and Fd regions, and with the flexible "hinge" region preserved.

Immunoglobulin G[1] consists of two heavy and two light polypeptide chains, which are held together both by disulfide bonds and by noncovalent interactions. These attractive forces can be broken in several ways, and the chains can be separated from each other (Edelman, 1959; Edelman and Poulik, 1961; Fleischman *et al.*, 1962; Utsumi and Karush, 1964). The properties of the isolated chains can then be studies. Such studies are of great importance for the evaluation of the contributions of the individual chains to the structure and activity of the whole immunoglobulin molecule. Extensive studies of amino acid sequences (Lennox and Cohn, 1967; Edelman and Gall, 1969) and of antigen-binding properties (Fleischman, 1966; Porter and Weir, 1966; Haber and Richards, 1966; Yoo *et al.*, 1967) of the isolated chains have been performed, but the molecular size and shape and the conformation of free chains have not been well investigated. The purpose of this work has been to characterize isolated heavy chains in these respects. Rabbit IgG was chosen as the parent protein so that these investigations might be correlated with other studies on the antigen-binding properties of heavy chain from rabbit antibodies. Our results indicate that free heavy chain is a dimer, stabilized by attractive forces not only between the two Fc regions, as would be expected from the structure of native IgG, but also between the two Fd segments, which are associated with light chain in the original IgG molecule. Moreover, the flexible "hinge" region, which exists in heavy chain when it forms part of whole IgG, seems to be retained in the isolated chain.

Materials and Methods

IgG was isolated from pooled rabbit serum by precipitation with 35% saturated ammonium sulfate and subsequent chromatography on DEAE-cellulose (Levy and Sober, 1960). It was stored at −20° as a precipitate in 80% ammonium sulfate. Immunoelectrophoresis (see details below) gave only one precipitin arc, but sedimentation velocity studies showed the presence of about 5% of a component sedimenting faster than the main 6.7S peak.

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[1] The nomenclature of and the abbreviations for the immunoglobulins and their subunits produced by reduction and proteolysis are those recommended by the World Health Organization (1964).

Mild reduction of IgG was performed in 0.5 M Tris buffer (pH 8.2) with 0.2 M mercaptoethanol for 2 hr at room temperature, followed by carboxymethylation with an equimolar amount of recrystallized iodoacetamide at 0° for 1 hr. The pH was kept at 8.2 by the addition of 1 M NaOH. The solution was then dialyzed, first against 0.1 M NaCl and then extensively against distilled water at 4°, and was stored frozen at −20°.

Heavy chain was isolated by gel chromatography in 1 M propionic acid (Fleischman *et al.*, 1962). A solution of 200 mg of reduced and carboxymethylated IgG in 10 ml of water was made 1 M in propionic acid by the addition of concentrated acid. After 2 hr the solution was applied to a 2.5 × 90 cm column of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, N. J.) in 1 M propionic acid and the column was eluted at a rate of 15 ml/hr. Fractions of 5 ml were collected. The time the solution was kept in 1 M propionic acid before it was applied to the column was increased in some experiments in order to demonstrate the effect of propionic acid on the elution position of heavy chains.

The heavy chain prepared in this manner was "renatured" by dialysis of the solution (about 50 ml) against three changes of 2000 ml of 0.01 M sodium acetate buffer (pH 5.5) followed by 2000 ml of 0.02 M sodium acetate buffer (pH 5.5) containing 0.1 M sodium chloride. It was then concentrated by ultrafiltration through a Diaflo UM 20E membrane (Amicon Corp., Lexington, Mass.) to a volume of about 10 ml and, in order to remove aggregated material, the solution was applied to a column (2.5 × 90 cm) of Sephadex G-200 in 0.02 M sodium acetate buffer (pH 5.5) plus 0.1 M NaCl. The elution rate of this column was 20 ml/hr and 5-ml fractions were collected. The purified heavy chain was finally concentrated by ultrafiltration for subsequent analyses.

All preparative procedures were carried out at 5°.

Double diffusion and immunoelectrophoresis were done on microscope slides in agar using the LKB Immunophor Model 6800A (LKB Instruments, Rockville, Md.) according to the standard LKB procedures. Goat antisera to rabbit IgG, to its Fab and Fc portions, obtained by papain digestion (Porter, 1959), and to rabbit light chain were prepared in the following manner. A primary immunization of 20 mg of protein in Freund's complete adjuvant was followed 1 month later by a similar injection. Injections were intramuscular and at several sites. Blood was collected from the jugular vein 2 weeks after the second course, and the serum was stored frozen at −20° until used. One goat antiserum directed against rabbit light chain was kindly donated by Dr. H. Eisen. Immunoelectrophoresis of whole rabbit serum against the goat anti-rabbit IgG showed a single precipitin arc corresponding to IgG. This antiserum reacts well with both the Fab and Fc fragments, whereas the anti-Fab reacts only with Fab and the anti-Fc only with the Fc fragment.

Sedimentation velocity experiments at different protein concentrations were performed in a Spinco Model E ultracentrifuge (Beckman Instruments, Palo Alto, Calif.) equipped with a phase-plate schlieren optical system. Both a 12-mm 4-deg single-sector cell in an AnD rotor at 56,100 rpm and a 30-mm 2.5-deg double-sector cell in an AnE rotor at 50,740 rpm were used. The cells were filled at 5° and slowly brought up to room temperature in order to avoid aggregation of heavy chain. The temperature was regulated at 25° during the run. The sedimentation coefficient was calculated from the slope of a plot of ln x (where x is the distance from the center of rotation to the peak) *vs.* time, and this observed value was converted to $s_{20,w}$ in the usual manner (Svedberg and Pedersen, 1940).

Molecular weights were determined in sedimentation equilibrium experiments using the meniscus depletion method described by Yphantis (1964). A Spinco Model E ultracentrifuge with an interference optical system was used. In all except a few initial experiments the temperature was 5°. Several runs were made with varying speeds, protein concentrations and solvents. Proteins in dilute salt solutions were dialyzed for 16 hr, but experiments performed in 6 M Gdn·HCl[2] (obtained from Heico Inc., Delaware Water Gap, Pa.) required at least 3-days dialysis. A 2.5-deg double-sector cell and a six-channel cell (Yphantis, 1964), both with sapphire windows, were used and were filled to a column height of 3 mm. Equilibrium times were estimated from the semi-empirical equation given by Yphantis (1964), and attainment of equilibrium was checked by measuring the fringe displacements at several radial distances of two successive exposures taken 2–4 hr apart. Measurements of fringe displacement (f) against distance from the center of rotation (r) were made with a Gaertner microcomparator (Gaertner Scientific Corp., Chicago, Ill.), and molecular weights (M) were calculated from the standard equation

$$\frac{\mathrm{d} \ln f}{\mathrm{d}r^2} = \frac{M(1 - \bar{v}\rho)\omega^2}{2RT}$$

i.e., from the slope of a plot of ln f *vs.* r^2. The remaining symbols in this equation have their usual meaning (Yphantis, 1964).

The value for the partial specific volume ($\bar{v}$) was calculated from the amino acid composition of rabbit heavy chain (Crumpton and Wilkinson, 1963) and was found to be 0.73. Since this is only an approximation, the same value was also used for the effective partial specific volume in 6 M Gdn·HCl. Pycnometric measurements of $\bar{v}$ were not feasible because of the rather low solubility of heavy chain.

Optical rotatory dispersion and circular dichroism spectra were measured with a Cary Model 60 recording spectropolarimeter (Cary Instruments, Monrovia, Calif.) equipped with a Model 6001 circular dichroism attachment. The proteins studied were dissolved in 0.01 M NaCl, pH 5.5 or 2.5. The optical rotatory dispersion spectra were recorded at 25.0 or 5.0°, using protein concentrations of 0.05–1.0 mg/ml and cells with from 5-cm to 0.5-mm path length. The optical density was never allowed to exceed 1.5. The circular dichroism spectra were measured only at 25–27°, since no thermostatted cell holder was available, and the optical density was always kept below 1.0. The results are given as plots of reduced mean residue rotation $[m']_\lambda$ or reduced mean residue ellipticity $[\theta']_\lambda$ *vs.* wavelength. These two parameters are obtained from

$$[m']_\lambda = \frac{3}{n_\lambda^2 + 2} \frac{M_0 \alpha_\lambda}{100dc}$$

$$[\theta']_\lambda = \frac{3}{n_\lambda^2 + 2} \frac{M_0 \theta_\lambda}{100dc}$$

where α_λ is the observed rotation and θ_λ the observed ellipticity, both at wavelength λ, d is the cell path length in deci-

[2] Abbreviation used is: Gdn·HCl, guanidine hydrochloride (this is not the preferred abbreviation of the authors).

meters, c the protein concentration in grams per cubic centimeters, and n_λ the refractive index of the solvent at the wavelength in question (the values for water were used for the latter parameter). The mean residue weight, M_0, was taken as 108 for both nonspecific rabbit IgG and heavy chain (Dorrington *et al.*, 1967).

Gel chromatography of fully reduced and carboxymethylated proteins in 6 M Gdn·HCl was performed as described by Fish *et al.* (1969). After reduction of the proteins for 4 hr with 0.2 M mercaptoethanol at pH 8.6, followed by alkylation with a slight excess of iodoacetic acid of the same pH, about 2.5 mg of protein in 0.15–0.20 ml of solution was applied to a column (1.5 × 85 cm) of Bio-Gel A-5M (Bio-Rad Laboratories, Richmond, Calif.; agarose content 6%) in 6 M Gdn·HCl. The effluent was monitored by absorbance at 280 mμ. Calibration experiments with the same proteins of known molecular weights as used by Fish *et al.* (1969) were run periodically. The results of these were plotted as the distribution coefficient, K_d, *vs.* the logarithm of the molecular weight. The approximate molecular weight of an unknown polypeptide chain can be estimated from its K_d by interpolation in such a standard curve.

Papain digestion of heavy chains was performed after a 5-hr dialysis at 5° of the protein solutions (2 mg/ml) against 0.1 M phosphate buffer (pH 7). The solution was slowly brought to room temperature and EDTA and mercaptoethanol were added to final concentrations of 0.002 and 0.01 M, respectively. In order to avoid precipitation of the heavy chains, papain (Worthington Biochemicals, Freehold, N. J.) was added at room temperature and the solution was then placed in a water bath at 37°. A papain to protein ratio of 1:200 (wt/wt) was used. The digestion was allowed to continue for 15 min and was stopped by cooling and by the addition of iodoacetamide to a concentration of 0.011 M. The solution was then lyophilized for gel chromatography in Gdn·HCl, or dialyzed for 24 hr against 0.02 M sodium acetate buffer, pH 4.5, +0.1 M NaCl, and concentrated for gel chromatography on Sephadex G-100. The latter was performed in a 2.5 × 55 cm column, whose flow rate was 15 ml/hr; 3-ml fractions were collected.

All protein concentrations were determined spectrophotometrically in a Cary 15 spectrophotometer (Cary Instruments, Monrovia, Calif.). Extinction coefficients of both rabbit IgG and its heavy chain were determined after 48-hr dialysis against 0.01 M acetate buffer (pH 5.5). Aliquots of the protein solution and the dialysate were dried at 105° to constant weight, and the amount of protein in solution was obtained as the difference between these two values. About 8 mg of protein was used for each determination. Other aliquots of the protein solution were diluted and the absorbance was determined. The extinction coefficient at 280 mμ of a 1% (wt/v) solution in a 1-cm cell was found to be 13.8 ± 0.1 (5 determinations) for rabbit IgG and 14.4 ± 0.1 (9 determinations) for rabbit heavy chain.

Results

Preparation. Heavy chain shows a pronounced tendency to aggregate and even precipitate in dilute salt solution around neutral pH. This complication can partly be overcome by polyalanylation (Fuchs and Sela, 1965), which renders the heavy chain soluble at high concentrations. Preliminary experiments, however, showed that this treatment did not satisfactorily prevent the polymerization of the chains. It was also felt that the introduction of poly-D,L-alanine chains on the surface of the molecule would unnecessarily complicate the interpretation of hydrodynamic data. We have therefore developed conditions, under which unmodified heavy chain can be isolated and studied with minimum aggregation.

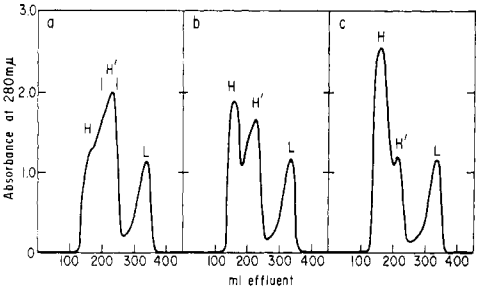

FIGURE 1: Gel chromatography of mildly reduced and carboxymethylated IgG on Sephadex G-100 in 1 M propionic acid. 10-ml sample (20 mg/ml) on 2.5 × 90 cm column. Sample applied (a) 2 hr, (b) 15 hr, and (c) 60 hr after the addition of propionic acid. The vertical lines indicate those fractions used for further analyses.

Figure 1 shows that prolonged exposure to 1 M propionic acid during the separation of the heavy chain from the light chain by gel chromatography in this solvent has to be avoided. Two heavy chain peaks (called H and H′ by Metzger and Mannik, 1964) are seen in all three chromatograms, but the first one of these, H, progressively increases in amount as the reduced and carboxymethylated IgG is stored in 1 M propionic acid before it is applied to the column. In all three chromatograms the proportions between the combined heavy-chain peaks and the light-chain peak (L) is constant, *i.e.*, slightly larger than 2:1, when differences in extinction coefficient are taken into account. The first peak, H, presumably contains polymers of heavy chain (Olins and Edelman, 1964; Metzger and Mannik, 1964). When renatured and concentrated for subsequent gel chromatography at pH 5.5 (see below), 75% of the material in this peak precipitated and about half of the material still in solution was in the form of heavy-chain polymers. Under the same conditions only about 15–25% of the second peak, H′, precipitated and the supernatant contained only about 15–20% of heavy-chain aggregates. Routinely, therefore, chromatography of the reduced and carboxymethylated IgG was started 1–2 hr after the addition of propionic acid. Only that part of peak H′ indicated by the vertical lines in Figure 1 was used for all further studies, and renaturation (see below) was started as soon as this material had emerged from the gel chromatography column.

Renaturation of the heavy chain, *i.e.*, removal of the propionic acid, was routinely accomplished by extensive dialysis against 0.01 M sodium acetate buffer (pH 5.5). At this ionic strength and pH aggregation is minimized, and the heavy chain is soluble at fairly high concentrations, *i.e.*, 5–10 mg/ml. An increase in either pH or ionic strength causes more rapid formation of aggregates and subsequently precipitation occurs. However, renaturation at low ionic strength followed by an increase of the ionic strength resulted in significantly less aggregation than removal of the propionic acid directly at the higher ionic strength.

Since several physicochemical measurements must be performed at an ionic strength of at least 0.1 in order to avoid charge effects, the aggregates formed under these conditions must be removed. This was accomplished by gel chromatography of the renatured heavy chain on Sephadex

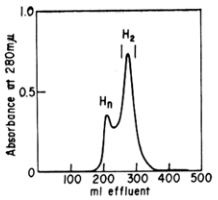

FIGURE 2: Gel chromatography of renatured heavy chain (peak H′ from Figure 1a) on Sephadex G-200 in 0.02 M sodium acetate buffer (pH 5.5) containing 0.1 M NaCl. 10-ml sample (3.8 mg/ml) on 2.5 × 90 cm column. The vertical lines indicate those fractions used for further analyses.

G-200 in 0.02 M acetate buffer (pH 5.5) and 0.1 M NaCl (Figure 2). The recovery in such a chromatography was 95–99%. The first peak, called H_n, contained various polymers of heavy chains as was evident from sedimentation velocity experiments. These showed one major peak with a sedimentation coefficient of about 13 S, but it was markedly skewed toward the bottom of the cell, indicating the presence of even higher aggregates. These polymers are noncovalently linked since 6 M Gdn·HCl reduced the molecular weight of peak H_n to about 55,000 as measured in a sedimentation equilibrium experiment. The second peak, designated H_2, contained heavy chain dimers (which will be further substantiated below), and it was used for all subsequent measurements. No peak of smaller molecular size was seen. When the dimer was concentrated and rechromatographed immediately, it appeared as one peak at its original place. When the concentrated solution (~3 mg/ml) was stored for 2–3 days and then rechromatographed, about 5–10% of aggregates reappeared. It was therefore essential to perform all measurements as soon as possible after this chromatographic step.

All preparative procedures were carried out at 5°. This is essential since exposure to higher temperatures tends to cause aggregation and even slow formation of visible precipitates.

The purification procedure described above entails some losses of material by precipitation and aggregation, and the final purified product represents only about 50% of the heavy chains originally present in the starting material. It is possible that some selectivity occurred in this way and that the results obtained may not be entirely representative of the totality of heavy polypeptide chains present in nonspecific rabbit IgG.

Purity. The purity of the heavy-chain preparation was investigated by immunodiffusion against goat anti-light-chain serum (Figure 3). At a concentration of 3 mg/ml, heavy chain gave no precipitin line, whereas both light-chain and native IgG gave clearly visible reactions at the highest dilutions

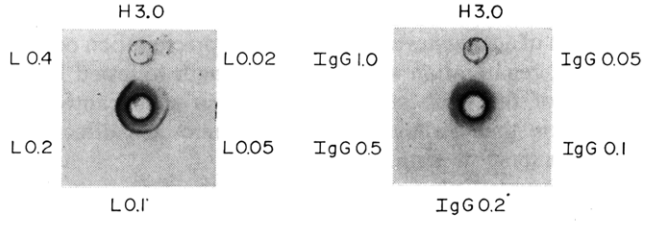

FIGURE 3: Immunodiffusion of rabbit heavy chain (H), light chain (L), and IgG against goat antirabbit light chain. The numbers indicate protein concentrations in milligrams per milliliter.

TABLE I: Molecular Weights of Heavy Chain under Different Conditions.

Solvent	Concn (mg/ml)	Speed (rpm)	Temp (°C)	Mol Wt
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.12	15,220	5	108,000
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.12	17,980	5	113,000
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.34	17,980	5	109,000
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.12	20,410	5	117,000
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.28	20,410	5	111,000
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.18	17,980	25	111,000[a]
0.02 M phosphate (pH 7.0) + 0.1 M NaCl	0.12	17,980	5	107,000[b]
0.02 M Tris (pH 8.5) + 0.1 M NaCl	0.12	17,980	5	112,000[b]
0.1 M NaCl (pH 2.5)	0.12	27,690	5	62,000
0.1 M NaCl (pH 2.5) + 2% sucrose	0.14	25,980	5	58,000
6 M Gdn·HCl (pH 7.0)	0.12	36,000	25	52,000

[a] Markedly curved plot. Molecular weight computed from initial slope. [b] Very slightly curved plot. Molecular weight computed from initial slope.

employed. By this analysis the heavy-chain preparation used should contain less than about 1% light chain and less than about 2% IgG. Another antiserum (donated by Dr. H. Eisen), however, gave evidence for the presence of about 3% light chain. The material in peak H_2 was also fully reduced and carboxymethylated and was passed through a column of 6% agarose in 6 M Gdn·HCl (Fish *et al.*, 1969). Under these conditions all polypeptide chains behave as linear random coils and determination of elution position off the column gives a theoretically valid and fairly accurate estimation of the molecular weight of the chain. The major part of the heavy-chain sample eluted as expected, *i.e.*, at a molecular weight of about 50,000, but 3–7% of the applied protein appeared in a peak with a molecular weight of about 25,000. Part of this material probably is light chain and the rest may be split heavy chain (Weir and Porter, 1966a). The presence of this small impurity should not have affected the results of subsequent measurements to any significant degree.

Molecular Weight. The molecular weight of peak H_2 was determined in sedimentation equilibrium experiments using the meniscus depletion method. Values ranging from 107,000 to 117,000 were obtained under different conditions (Table I). The plots of the logarithm of fringe displacement *vs.* r^2 for runs at pH 5.5 and at 5° were all linear from 50-μ displacement to the bottom of the cell, as shown in Figure 4a. This indicates that the samples were homogeneous, and that no appreciable aggregation had occurred. A few initial runs were performed at 25°, but the plots obtained were all markedly curved (Figure 4b), indicating that pronounced aggregation had taken place at this temperature. In spite of this, the molecular weight of the smallest component

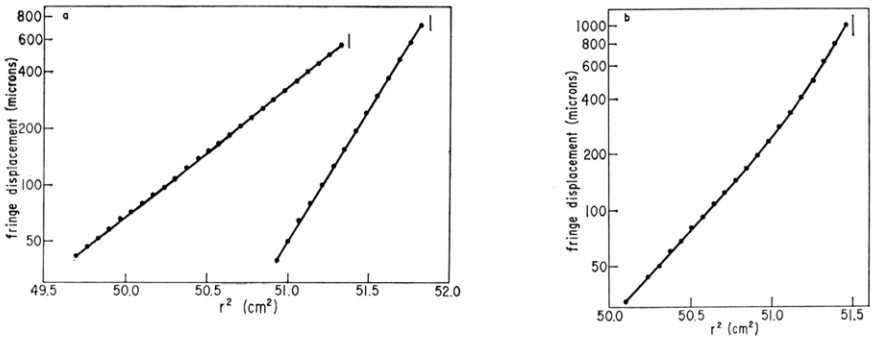

FIGURE 4: Sedimentation equilibrium of heavy chain in 0.02 M sodium acetate buffer (pH 5.5) containing 0.1 M NaCl. (a) 5°, 0.12 mg/ml; 15,200 rpm (left) and 20,410 rpm (right). (b) 25°, 0.18 mg/ml, 17,980 rpm. The vertical lines indicate the cell bottoms.

present (*i.e.*, the molecular weight calculated from the initial slope) was similar to the values obtained at the lower temperature. Experiments at higher pH values than 5.5 (at 5°) gave evidence for a low degree of aggregation with increasing pH.

It should be noted that the value for $\bar{v}$ used (0.73) had to be estimated from the amino acid composition, and thus it is only approximate. Evidence for this is the fact that the same calculated value was obtained for light chain, whereas the measured value for whole IgG is higher, *i.e.*, 0.738. No allowance for carbohydrate content was made in these calculations; if the carbohydrate were taken into account the value for $\bar{v}$ would be lower, but a quantitative estimate is difficult to make. The molecular weights given may therefore be reliable only to within ±10%.

Shape. Sedimentation coefficients of the heavy-chain dimer were determined at several protein concentrations and have been plotted as a function of concentration in Figure 5. The data extrapolate to a value of 5.7 S at zero protein concentration. The slope of the regression line is positive, which indicates a tendency of the protein to aggregate as the concentration increases. This is presumably at least partly due to the fact that the experiments were performed at 25°. The schlieren patterns showed one symmetrical peak, indicating homogeneity, and only in a few instances, such as in Figure 6a was a few per cent of a faster sedimenting material evident.

The value for $s^0_{20,w}$ can be used together with the molecular weight and the partial specific volume to calculate the frictional ratio, f/f_{min}, *i.e.*, the ratio of the observed frictional coefficient, f, to the minimal frictional coefficient the molecule can have, f_{min}. A sedimentation constant of 5.7 S, a molecular weight of 110,000 and a partial specific volume of 0.73 gives a value for f/f_{min} of 1.44. Allowing reasonable variations of the molecular weight from 100,000 to 110,000 and $\bar{v}$ from 0.725 to 0.735 gives values from 1.37 to 1.48. These values are clearly larger than for typical globular proteins, which have values for f/f_{min} of 1.1–1.25 (Tanford, 1961).

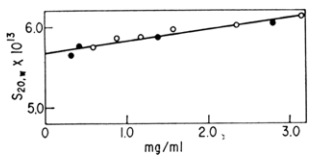

FIGURE 5: Sedimentation velocity of heavy chain dimer in 0.02 M sodium acetate buffer (pH 5.5) containing 0.1 M NaCl, at 25°. Filled circles denote values obtained in a 30-mm cell at 50,750 rpm, and open circles values obtained in a 12-mm cell at 56,100 rpm.

Optical Rotatory Dispersion and Circular Dichroism Spectra. The optical rotatory dispersion spectrum of heavy-chain dimers is given in Figure 7. This spectrum was obtained at 5° with a protein solution of concentration 1 mg/ml and a pH of 5.5. The low temperature was necessary to prevent precipitation in the cell during the scans. At 25° only protein concentrations of less than about 0.1 mg/ml could be used, but the results obtained were identical with those at higher concentrations and 5°. Experiments at pH 7.0 and 8.5 also gave spectra identical within experimental error to the one shown. The well-established optical rotatory dispersion spectrum of native IgG is given in the figure for comparison. The rotation of heavy chain is more negative than that of IgG throughout the wavelength range, but the main conformational features of IgG are preserved, namely the Cotton effect centered around 240 mμ and the minima at 231 and 225 mμ, the latter being reduced to a shoulder in the heavy-chain spectrum due to the dominating 231-mμ trough.

The circular dispersion spectrum in Figure 8 shows the same general features as the optical rotatory dispersion. Also in this figure the spectrum of IgG is given for comparison. Both heavy chain and IgG exhibit main troughs at 217 mμ of approximately equal magnitude, and the differences between them are confined to the wavelength range above about 225 mμ. The shoulders in the IgG spectrum around 238 and 228 mμ (each presumably due to the presence of both a negative and a positive circular dichroism band) are discernible also in the spectrum of heavy chain, but the ellipticity of the latter is more negative. The spectrum in the region above 260 mμ is of low magnitude (Cathou *et al.*, 1968), and could not be measured in the case of heavy chain,

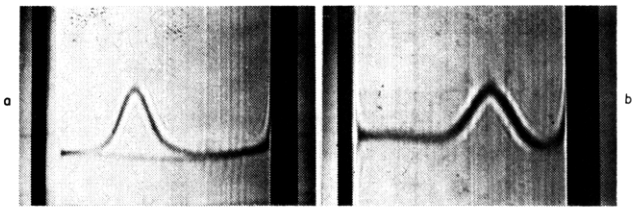

FIGURE 6: Sedimentation velocity patterns. (a) Of heavy-chain dimer in 0.02 M sodium acetate buffer (pH 5.5) containing 0.1 M NaCl at 50,740 rpm and 25°. 2.8 mg of protein/ml; 30-mm double-sector cell; bar angle 50°. The picture was taken 65 min after top speed was reached. (b) Of peak I from a gel chromatography on Sephadex G-100 of papain-digested heavy chain (Figure 9), in 0.02 M sodium acetate buffer (pH 4.5) containing 0.1 M NaCl, at 56,100 rpm and 25°. 6 mg of protein/ml; 12-mm single-sector cell; bar angle 40°. The picture was taken 154 min after top speed was reached.

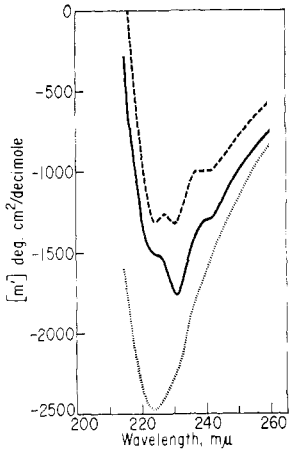

FIGURE 7: Optical rotatory dispersion spectra of heavy chain in 0.01 M NaCl (pH 5.5) (solid line), of heavy chain in 0.01 M NaCl (pH 2.5) (dotted line), and of IgG in 0.01 M NaCl (pH 5.5) (broken line). All experiments were performed at 5° and with protein concentrations of 0.8–1.0 mg/ml.

since the experiments had to be performed at room temperature and again only concentrations of less than 0.1 mg/ml could be used.

Dissociation. The heavy-chain dimer is completely dissociated to monomer in 6 M Gdn·HCl, as is evident from Table I, where the molecular weight in this solvent is given as 52,000. Thus the bonds holding the two chains together are wholly of a noncovalent nature, *i.e.*, all interchain disulfide bonds have been reduced and alkylated.

Dissociation of the heavy-chain dimer at acid pH was also studied. The molecular weight at pH 2.5 at either high or low ionic strength was found to be about 60,000 (Table I). No significant size heterogeneity was apparent from the plots of the logarithm of fringe displacement *vs.* r^2. The high molecular weight, however, is explained by the results of sedimentation velocity experiments in 0.1 M NaCl (pH 2.5) which showed that 5–10% of the material remained undissociated at this pH. The sedimentation coefficient (at a protein concentration of 3 mg/ml) of the main peak was 2.9 S. Lowering of the pH to 1.8 did not cause further dissociation; thus the resistant dimers seem to be very stable.

The lower, dotted curve in Figure 7 shows the optical rotatory dispersion spectrum of heavy chain at pH 2.5.

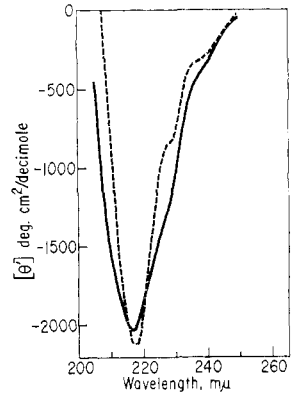

FIGURE 8: Circular dichroism spectra of heavy chain (solid line) and of IgG (broken line) in 0.01 M NaCl (pH 5.5) at 25–27°. Protein concentrations 0.05–0.2 mg/ml.

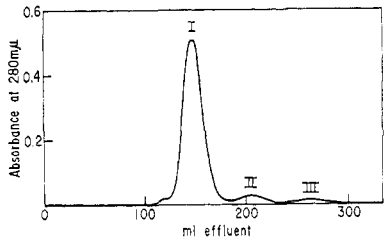

FIGURE 9: Gel chromatography of papain-digested heavy chain on Sephadex G-100 in 0.02 M sodium acetate buffer (pH 4.5), containing 0.1 M NaCl. 1.8-ml sample (5 mg/ml) on 2.5 × 55 cm column.

The fine structure has disappeared, and only one minimum at 225 mμ, with a rotation about 40% more negative than at neutral pH, is evident. Obviously the dissociation of the heavy-chain dimer at acid pH is accompanied by a conformational change. Whether these two changes occur simultaneously or one precedes the other has not yet been investigated.

Papain Digestion. A further investigation of the structure of heavy chain was performed by digesting it with papain. This was done at 37° in phosphate buffer (pH 7.0) for 15 min with 0.5% (wt/wt) crystalline papain. After this brief digestion the solution was dialyzed against 0.02 M acetate buffer (pH 4.5) containing 0.1 M NaCl, in order to prevent the crystallization of some material that occurred at higher pH values. The recovery in such a dialysis was 90–95%, which, in view of unavoidable loses during handling, indicates that very little material had been digested to small dialyzable peptides. The solution was applied to a column of Sephadex G-100 at pH 4.5, and the elution pattern in Figure 9 was obtained. Three well-separated peaks are evident. The first and major of these, designated peak I, accounts for about 90% of the applied material and was studied further. A sedimentation velocity experiment gave only one boundary (Figure 6b) with a sedimentation coefficient at 6 mg/ml of 3.7 S, which is much lower than can be estimated for undigested heavy chain by extrapolation of the straight line in Figure 5 to that concentration. Table II shows the molecular weights of peak I obtained in sedimentation equilibrium experiments under different conditions. All plots of the logarithm of fringe displacement *vs.* r^2 were linear from displacements of about 70 μ to the bottom of the cells, indicating homogeneity of the samples. The molecular weight in dilute salt solutions of about 50,000 (Table II)

TABLE II: Molecular Weights of Peak I from Gel Chromatography of Papain-Digested Heavy Chain.[a]

Solvent	Concn (mg/ml)	Speed (rpm)	Mol Wt
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.12	23,150	50,000
0.02 M acetate (pH 5.5) + 0.1 M NaCl	0.12	29,500	50,000
0.02 M phosphate (pH 7.0) + 0.1 M NaCl	0.14	29,500	47,000

[a] All experiments were performed at 25°.

was reduced to about 24,000 in 6 M Gdn·HCl. This was determined on a calibrated agarose column in 6 M Gdn·HCl according to the method of Fish *et al.* (1969). As stated earlier this method gives a valid estimation of the molecular weight of the polypeptide chain. Several runs were made, all giving the same molecular weight; in none of these were more than one elution peak seen.

Immunodiffusion experiments of peak I (J. S. Huston, I. Bjork, and C. Tanford, to be published) showed that it reacted well with goat anti-Fc, giving a line of identity with Fc prepared by Porter's method (Porter, 1959). It also gave a reaction with anti-Fab, and in this case the precipitin line fused with that formed by Fd prepared according to the procedure described by Fleischman *et al.* (1963).

The peak designated as II in Figure 9 was found to have a molecular weight in 6 M Gdn·HCl (also as determined by gel chromatography in this solvent) of about 12,000. Its molecular weight in dilute salt solution was not determined. Peak III contains low molecular weight peptides. These probably represent a larger amount of the original digest than is apparent from this elution pattern, since the losses during dialysis, about 10%, should primarily have affected this peak. These small peptides increased in amount if the digestion was continued for longer periods of time, as was evident from direct chromatography in 6 M Gdn·HCl of aliquots of a digestion mixture removed at different times.

Discussion

The results presented in this paper suggest a gross conformation of free heavy chain like the one schematically presented in Figure 10. The essential features of this proposed structure are the following. The heavy chain exists as a dimer at near-neutral pH, the flexible hinge region present in the original IgG molecule is preserved, the two Fd segments of the dimer interact pairwise with each other in a way resembling the Fd–L interactions in intact IgG, and the Fc region has a conformation similar to the one it has in intact IgG. The evidence for these conclusions will be discussed below.

No reliable "native" molecular weight of heavy chain has been reported in the literature due to problems with aggregation. Using the careful procedures for preparation and analysis that were outlined in the earlier part of this paper, we have determined a molecular weight of rabbit heavy chain in dilute salt solutions of about 110,000. This result was reproducible under conditions of different protein concentrations, pH, and rotor speeds. In 6 M Gdn·HCl, however, the molecular weight was reduced to 52,000. This value agrees well with results of several similar measurements in dissociating solvents by other investigators (Marler *et al.*, 1964; Pain, 1963; Small and Lamm, 1966). The uncertainty of these two molecular weights is rather large, maybe ±10%, due to the fact that the partial specific volume was only estimated from the amino acid composition, and because this value was also used for the effective partial specific volume in 6 M Gdn·HCl. Because of preferential binding of Gdn·HCl, the latter parameter may actually differ from $\bar{v}$ by 0.01–0.02 (Hade and Tanford, 1967; Reisler and Eisenberg, 1969). Nevertheless, the errors are not sufficiently large to invalidate the obvious conclusion that heavy chain must be a dimer under nonaggregating conditions at near-neutral pH.

The presence of a flexible hinge region in intact IgG, located in the middle of the heavy chain between the Fd and Fc portions, has been inferred by many authors from several different types of evidence, *e.g.*, hydrodynamic properties

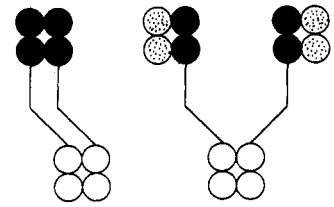

FIGURE 10: Schematic representation of the gross conformation of free heavy chain (left), compared to the structure proposed by Edelman *et al.* (1969) for whole IgG (right). The circles represent the domain regions; dotted circles denote light chain, black, the Fd segments and white, the Fc segments.

and susceptibility to proteolysis (Noelken *et al.*, 1965), fluorescence depolarization measurements (Zagyanski *et al.*, 1969; Yguerabide *et al.*, 1970), and electron microscopic studies (Valentine and Green, 1967). The suggestion that this hinge region is preserved in free heavy chain is based on two main facts, namely the magnitude of the frictional ratio calculated from the sedimentation constant, and the susceptibility of heavy chain to papain digestion. The frictional ratio was found to be about 1.40, which is larger than for typical globular proteins. Comparison to the value for intact IgG, 1.47 (Noelken *et al.*, 1965), suggests an extended shape of the heavy-chain dimers, possibly a shape similar to that proposed for IgG, with two or three main globular regions linked by a flexible portion of the chain. Further confirmation of the deviation from a compact globular shape would have been expected to be given by viscosity measurements, but these could not be carried out due to the low solubility of heavy chain. Fluorescence depolarization measurements should be technically feasible and could add some further support for the nonspherical shape and possibly also for the flexibility of heavy chains. A rigid asymmetric molecule, however, cannot be definitely ruled out by these hydrodynamic data.

As in the case of IgG, a most convincing support for the existence of a flexible portion of free heavy chain is the fact that proteolytic cleavage occurs in a very limited, but easily accessible, region in the middle of the chain. The main product purified by gel chromatography from a papain digest of heavy chain was found to be a fraction with a molecular weight in a dissociating solvent (6 M Gdn·HCl) of about 24,000. This fraction clearly represents material with a molecular weight corresponding to that of half the heavy chain, suggesting proteolytic cleavage somewhere near the center of the chain. Such a cleavage should give the Fd and Fc portions of heavy chain as the resulting products. The presence of both of these in the main gel chromatographic fraction of the papain digest has been clearly established. Firstly, the total recovery of this fraction is 70–80% and thus neither of the Fd or Fc parts, being of equal size, could have completely disappeared by digestion to smaller pieces. Moreover, the presence of both Fd and Fc have been unequivocally demonstrated by immunological techniques. Thus proteolytic cleavage does seem to have occurred near the middle of the heavy chain, *i.e.*, at about the same place as in intact IgG. Similarly, Jaton *et al.* (1968) have reported cleavage of rabbit heavy chain (in this case polyalanylated) into two parts by the action of cyanogen bromide. These data indicate the preservation of the hinge region in isolated heavy chain.

Further conclusions can be drawn from the results of these proteolytic digestion experiments. The "native" molecular

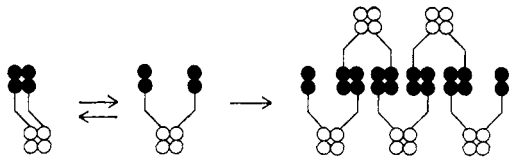

FIGURE 11: Proposed mechanism for the polymerization of heavy chain. See further details in text.

weight (in dilute salt solution) of the main gel chromatographic fraction, containing both the Fd and the Fc portions of the heavy chain, was about 50,000. A high degree of molecular size homogeneity of this fraction was concluded both from sedimentation velocity and sedimentation equilibrium experiments. As mentioned earlier, the molecular weight was reduced to about 24,000 in 6 M Gdn·HCl, still with no size heterogeneity evident. These observations must mean that both Fd and Fc predominantly exist as dimers in their native state. Application of this finding to the structure of heavy chain suggests that heavy chain dimers are held together not only by attractive forces in the Fc region, as in intact IgG, but also by noncovalent interaction between the two Fd parts. The interactions between Fd and light chain in the native IgG molecule thus seem to have been replaced by similar interactions between two adjacent Fd segments when light chain is absent. An objection to this conclusion can be raised, namely that the two Fd portions may not interact in undigested heavy chain, and that the Fd dimers accordingly are formed only when the hinge region is cleaved. This question cannot be answered unequivocally, but some circumstantial evidence supports our conclusion. Certain IgA molecules contain light chains that are disulfide linked to each other and not to the heavy chains, but still noncovalently bound to the latter (Abel and Grey, 1968). A similar structure can also be artifically produced in the case of IgG by recombination of mildly reduced and carboxymethylated heavy chains with reduced, but not alkylated, light chains. During subsequent air oxidation, disulfide bonds form between the two light chains (Grey, 1969). In order for this to happen the light chains must be in close proximity to each other, and in the model proposed to explain these phenomena, they were actually placed facing each other inside the two Fd fragments (Abel and Grey, 1968). This model would predict that when the light chains are removed, the two Fd segments could interact to form a dimer, as suggested in this paper, since the sites previously occupied by the light chains would be facing each other and thus could participate in mutual noncovalent interactions. This more or less static model, however, is not the only one that can account for the previous observations. Rotational freedom of the Fab region, and also of the unassociated Fd segments, around the "hinge" region would satisfactorily explain both the possibility of formation of disulfide-linked light-chain dimers and the proposed attractions between the two Fd segments in free heavy chain. Givol and DeLorenzo (1968) have indicated that the flexible region of the heavy chain in IgG, *i.e.*, that segment accessible to proteolytic enzymes, comprises about 25–30 residues. This length of flexible chain would seem to allow sufficient rotation and bending both to bring the light chains of the two Fab regions of IgG close together and to permit two Fd segments of free heavy chain to associate with each other.

Acid pH dissociates rabbit IgG into two halves by breaking the noncovalent bonds between the Fc segments, but does not seem to similarly affect the Fab regions (Palmer *et al.*, 1963). In contrast, acid pH results in almost complete dissociation of rabbit heavy-chain dimers into monomers; thus the attractive forces between the two chains must have been broken in both the Fd and the Fc regions. This indicates that the interactions between two Fd portions of a heavy-chain dimer may be more easily broken and therefore probably are weaker than the corresponding interactions between Fd and light chain in the Fab part of the whole IgG.

The optical rotatory dispersion and circular dichroism spectra lend some additional support to the proposed model for the structure of free heavy chain. The optical rotatory dispersion spectrum of heavy chain presented here, and that of light chain, given in a subsequent publication (Björk and Tanford, 1971a), are both more negative than the corresponding spectrum of native IgG. This must mean that some conformational change has accompanied dissociation of IgG into its component chains. If this were not the case, the curve for the native protein would be a weighted average of the curves for the two separated chains, and this clearly cannot be true (see further Björk and Tanford, 1971b). This conclusion was also reached by Dorrington *et al.* (1967), although their optical rotatory dispersion spectra were not as accurate as those presented here. Nevertheless, the characteristic features of the dispersion curve of IgG are also given by heavy chain, even though they are superimposed on a more negative background rotation. It can therefore be assumed that free heavy chain has a conformation not drastically different from the one it has when combined with light chain in native IgG, and that consequently large regions of ordered structure are preserved. This probably holds true for both the Fd and Fc parts. The 231-mμ trough has been shown by Steiner and Lowey (1965) to be predominantly given by the Fc region in intact IgG; in heavy chain this trough should be expected to dominate, as it does, since the relative concentration of Fc is higher than in IgG. The two other features, namely the 240-mμ Cotton effect and the 225-mμ minimum are given by the Fab regions of intact IgG (Steiner and Lowey, 1965), and probably arise from the Fd portions of isolated heavy chain. The circular dichroism spectrum of heavy chain, also being reminiscent of that of IgG, further supports the conclusion that a rather limited conformational change occurs upon chain separation.

The domain hypothesis, originally proposed by Edelman *et al.* (1969), has been incorporated both for the structure of IgG and in the model proposed for heavy chain in Figure 10. This hypothesis is based on the existence of a linear periodic arrangement of intrachain disulfide bonds in whole IgG (Edelman *et al.*, 1969; O'Donnell *et al.*, 1970), and also on evidence for evolution of IgG by gene duplication (Hill *et al.*, 1966). The hypothesis suggests that in both heavy and light chains each segment that contains one of these intrachain disulfide bonds, *i.e.*, each of the partly homologous segments of about 110 residues that have arisen by gene duplication, is folded into a compact domain. Between these globular domains there are loose connecting regions, which, however, are shorter, less flexible and less accessible than the hinge region in the center of the molecule. Supporting evidence for this theory has come from the demonstration of an enzyme-susceptible site in the middle of the Fc fragment (Turner and Bennich, 1968). Cleavage by pepsin and papain at this point produces fragments corresponding to half the Fc portion, but the fission proceeds at a much slower rate than at the central hinge region. The suggestion that the compact domains also exist in free heavy chain is based on

similar evidence. Firstly, the intrachain disulfide bonds presumably have not been broken by the mild reduction procedures employed and the same linear arrangements of these bonds as in intact IgG should exist in isolated heavy chain. Furthermore, digestion of heavy chain with papain resulted in the appearance of a smaller amount of material with a molecular weight corresponding to quarters of heavy chain. However, it is not known whether these fragments were formed only from the Fc region, as might be expected from the work of Turner and Bennich (1968), or also from the Fd region. Further studies to elucidate this point are in progress.

One of the main obstacles to all studies of free heavy chains has been their tendency to aggregate and precipitate when in solution at near-neutral pH. This behaviour can be explained on the basis of the model we have suggested above. There might exist an equilibrium in solution between a predominant number of heavy-chain dimers, which interact pairwise in both the Fd and Fc regions as indicated earlier (Figure 11a), and a small number of dimers that are dissociated only in the Fd region (Figure 11b). These "free" Fd segments can then interact with other Fd segments of similarly dissociated heavy chains, and large polymers may ultimately form (Figure 11c) and precipitate out of solution.

A large number of experiments have demonstrated that isolated heavy chains retain most of the antibody activity of the parent antibody molecule (for reviews, see Fleischman, 1966, and Porter and Weir, 1966). Our results, and the model we have proposed, may provide a plausible explanation for these observations. The two Fd segments of a heavy-chain dimer may be able to interact to form an antigen-binding site similar to that formed in native IgG by interaction between Fd and light chain. A binding site formed in this way would be expected to have lower affinity for antigen than the site in the native molecule, and this is indeed the usual experimental observation. (Fleischman *et al.*, 1963; Utsumi and Karush, 1964; Haber and Richards, 1966; Weir and Porter, 1966b; Jaton *et al.*, 1968). Moreover, this concept further means that one heavy chain dimer should possess only one antigen-binding site. This value has actually been demonstrated in one case (Utsumi and Karush, 1964), but the usual value found in the literature is about 0.5 binding site dimer (Weir and Porter, 1966b; Haber and Richards, 1966; Jaton *et al.*, 1968). This low value might be due to experimental difficulties, such as irreversible denaturation during the preparative procedures, so that a major part of the molecules were rendered incapable of binding any antigen at all. Alternatively, a large number of low-affinity sites were not saturated under the conditions employed for the measurements. This interesting problem obviously requires further investigations.

After this manuscript was completed, we were made aware of a paper by Stevenson (1968), showing that stable dimers of the heavy chain of human IgG can be obtained under conditions very similar to those employed in this paper for rabbit IgG. Stevenson and Dorrington (1970) have described physical studies on the human dimer that partially duplicate those described here, though there are some differences, notably in the tendency to form aggregates. Stevenson and Dorrington report that 60–70% of species with sedimentation coefficient near 17 S appeared when sedimentation velocity measurements were made in the presence of 0.1 M NaCl. Only very weak tendency to aggregate was observed by us under these conditions. All the sedimentation results reported in this paper were in fact measured in the presence of 0.1 M NaCl. It should also be noted that Stevenson and Dorrington's value of $s^0_{20,w}$ for the dimer, measured in 0.004 M acetate buffer, is 5.24 S, as compared to our value of 5.7 S.

References

Abel, C. A., and Grey, H. M. (1968), *Biochemistry 7*, 2682.

Björk, I., and Tanford, C. (1971a), *Biochemistry 10*, 1280.

Björk, I., and Tanford, C. (1971b), *Biochemistry 10*, 1289.

Cathou, R. E., Kulczycki, Jr., A., and Haber, E. (1968), *Biochemistry 7*, 3958.

Crumpton, M. J., and Wilkinson, J. M. (1963), *Biochem. J. 88*, 228.

Dorrington, K. J., Zarlengo, M. H., and Tanford, C. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 996.

Edelman, G. M. (1959), *J. Amer. Chem. Soc. 81*, 3155.

Edelman, G. M., Cunningham, B. A., Gall, W. E., Gottlieb, P. D., Rutishauser, V., and Waxdal, M. J. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 78.

Edelman, G. M., and Gall, W. E. (1969), *Annu. Rev. Biochem. 38*, 415.

Edelman, G. M., and Poulik, M. D. (1961), *J. Exp. Med. 113*, 861.

Fish, W. W., Mann, K. G., and Tanford, C. (1969), *J. Biol. Chem. 244*, 4989.

Fleischman, J. B. (1966), *Annu. Rev. Biochem. 35*, 835.

Fleischman, J. B., Pain, R. H., and Porter, R. R. (1962), *Arch. Biochem. Biophys., Suppl. 1*, 174.

Fleischman, J. B., Porter, R. R., and Press, E. M. (1963), *Biochem. J. 88*, 220.

Fuchs, S., and Sela, M. (1965), *J. Biol. Chem. 240*, 3558.

Givol, D., and DeLorenzo, F. (1968), *J. Biol. Chem. 243*, 1886.

Grey, H. M. (1969), *J. Immunol. 102*, 848.

Haber, E., and Richards, F. F. (1966), *Proc. Roy. Soc., Ser. B 166*, 176.

Hade, E. P. K., and Tanford, C. (1967), *J. Amer. Chem. Soc. 89*, 5034.

Hill, R. L., Delaney, R., Fellows, Jr., R. E., and Lebowitz, H. E. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 1762.

Jaton, J. C., Klinman, N. R., Givol, P., and Sela, M. (1968), *Biochemistry 7*, 4185.

Lennox, E., and Cohn, M. (1967), *Annu. Rev. Biochem. 36*, 365.

Levy, H. B., and Sober, H. A. (1960), *Proc. Soc. Expt. Biol. Med. 103*, 250.

Marler, E., Nelson, C. A., and Tanford, C. (1964), *Biochemistry 3*, 279.

Metzger, H., and Mannik, M. (1964), *J. Exp. Med. 120*, 765.

Noelken, M. E., Nelson, C. A., Buckley, III, C. E., and Tanford, C. (1965), *J. Biol. Chem. 240*, 218.

O'Donnell, I. J., Frangione, B., and Porter, R. R. (1970), *Biochem. J. 116*, 261.

Olins, D. E., and Edelman, G. M. (1964), *J. Exp. Med. 119*, 789.

Pain, R. H. (1963), *Biochem. J. 88*, 234.

Palmer, J. L., Nisonoff, A., and van Holde, K. E. (1963), *Proc. Nat. Acad. Sci. U. S. 50*, 314.

Porter, R. R. (1959), *Biochem. J. 73*, 119.

Porter, R. R., and Weir, R. C. (1966), *J. Cell. Physiol., 67, Suppl. 1*, 51.

Reisler, E., and Eisenberg, H. (1969), *Biochemistry 8*, 4572.

Small, P. A., and Lamm, M. E. (1966), *Biochemistry 5*, 259.

Steiner, L. A., and Lowey, S. (1966), *J. Biol. Chem. 241*, 231.

Stevenson, G. T. (1968), *Bibl. Haematol. 29*, 537.

Stevenson, G. T., and Dorrington, K. J. (1970), *Biochem. J. 118*, 703.

Svedberg, T., and Pedersen, K. O. (1940), The Ultracentrifuge, Oxford, Clarendon Press, p 35.
Tanford, C. (1961), Physical Chemistry of Macromolecules, New York, N. Y., Wiley, p 358.
Turner, M. W., and Bennich, H. (1968), *Biochem. J. 107*, 171.
Utsumi, S., and Karush, F. (1964), *Biochemistry 3*, 1329.
Valentine, R. C., and Green, N. M. (1967), *J. Mol. Biol. 27*, 615.
Weir, R. C., and Porter, R. R. (1966a), *Biochem. J. 100*, 63.
Weir, R. C., and Porter, R. R. (1966b), *Biochem. J. 100*, 69.
World Health Organization (1964), *Bull. W. H. O. 30*, 447.
Yguerabide, J., Epstein, H. F., and Stryer, L. (1971), *J. Mol. Biol.* (in press).
Yoo, T. J., Roholt, O. A., and Pressman, D. (1967), *Science 157*, 707.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.
Zagyanski, Yu, A., Nezlin, R. S., and Tumerman, L. A. (1969), *Immunochemistry 6*, 787.

Gross Conformation of Free Polypeptide Chains from Rabbit Immunoglobulin G. II. Light Chain*

Ingemar Bjork† and Charles Tanford‡

ABSTRACT: Rabbit light chains were isolated in the usual manner by gel chromatography in 1 M propionic acid. The preparation thus obtained, when returned to neutral pH, consisted of apparently stable monomers and dimers and these were separated from each other by gel chromatography. The existence of monomeric and dimeric forms of rabbit light chains most probably is a result of intrinsic properties of two different classes of light-chain species. The monomer dimerizes reversibly at high protein concentrations, whereas a corresponding dissociation of the dimer form at low protein concentrations was not detected. Hydrodynamic parameters, such as frictional ratio and intrinsic viscosity, indicate that both forms deviate somewhat from a compact globular shape and that the dimer is slightly more extended than the monomer.

These results, and the cleavage of light chains into halves by proteolytic digestion, earlier reported for free human chains and confirmed here for rabbit light chains, lend some support to the proposed "domain" theory for the structure of immunoglobulins. A difference between the conformations of the monomer and dimer forms was deduced from optical rotatory dispersions and circular dichroism measurements, but it was not decided whether this difference is due to a conformational change accompanying dimerization or merely reflects different conformations of the two classes of light-chain species.

Investigations of the size, shape, and conformation of separated heavy and light chains of immunoglobulin G[1] are of great importance because of the significant contributions such studies can give to the elucidation of the relationship between structure and function of antibodies. In a companion paper (Björk and Tanford, 1971a) some of these physicochemical properties of isolated rabbit heavy chain were reported, and in this communication we describe similar properties of free rabbit light chain. As in the study of heavy chains, rabbit IgG was chosen as the parent protein, because of our ultimate aim to attempt to correlate the physicochemical properties of free chains from rabbit antibodies with their antigen-binding ability. The investigations show that some light-chain species exist in free form as apparently stable monomers and others as stable dimers, and that both forms have somewhat more extended shapes than typical globular proteins. This property and the reported cleavage of light chains into halves by proteolytic enzymes (Solomon and McLaughlin, 1969; Karlsson *et al.*, 1969), which was confirmed in this work for rabbit light chains, support the "domain" hypothesis, proposed by Edelman *et al.* (1969). An interesting finding, finally, is the fact that the light-chain monomer has a conformation, as measured by optical rotatory dispersion and circular dichroism, slightly different from that of the dimer.

Materials and Methods

Preparation of rabbit IgG and separation of its heavy and light chains was performed essentially as described in the preceding paper (Björk and Tanford, 1971a). The only modification was that the procedure was scaled up so that 1g of reduced and carboxylated IgG was applied to a 6 × 115 cm column of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, N. J.) in 1 M propionic acid. The separation between heavy and light chains on this column was equally good as, and in some instances even better than, that obtained on the smaller column used previously, and the recoveries were also comparable. The light-chain peak had completely emerged from the column after

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[1] The nomenclature of and the abbreviations for the immunoglobulins and their subunits produced by reduction and proteolysis are those recommended by the World Health Organization (1964).