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Change in Conformation of the Rabbit γG-Immunoglobulin Molecule with Various Chemical Treatments*

Carol Warner and Verne Schumaker

ABSTRACT: Changes in the three-dimensional structure of rabbit γG-immunoglobulin and rabbit anti-lactoside antibody have been studied using the technique of differential sedimentation. The molecules were subjected to reduction, reduction and alkylation, and pH change. The sedimentation coefficient of the anti-lactoside antibody was about 0.3 S smaller than that of the nonspecific γG-immunoglobulin. However, both molecules showed the same behavior when subjected to reduction, and to reduction and alkylation. Reduction caused a *decrease* in sedimentation coefficient, corresponding to an increase in frictional coefficient, whereas reduction and alkylation caused an *increase* in sedimentation coefficient, corresponding to a decrease in frictional coefficient. Change of pH, away from neutrality, caused a decrease in the sedimentation coefficient of γG-immunoglobulin. A model for γG-immunoglobulin structure, based on a flexible Y, was proposed to explain the observed changes in frictional coefficient. It was found that the frictional coefficient of the γG-immunoglobulin molecule increases as the angle between the arms of the Y increases.

The three-dimensional structure of the γG-immunoglobulin molecule is a problem which still eludes biochemists. It has been suggested (Noelken *et al.*, 1965) that the molecule is Y shaped, consisting of three compact globularr egions (the two Fab pieces and the Fc piece) connected by somewhat flexible regions. This model is supported by the hydrodynamic data which give a frictional coefficient ratio of 1.47 for the entire IgG molecule, but 1.24 for the Fab piece and 1.21 for the Fc piece (Noelken *et al.*, 1965). Also, the intrinsic viscosity is 6–8 cc/g (Jirgensons, 1962) which is higher than one would predict for a typical globular protein. This model is also supported by various fluorescence depolarization studies (Chowdhury and Johnson, 1961; Steiner and Edelhock, 1962) which show that the rotational relaxation time of a fluorescent dye attached to the IgG molecule is smaller than that predicted for a compact sphere, thus indicating that the

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IgG molecule is somewhat asymmetric. Moreover, the relaxation times appear to be greater (Weltman and Edelman, 1967; Wahl and Weber, 1967; Krause and O'Kouski, 1967; Ingram and Jerrard, 1962; Edsall and Foster, 1948) for the whole molecule than for the Fab or Fc pieces, indicating that rotation of these regions is probably not completely free. Also, the fact that the IgG molecule seems to have one site which is susceptible to attack by various, unrelated proteolytic enzymes, indicates a region which is exposed, perhaps as a short region of unfolded polypeptide chain. This region also seems to be the site of the "critical disulfide bond" described by Nisonoff (Palmer and Nisonoff, 1964) which is subject to reduction and alkylation under mild conditions.

Recent electron microscopic data can also be used to speculate about the shape of the antibody molecule. Feinstein and Rowe (Feinstein and Rowe, 1965) reported that the antibody molecule, when it was not combined with antigen, was only slightly asymmetric with a maximum dimension of 105 Å. However, when γG-immunoglobulin molecules were bound to ferritin they seemed to show a bend in the middle. The angle of the bend was seen to vary over a wide range, but the maximum length of the antibody never exceeded 200 Å. Valentine and Green (Valentine and Green, 1967) have looked at an antibody–hapten complex in the electron microscope and have seen the formation of regular complexes with each molecule having the Y-shaped configuration. Preliminary X-ray data (Kratky and Paletta, 1955; Edelman and Gally, 1964) are consistent with an elongated model or a more compact Y-shaped model.

It is the purpose of this paper to correlate data on the changes in frictional coefficient of the γG-immunoglobulin molecule upon reduction, reduction and alkylation, and pH change, with a model for the γG-immunoglobulin molecule based on a flexible Y. The technique of differential sedimentation developed by Schumaker and Adams (1968) is used to obtain data on the change in sedimentation coefficient of the molecule upon various chemical treatments. These changes can in turn be interpreted as changes in frictional coefficient of the molecule as described by Schumaker (1968). Then, the Kirkwood (1954) theory as applied by Bloomfield (Bloomfield *et al.*, 1967a,b) to macromolecules, is used to calculate frictional coefficients of the molecule in various states, to explain the experimentally observed changes in frictional coefficient.

Materials and Methods

Preparation of Rabbit Immunoglobulin G. Young adult New Zealand white rabbits were injected intravenously with 1 ml of a 1% solution of bovine serum albumin in 0.15 M NaCl, three times per week for 6 weeks (Campbell *et al.*, 1964). The rabbits were then bled, by cardiac puncture, and the usefully positive sera pooled. The total IgG was then isolated by a three times Na_2SO_4 precipitation according to the method of Kekwick (1940). This preparation is hereafter referred to as "new" IgG. A nonspecific preparation of rabbit IgG was obtained from Pentex, Kankakee, Ill., and is hereafter referred to as Pentex IgG or IgG. Purified anti-lactoside rabbit IgG was kindly given to us by Dr. Fred Karush, and is referred to as "Lac Ab" throughout the text. All samples were dissolved in appropriate buffers and dialyzed overnight before use. The concentration was determined by $OD_{280\,m\mu}$, assuming an ϵ value of 1.36 ml/mg as reported by Small and Lamm (1966).

Differential Sedimentation. The technique of differential sedimentation (Schumaker and Adams, 1968) has been used in an attempt to gain some knowledge of the conformational changes which occur in the IgG molecule in various chemical environments. Basically, we run two samples simultaneously in the ultracentrifuge and process the data so that we can detect any change in sedimentation coefficient as small as ±0.016 S. In this way we minimize effects of fluctuation in temperature and rotor speed and can compensate directly for the inverse square law of radial dilution. A series of ten schlieren photographs is taken at 8-min intervals.

Viscosity and density corrections also become very important in determining small differences in sedimentation coefficient, so all values are carefully corrected to the viscosity and density of water at 20°. Viscosity measurements were made either in a three-bulb Ubblelohde viscometer or an Ostwald viscometer depending on the size of the available sample. Likewise, density measurements were either made in a 25- or 1-ml pycnometer.

Reduction. A. EFFECT OF CYSTEINE. "New" IgG and Pentex IgG, in a 0.075 M NaCl–0.025 sodium phosphate–2×10^{-3} M EDTA (pH 7.0), were treated with solid L-cysteine to give a final concentration of 0.01 M (Gergely *et al.*, 1966). The antibody was allowed to incubate 2 hr in this reducing medium before centrifugation. However, the centrifuge cell was filled directly after the addition of the cysteine to eliminate any problems of oxidized cysteine (cystine) precipitating out of solution.

B. EFFECT OF MERCAPTOETHANOL. The solutions were treated with 2-mercaptoethanol essentially as described by Utsumi and Karush (1964) and by Palmer and Nisonoff (1964). IgG (0.5 ml) at 15 mg/ml in 0.1 M sodium acetate (pH 5.0) was mixed with 1.0 ml of 0.015 M 2-mercaptoethanol. Nitrogen was flushed through the system for 30 min before mixing the solutions, then the mixed solutions were incubated under nitrogen for an additional 60 min. The centrifuge cell was flushed with nitrogen and filled at this time. The same procedure was repeated for Lac Ab, except that the original buffer in which 15 mg/ml of Lac Ab was present, was 0.15 M NaCl–0.02 M sodium phosphate (pH 5.0). In this way, the final concentration of 2-mercaptoethanol, in both the IgG and Lac Ab solutions, was 0.01 M.

Reduction and Alkylation. The reduction and alkylation was performed for both IgG and Lac Ab in separate two-arm Warburg flasks. The center well was filled with 0.4 ml of 15 mg/ml of appropriate antibody solution, as described above. One arm contained 0.4 ml of a 0.030 M 2-mercaptoethanol solution and the other arm, 0.4 ml of a 0.30 M iodoacetic acid solution (recrystallized from hexane just prior to use). The system was flushed with nitrogen for 30 min; then the mercaptoethanol was tipped into the center well and reduction allowed to proceed for 60 min; finally, the iodoacetate was tipped into the center well, and alkylation allowed to proceed overnight. The solution was then dialyzed against two changes of 0.025 M NaCl, and used in the centrifugation studies.

pH Studies. The IgG used in all the pH studies was in 0.15 M NaCl which had been adjusted to the appropriate pH with small quantities of NaOH or HCl. In the differential sedimentation runs, IgG in 0.15 M NaCl (pH 7.0) was always used as a reference solution in the 1° positive cell, while the experimental solution was always put in the 1° negative cell.

TABLE I: Sedimentation Coefficients of Various Rabbit IgG Preparations.

Run No.	Description	Concn[a] (mg/ml)	$s^0_{20,w}$[c] (S)	$\delta s^0_{20,w}$[b,c] (S)
490	"New" IgG	2.88	6.748 ± 0.008	−0.011 ± 0.011
490	"New" IgG	2.88	6.737 ± 0.008	
542	Pentex IgG	4.19	6.795 ± 0.013	−0.016 ± 0.007
542	Pentex IgG	4.19	6.812 ± 0.017	
491	Pentex IgG	3.51	6.748 ± 0.011	−0.005 ± 0.011
491	"New" IgG	2.88	6.743 ± 0.015	
543	Pentex IgG	4.19	6.748 ± 0.014	−0.329 ± 0.016
543	Lac Ab	3.28	6.419 ± 0.023	

[a] Concentrations were determined either from $OD_{280\,m\mu}$ or from the area under the schlieren peaks. [b] A negative sign indicates that the solution in the 1° negative cell is sedimenting slower than the solution in the 1° positive cell to which it is compared. In each paired run, the contents of the 1° positive cell is listed first. [c] The plus and minus variations listed in this table represent 67% confidence limits on the slopes of the least-square lines through the experimental data.

TABLE II: Change in Sedimentation Coefficient of the Antibody Molecule upon Reduction.

Run No.	Description	Concn[a]	$s^0_{20,w}$[c] (S)	$\delta s^0_{20,w}$[b,c] (S)
492	Pentex IgG plus 0.01 M cysteine	3.51	6.745 ± 0.011	+0.093 ± 0.008
492	"New" IgG	2.88	6.838 ± 0.017	
493	Pentex IgG	3.51	6.768 ± 0.006	−0.117 ± 0.023
493	"New" IgG plus 0.01 M cysteine	2.88	6.651 ± 0.018	
558	Pentex IgG	4.33	6.611 ± 0.008	−0.049 ± 0.003
558[d]	Pentex IgG plus 0.01 M mercaptoethanol	4.39	6.569 ± 0.005	
559	Pentex IgG	4.33	6.685 ± 0.027	−0.434 ± 0.019
559	Lac Ab plus 0.01 M mercaptoethanol	3.84	6.251 ± 0.040	

[a–c] See corresponding footnotes to Table I. [d] The presence of 0.01 M mercaptoethanol causes the molecules to sediment 0.011 S slower (due to viscosity and density effects) than in the absence of mercaptoethanol. This has been taken into account in calculating $\delta s^0_{20,w}$.

Results

The results presented in this section are the results of paired differential sedimentation runs on the various antibody preparations after the prescribed chemical treatments. Table I shows the results of paired runs on the antibody preparations prior to any chemical treatment. It is noted that the sedimentation coefficients of the "new" IgG and the Pentex IgG are identical, while the Lac Ab is 0.329 S slower than IgG. For this reason, Pentex IgG was used as a reference protein in the 1° positive cell in all future runs.

The results of reduction with cysteine and with mercaptoethanol are given in Table II. It is seen that both reducing agents seem to cause a small but significant decrease in sedimentation coefficient of the molecule, which averages −0.091 S.

The results of reduction and alkylation of the antibody molecule are presented in Table III. Reduction and alkylation together seem to cause an increase in sedimentation coefficient of the molecule, which averages +0.095 S.

The pH of Pentex IgG was varied from 2 to 12 with the results shown in Table IV. The molecule retains an apparently symmetrical schlieren peak until pH 12, at which point it breaks up into half-molecules with time (Spencer and Schumaker, 1967). At pH 12, the $\delta s^0_{20,w}$ is given for the "intact" molecule peak. The change in sedimentation coefficient with pH is shown graphically in Figure 1.

Discussion

The main conclusion from this work is that changes in the environment of the γG-immunoglobulin molecule can cause a change in the frictional coefficient of the molecule. Table V correlates the change in sedimentation rate of the macromolecule with the corresponding per cent change in the frictional ratio of the molecule. Reduction causes an increase in frictional

TABLE III: Change in Sedimentation Coefficient of the Antibody Molecule upon Reduction and Alkylation.

Run No.	Description	Concn[a]	$s^0_{20,w}$[c] (S)	$\delta s^0_{20,w}$[b,c] (S)
561	Pentex IgG	4.33	6.628 ± 0.011	+0.108 ± 0.008
561	Reduced and alkylated Pentex IgG	4.21	6.736 ± 0.016	
562	Pentex IgG	4.33	6.679 ± 0.012	−0.248 ± 0.014
562	Reduced and alkylated Lac Ab	2.26	6.431 ± 0.019	

[a-c] See corresponding footnotes to Table I.

TABLE IV: Change in Sedimentation Coefficient of the Antibody Molecule with pH Variation.

Run No.	Description[d]	Concn[a]	$s^0_{20,w}$[c] (S)	$\delta s^0_{20,w}$[b,c] (S)
697	pH 7.0	3.98	6.793 ± 0.009	−0.912 ± 0.013
697	pH 2.0	4.31	5.882 ± 0.008	
730	pH 7.0	3.98	6.785 ± 0.023	−0.611 ± 0.016
730	pH 3.0	4.79	6.175 ± 0.024	
698	pH 7.0	3.98	6.845 ± 0.005	−0.115 ± 0.007
698	pH 4.0	3.98	6.729 ± 0.005	
696	pH 7.0	3.98	6.783 ± 0.008	−0.011 ± 0.014
696	pH 6.0	4.27	6.772 ± 0.014	
692	pH 7.0	3.98	6.750 ± 0.005	−0.001 ± 0.009
692	pH 7.0	3.98	6.749 ± 0.006	
693	pH 7.0	3.98	6.753 ± 0.008	+0.021 ± 0.007
693	pH 8.0	3.48	6.774 ± 0.009	
694	pH 7.0	3.98	6.789 ± 0.013	−0.041 ± 0.011
694	pH 10.0	3.98	6.746 ± 0.018	
695	pH 7.0	3.98	6.792 ± 0.008	−1.095 ± 0.008
695	pH 12.0	~2	5.718 ± 0.011	

[a-c] See corresponding footnotes to Table I. [d] Pentex IgG was used in each experiment.

coefficient of the molecule. The effect is qualitatively the same for both mercaptoethanol and cysteine. This was to be expected since the conditions used were known to reduce the inter-heavy-chain disulfide bond of the molecule (Palmer and Nisonoff, 1964; Gergely *et al.*, 1966; Edelman, 1959; Edelman and Poulik, 1961; Fleischman *et al.*, 1962). It has been shown that rabbit IgG's have only one inter-heavy-chain disulfide bond (Hong and Nisonoff, 1965) whereas the complete sequence of a human γG_1 (Eu) myeloma protein (Edelman *et al.*, 1969) reveals two inter-heavy-chain disulfide bonds. However, the amino acid sequence of rabbit heavy chains in the region of the disulfide bond seems to be very similar to that in human γG_1 (Smyth and Utsumi, 1967; Cebra *et al.*, 1968) with the single disulfide bond in the rabbit protein corresponding to the first inter-heavy-chain bond in human γG_1 (Hill *et al.*, 1967). We might note, even though Lac Ab apparently has a different average molecular weight than IgG (*i.e.*, Lac Ab has a smaller sedimentation coefficient than IgG), it behaves in the same manner upon reduction, perhaps indicating homologous disulfide chemistry of the two molecules. Reduction and alkylation cause a decrease in the frictional coefficient of both IgG and Lac Ab, emphasizing again, the similar behavior of the molecules.

Changing the pH of the IgG molecule, in either direction away from pH 7, causes an increase in the frictional coefficient of the molecule. This agrees with the results of a pH study by Charlwood and Utsumi (1969). It is important to note that the molecule maintains the same frictional coefficient from pH 6 to 8, so that when using physiological conditions, one need not worry about pH changing the sedimentation properties of the molecule.

The observed changes in frictional coefficient may be interpreted as conformational changes in the three-dimensional structure of the antibody molecule. The decrease in frictional coefficient of the molecule upon reduction and alkylation might indicate a more compact molecule, while the increase in frictional coefficient upon reduction, or change in pH, might reflect a general expansion of the molecule. We would like to propose a conformational change in γG-immunoglobulin structure which could account for these changes.

The model is based on the idea of a somewhat flexible Y which has recently become popular (Noelken *et al.*, 1965;

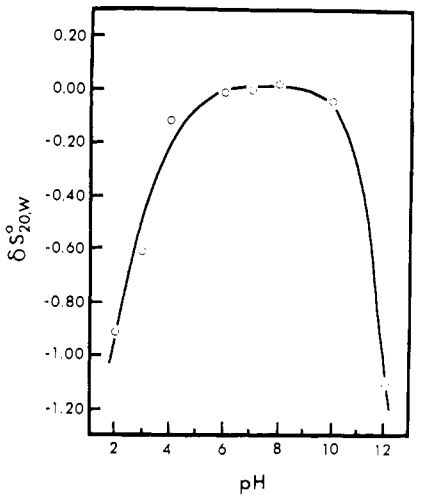

FIGURE 1: A plot of $s^0_{20,w}$ *vs.* pH. The values for $\delta s^0_{20,w}$ were calculated from paired differential sedimentation runs as described in the text.

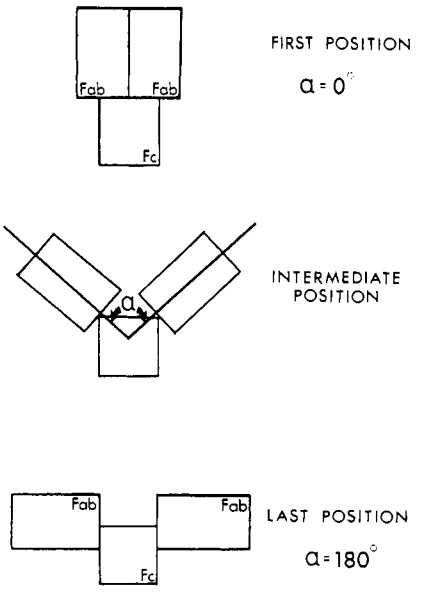

FIGURE 2: A model for γG-immunoglobulin structure based on a flexible Y. α is defined as the angle between the Fab pieces; α is formed by the intersection of the two lines drawn through the center of each Fab piece and parallel to the sides of the schematic rectangles representing the Fab pieces. This means that the initial position corresponds to $\alpha = 0°$, and the final position corresponds to $\alpha = 180°$.

Valentine and Green, 1967). Figure 2 is a model in which the Y is considered to be flexible, in that the two arms may rotate in a plane from an angle, α, of 0° for the completely closed structure, to an angle of 180° for the completely open structure. Rectangles schematically represent the Fab and Fc pieces as the arms of the Y. The frictional coefficient of the entire structure can be calculated from eq 1 as described by Bloomfield

$$f = \left(\sum_{i=1}^{n}\zeta_i\right)\left[1 + \left((1/6\pi\eta)\sum_{i=1}^{n}\zeta_i\right)\sum_{i=1}^{n}\sum_{j=1}^{n}{}'\zeta_i\zeta_j\langle R_{ij}^{-1}\rangle\right]^{-1} \quad (1)$$

where f = translational frictional coefficient of the entire macromolecule, ζ = translational frictional coefficient of each subunit i, where there is a total of n subunits, η = solvent viscosity (taken as 0.01 P for water in all calculations), Σ' = the prime sign of the summation denotes omission of the term

TABLE V: Actual Change in $s^0_{20,w}$ of Antibody with Various Treatments and Corresponding Change in Frictional Ratio of the Moleucle.

Operation	Actual Change in $s^0_{20,w}$ of Macromolecule	% Change in Frictional Ratio
Reduction of Pentex IgG with cysteine	−0.093	+1.41
Reduction of "new" IgG with cysteine	−0.117	+1.78
Reduction of IgG with mercaptoethanol	−0.049	+0.74
Reduction of Lac Ab with mercaptoethanol	−0.105	+1.67
Reduction and alkylation of IgG	+0.108	−1.59
Reduction and alkylation of Lac Ab	+0.081	−1.25
pH 2.0	−0.912	+15.8
pH 3.0	−0.611	+10.0
pH 4.0	−0.115	+1.75
pH 10.0	−0.041	+0.61
pH 12.0	−1.095	+19.5

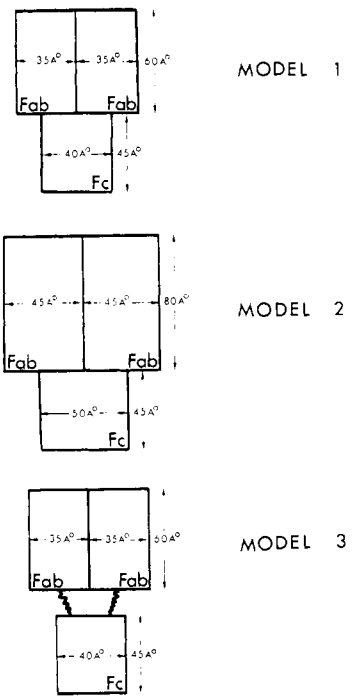

FIGURE 3: Models 1, 2, and 3, as described in the text, showing the dimensions of each.

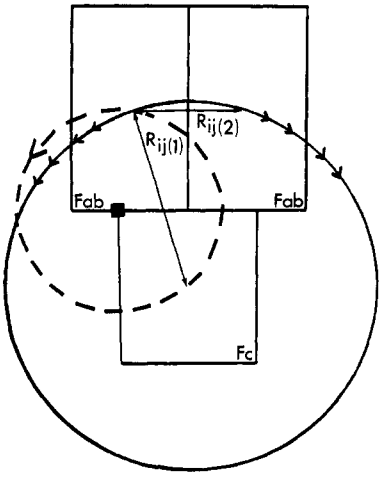

FIGURE 4: Two possible paths of motion of the Fab pieces. The small dashed circle is the path of motion with $R_{ij(1)}$ variable. The large solid circle is the path of motion with $R_{ij(1)}$ constant.

with $j = 1$, R_{ij} = the distance between any two subunits, and $\langle\ \rangle$ = this denotes that the average is taken over internal coordinates only.

Our basic aim is to predict what will happen to the frictional coefficient of the γG-immunoglobulin molecule under various conditions so that we can better understand the changes in sedimentation coefficient seen by the technique of differential sedimentation.

The dimensions of the molecule are estimated from the electron micrographs. Three models will be considered: the first has the dimensions assumed by Valentine and Green (1967); the second has the maximum dimensions possible from their electron micrographs. (This model is included since the electron micrographs are of dehydrated particles, possibly appearing smaller than they are in solution. However, it does not matter if these are not the true dimensions of the molecule, for we are only interested in calculating percentage changes in frictional coefficient, at various angles, α, and these are not strong functions of the absolute dimensions.) The third model varies the distance between the subunits, using the same dimensions for the subunits as in model 1. The three models are shown in Figure 3. The path of motion of the Fab subunits determines the shape of the plot of the frictional coefficient of the molecule *vs.* α. Two possible paths of motion are shown in Figure 4. The small dotted circle is the result of a path of motion described by a hinge point at the small, solid black square. The large solid circle is a path of motion described by a circle with radius = $R_{ij(1)}$, *i.e.*, the distance between the center of the Fab pieces and the Fc piece remains constant.

Of course, the molecule is not composed of rectangles, but is simply represented this way for convenience. The first approximation, in the model, is that each of the rectangles is actually a rectangular solid with a depth equal to the width as shown in Figure 3. The second approximation is that each of the rectangular solids can be represented by an ellipsoid of revolution with equal volume to the rectangular solid. It is true that perhaps a better approximation would be to call the subunits cylinders, but no exact method exists to calculate the theoretical frictional coefficient of a cylinder, while this quantity can be calculated for an ellipsoid using the Perrin (1936)

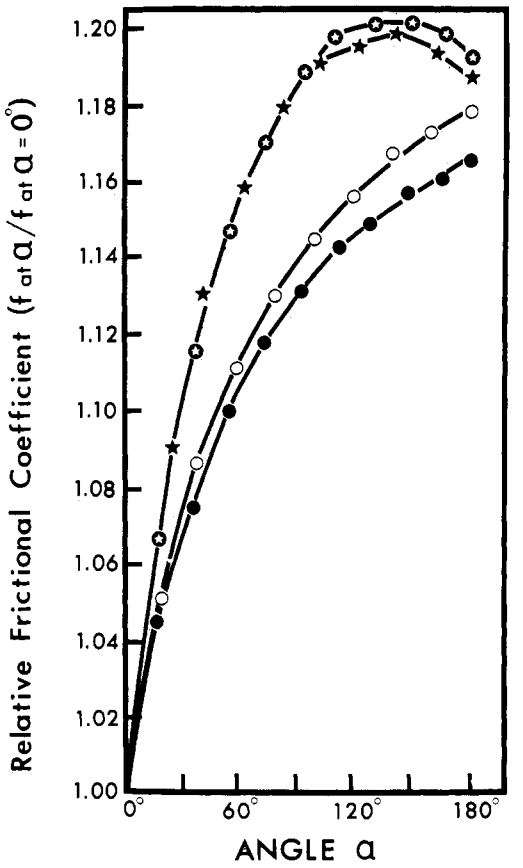

FIGURE 5: A plot of the relative frictional coefficient (f at α/f at α = 0°) *vs.* the angle α. The path of motion shown in Figure 4 as the small dashed circle gives the curves depicted by (★) for models 1 and 3, and by (✪) for model 2. The path of motion shown in Figure 4 as the large solid circle gives the curves depicted by (○) for models 1 and 3, and by (●) for model 2.

equations. To further simplify the calculations, each ellipsoid can be converted into its frictionally equivalent sphere and eq 1 simplifies to eq 2, using the relationship $\zeta = 6\pi\eta r_i$. Table

$$f = \left(6\pi\eta\sum_{i=1}^{n} r_i\right)\left[1 + \left(1\Big/\sum_{i=1}^{n} r_i\right)\sum_{i=1}^{n}\sum_{j=1}^{n}{}' r_i r_j \langle R_{ij}^{-1}\rangle\right]^{-1} \quad (2)$$

VI gives the radii of the frictionally equivalent spheres for each subunit in each model shown in Figure 3. These values are then used in eq 2 to calculate f, the frictional coefficient.

A plot of the relative frictional coefficient (defined as the frictional coefficient at any α divided by the frictional coeffi-

TABLE VI: Values for the Radii of the Frictionally Equivalent Spheres in Models 1–3.

Radii	Model 1	Model 2	Model 3
r_i 1	26.7	34.8	26.7
r_i 2	26.7	34.8	26.7
r_i 3	25.8	30.0	25.8

cient at $\alpha = 0$) *vs.* the angle α between the two Fab pieces is shown in Figure 5. Careful surveyance of eq 2 will reveal that the per cent change in frictional coefficient with angle α (the only parameter in which we are really interested) is the same for models 1 and 3, so they will be considered together, *i.e.*, the shape of the curves for the two models is identical. Figure 5 shows the result of the path of motion defined in Figure 4 by the large solid circle (*i.e.*, $R_{ij(1)}$ is constant), and the result of the path of motion defined by a hinge point (*i.e.*, $R_{ij(1)}$ is variable) and depicted in Figure 4 as the small dashed circle. It is seen that variation in the dimensions of the subunits gives curves which are quite similar.

The main conclusion from this data is that the frictional coefficient of the γG-immunoglobulin molecule increases as the angle, α, between the two Fab pieces increases. It is clear from Figure 5 that the assumption that $R_{ij(1)}$ is constant gives a different graph from the graph where $R_{ij(1)}$ varies. Thus, the path of rotation of the subunits determines how the frictional coefficient will vary. However, the general shape of the curves for both paths of motion is very similar. The main difference is that in the second path of motion the frictional coefficient reaches a maximum and then decreases slightly. Of course, if the molecule were in the conformation with this maximum angle, α, we would have no way to tell if an observed decrease in frictional coefficient corresponded to a closing or an opening of the arms of the Y. Another notable thing about the curves is that the frictional coefficient increases at a greater rate for small values of α, than for large values of α. The maximum per cent increase for all three models is about 16–18% for the first path of motion and about 20% for the second path of motion. Thus, if a change in the environment of the molecule produced a change in the angle, α, this could easily be detected by differential sedimentation. Of course, only the X-ray structure will solve the average value of α for the "native" molecule. Depending on the angle, α, of the original molecule, the changes in sedimentation coefficient we have observed can perhaps be explained by variations in the angle between the arms of the Y in the γG-immunoglobulin molecule.

Since this manuscript was completed, Pilz *et al.* (1970) have published the small angle X-ray data for the human γG_1 (Eu) myeloma protein, of known primary structure. Their data are consistent with the model of the γG-immunoglobulin molecule as a flexible Y. However, their data are best explained by a larger molecule in solution, than had been indicated in the electron micrographs. We, too, found this to be true in calculating frictional coefficients for the γG-immunoglobulin molecule. This is the main reason that we chose to consider models 2 and 3 (more extended molecules), in addition to model 1, in our calculations of the change in frictional coefficient of the molecule with the angle between the arms of the Y.

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