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Immunology

Three-dimensional structure of an intact human immunoglobulin

(antibodies/x-ray diffraction/glycoprotein/carbohydrate)

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ABSTRACT **We have examined the low-resolution structure of a complete human IgG1 using known domain coordinates from crystallographic investigations of immunoglobulin fragment structures. Our results indicate that the Fc portion of this molecule has a structure similar to that of an isolated Fc fragment, with the carbohydrate moiety playing a central role as the principal contact between the C_H2 domains. Carbohydrate also forms a large part of the interface between the Fc and Fab regions. The relative orientations of the variable and constant portions of the Fab regions are intermediate between those reported previously, emphasizing the flexibility of the switch region. These data do not support a two-state allosteric model such as has been proposed for antibody effector functions.**

Most of the information available at present on the three-dimensional structure of immunoglobulins (Igs) has come from studies of isolated fragments (1–3). Progress in the determination of structure of intact Igs has been generally disappointing. The protein Dob, a human IgG1(κ) cryoglobulin, was the first crystalline intact Ig to be analyzed by x-ray diffraction (4). The crystals are sensitive to x-irradiation and show evidence of disorder in the diffraction pattern, with few useful reflections beyond 6 Å. An electron density map at this resolution showed the overall molecular boundary, on the basis of which a T-shaped model was proposed (5). This model was independently supported by an analysis of electron micrographs of sections of the Dob crystals (6, 7).

The human myeloma protein Mcg [IgG1(λ)] has also been crystallized, and the crystals diffract to a resolution of 3.5 Å (8), but there has been no reported solution of its three-dimensional structure.

A third human myeloma protein (IgG1), Kol, has been crystallized and shown to diffract to spacings of 3.5 Å (9). An electron density map at 5-Å resolution has been reported in which the two Fab arms are clearly visible with an angle of 120° between them (10). Unfortunately, the Fc part of the molecule is not at all visible in this map, probably because of disorder in the crystal.

Progress in the determination of structure of Ig fragments has been more rapid. the structures of two Fabs (11, 12), an Fc (13, 14), and a number of Bence-Jones proteins (15–17) have been reported. As a result, we now know separately the structures of all the component domains of an IgG molecule. When corresponding domains from different Igs have been compared, they have been found to be similar (refs. 10, 17, and 18; unpublished*). These observations, together with the results of amino acid sequence analyses, strongly suggest that, even in the variable parts of the molecule, the three-dimensional structures of the domains will be highly conserved.

The quaternary relationships between domains are not, however, invariant. Substantial differences have been observed in the relative orientation of the variable and constant regions (elbow bend) of the Fabs (ref. 10; unpublished*). Furthermore, solution studies have demonstrated a high degree of segmental flexibility in antibody molecules which has been interpreted as resulting from flexibility of the hinge region (19, 20).

On the basis of the x-ray diffraction results, an allosteric model has been previously proposed involving quaternary conformational changes between antigen-liganded and unliganded antibodies (21). In addition, the differences in quaternary structure between the Fabs of the fragment structures and Kol led to the proposal that the isolated Fabs would be in the liganded conformation. In order to test these hypotheses, it is clear that more data are needed, particularly on the domain interactions in intact Ig molecules.

In this paper, we describe a reinvestigation of the Dob 6-Å electron density map using a systematic computer search for individual domains. The search yielded the complete quaternary structure of the Dob IgG molecule.

METHODS

The general procedure was first to select a region of the Dob electron density map that we believed might correspond to a particular Ig domain. The coordinates of this domain were then systematically rotated and translated until they gave the best fit with this electron density.

The Dob electron density map used was that calculated previously from multiple isomorphous replacement data (22). The map was sampled at intervals of approximately 2 Å. The volume selected usually contained considerably more density than would be occupied by a single domain. The locations of electron density points in this region above a specified level were stored.

The coordinates of the corresponding domain were then systematically rotated and translated with respect to the electron density map; after each such transformation, they were compared with the density. The criterion of fit was the number of transformed coordinates that overlapped density. The rotations were initially carried out in increments of 15° through a wide range of Eulerian angles, decreasing in stages to 1° increments as the angular range of the search was reduced. At the same time, translations were applied in units of map grid-point intervals. These six-dimensional searches can be quite large, involving as many as 2 million transformations.

The resulting best fit can be quite convincing, as shown in Fig. 1 where one of the Eulerian angles has been varied through the optimum position for the C_H2 domain. Similar results were obtained by varying any of the other rotational or translational parameters for any of the other domains. The details of this procedure will be described elsewhere.

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Abbreviation: Ig, immunoglobulin.

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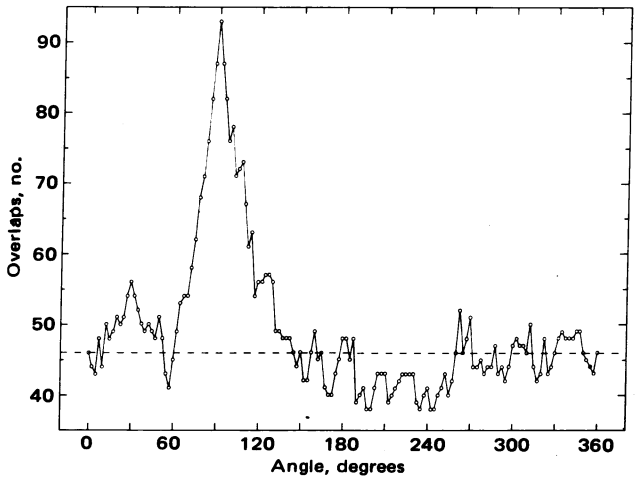


FIG. 1. Overlap of isolated Fc C_H2 α-carbon coordinates (including carbohydrate) on Dob electron density map-points as a function of a single Eulerian angle. The angle was varied systematically in 2.5° intervals over its full range, with all other rotation-translation parameters held constant at their maximum overlap values. The dashed line represents the mean of the overlap count calculated by varying all three Eulerian angles but again holding the three translation parameters constant. This mean value is taken as a measure of the background level of overlap. The standard deviation of the data in number of overlaps is 4.9; the peak itself is 9.7 SD above background. Note that the width of the peak at half-height is approximately 30°; a sampling at every 15° would ensure that we not miss this peak.

The Fc portion of the Dob electron density was the first to be fitted. [The Fc coordinates (α-carbons and carbohydrate hexose centers) were very kindly given to us in advance of publication by J. Deisenhofer and R. Huber.] In Dob, the two heavy chains in the Fc are related by an exact crystallographic 2-fold axis of symmetry. This fact greatly constrained the search space that had to be sampled in fitting coordinates. Initially, the searches were made with the pairs of C_H3 and C_H2 domains (including carbohydrate coordinates). Further searches were then made by using single C_H3 and C_H2 domains (with and without carbohydrates) so as not to constrain the results to the same relative orientation of domains as in the isolated Fc structure.

Once the Fc coordinates had been located in the electron density map, attempts were made to fit the Fab coordinates to nearby density. The Fab coordinates used were those from McPC 603 (12) and are available in AMSOM (23). The quality of the Dob map is poorer in the Fab region than in the Fc. Searches with individual Fab domains resulted in rather broad peaks (plotting overlap count versus angular range) so that only pairs of variable or constant domains were used. In the variable domain searches, the hypervariable loop atoms were not included. The Dob Fab regions are closely packed in the crystal. They form close contacts laterally with each other and are sandwiched at the ends between Fc regions. Because the Fab boundaries are not clear, the possibility of selecting the Fab region with the variable and constant portions interchanged was examined. Broad searches were made by using the α-carbon backbone of the constant domains in that part of the map thought to correspond to the variable part of the Dob Fab and vice versa. In each case, the overlap count was lower than the one obtained with the final correct designation. Peak heights above background differed by 23% for the constant domains and by 41% for the variable domains.

RESULTS

The three-dimensional structure of IgG Dob derived from the search procedure is shown in Figs. 2 and 3. The overall appearance of the molecule is T-shaped and corresponds to the solution preferred by Sarma *et al.* (5), although the boundary between the Fc and Fabs has been redefined.

The relative orientation of the Fc domains is similar to that in the isolated Fc structure (13, 14). Differences in orientation are within the accuracy of the search procedure. The distance between the centers of gravity of the two C_H3 domains across

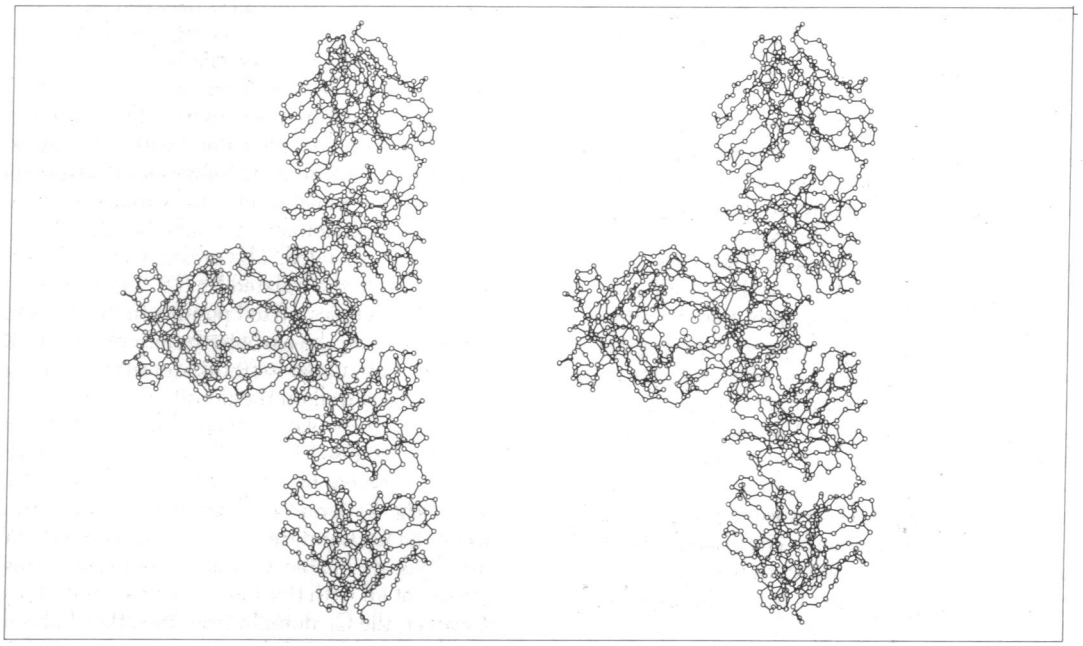


FIG. 2. Stereo view of the three-dimensional structure of Dob. The nomenclature used in describing the Ig molecule can be found in numerous texts and reviews (1, 2, 3, and 24). The smaller circles represent α-carbon atoms; the larger circles represent carbohydrate hexose units. The Fab arms of the molecule are aligned vertically, and a horizontal 2-fold axis of symmetry bisects the molecule through the Fc. In this view, the light chain is in the foreground of the upper Fab and the heavy chain is in the foreground of the lower Fab.

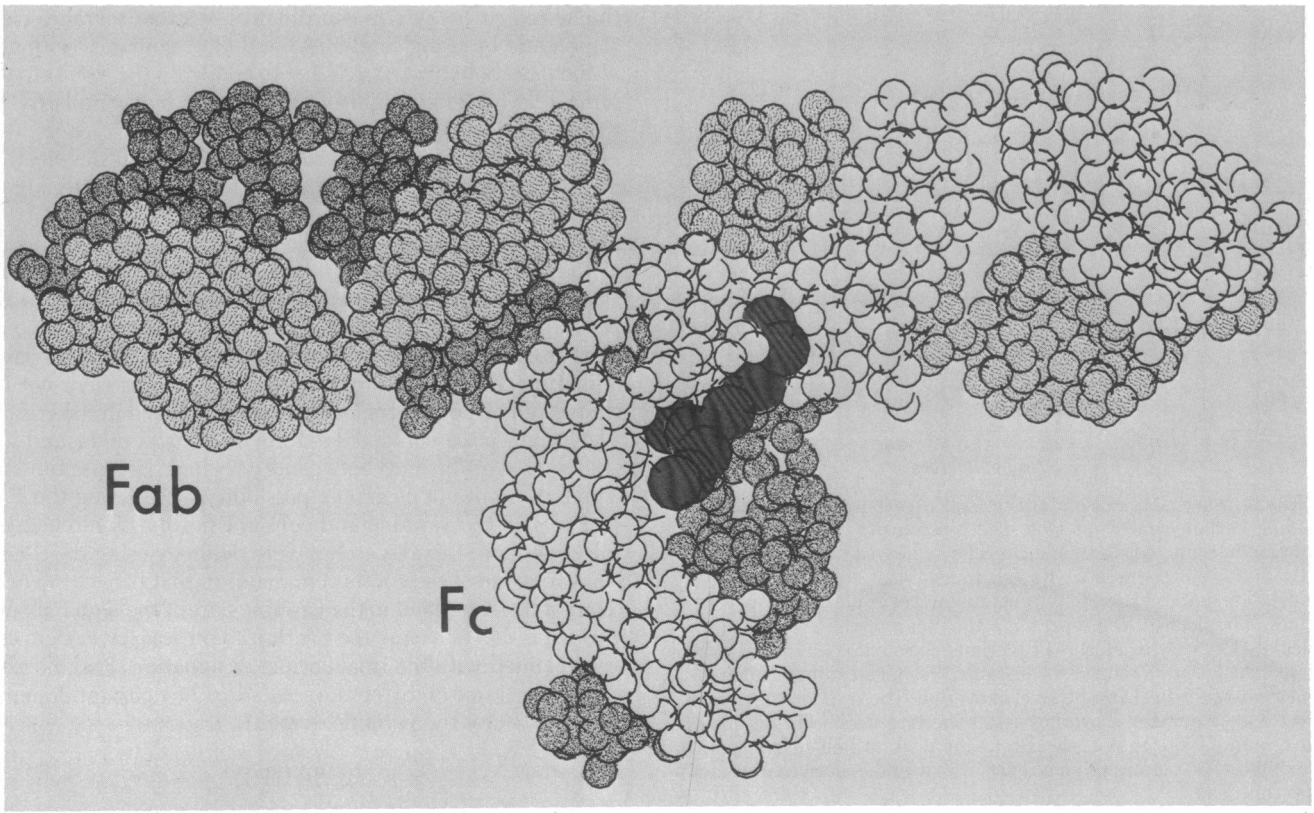


FIG. 3. Space-filling view of the Dob Ig molecule. One complete heavy chain is in white and the other is dark gray; the two light chains are lightly shaded. The large black spheres represent the individual hexose units of the complex carbohydrate. In this view, the 2-fold axis of symmetry is vertical. A crevasse is seen between the C_H2 of the white heavy chain and the C_L domain of the Fab on the left.

the dyad axis is 15.5 Å in Dob compared to 17.0 Å in the isolated Fc; for the C_H2 domains it is 37.1 Å for Dob and 37.8 Å for isolated Fc. The relationship between the C_H2 and C_H3 domains in the two structures was compared by superimposing the C_H3 domains and then comparing their relative C_H2 orientations. The differences amount to an overall rotation of 7° which is comparable in size to the width of the maximum overlap peak for the C_H3 domain and is not a significant difference (this is an exceptionally wide peak; in general, the overlap peak was about 3° wide).

The last residue in the Dob C_H2 and the first residue in the Dob C_H3 domain meet within 1 Å of their ideal distance and are easily connected. The disposition of the carbohydrate in Dob corresponds to a strong region of density in the map. The Dob C_H2 domains are separated by the carbohydrate chains. There are no direct contacts between C_H2 domains. The closest contact between carbohydrate units is 7.4 Å across the 2-fold axis.

The structures of the variable and constant Fab domains are sufficiently different to distinguish between these regions in the Dob map on the basis of overlap count. However, due to the pseudo-dyad symmetry that relates the light and heavy chains, no distinction could be made between V_H and V_L or between C_H1 and C_L solely on the basis of overlap counts. This ambiguity was resolved by making use of the fact that Dob has a disulfide bond between the COOH termini of the light chains (L. A. Steiner, personal communication). The distances between the C_L termini for the two possible solutions were 3.7 Å and 30 Å, respectively. The former is consistent with a disulfide bridge and was therefore assigned to the light chain.

Dob has a 15-residue deletion in the hinge region that connects the Fab and Fc (L. A. Steiner, personal communication), leaving only 7 residues in its shortened hinge. The terminal residue of the McPC 603 C_H1 chain corresponds to one of the deleted residues in Dob. Therefore, 6 extra residues were inserted to connect the end of the Dob C_H1 domain with the start of the Dob C_H2 domain. In placing these residues, an attempt was made to follow a stretch of electron density in this region of the Dob map, avoiding close contacts with other residues. Although the variable and constant regions of the Fab were fitted separately, the distances between the C_αs to be connected were 4.3 Å and 5.5 Å for the light and heavy chains, respectively, so that these atoms could be easily connected by rotating a few bonds in the switch region.

The carbohydrate coordinates obtained from Deisenhofer and Huber were included along with the α-carbon coordinates in fitting the C_H2 domain. Subsequent examination of the Dob map showed a branched chain of strong density wrapped partly around the C_H2 domain to which the carbohydrate coordinates roughly corresponded. The presence of strong density indicates that here, as in the isolated Fc (13, 14), the carbohydrate is ordered and occupies a fixed position in the molecule. A Kendrew model based on the carbohydrate sequence of Kornfeld *et al.* (25) was constructed to fit this density and, as shown in Fig. 4, superimposes well on the density.

Carbohydrate plays a major role in the interaction of the Fc and Fab regions (Fig. 3). The C_H1 domain is prevented from making close contacts with the C_H2 domain by the carbohydrate (Fig. 5). Thus, the closest contact distances are not between the C_H1 and the C_H2 domains directly but through the carbohydrate (Table 1). The C_L domain of this Fab does not interact at all with this C_H2 domain or with the carbohydrate. However, the C_L domain from the other Fab subunit interacts weakly with this C_H2 domain, covering part of its outer surface so as to form a crevasse 10 Å wide between the two domains.

Fabs are generally found to be bent. The angle subtended by the local dyads relating the variable and constant pseudo-

Table 1. Longitudinal contact distances between domains in the Fabs of McPC 603, Dob, and Kol and in the interface between Fc and Fab in Dob

Contact	McPC 603 (12) Contacts, no.	McPC 603 (12) Contact distance, Å	Dob Contacts, no.	Dob Contact distance, Å	Kol (21) Contacts, no.	Kol (21) Contact distance, Å
C_H1–V_H	6	<6.5	0	<7	0	<7
	41	<10	10	<10	5	<10
C_L–V_L	2	<6.5	0	<7	0	<7
	28	<10	20	<10	6	<10
C_L–C_H2			2	<6.5		
			43	<10		
C_H1–CHO			8	<6.5		
			24	<10		
C_H1–C_H2			0	<6.5		
			11	<10		

dimers (the elbow bend) has been found to vary from a maximum of 170° for Kol to a minimum of 113° (Table 2). The Dob Fab falls in the middle of the range with an elbow bend of 147°. The range of flexibility between variable and constant domains is also evident in a comparison of longitudinal contacts (<10 Å) between these domains in the light and heavy chains of McPC 603, Dob, and Kol Fabs (Table 1). McPC 603 Fab with an elbow bend of 134° has the largest number of longitudinal contacts and Kol Fab has the smallest. Dob Fab, with an intermediate elbow bend, has an intermediate number of longitudinal contacts.

DISCUSSION

The method of analysis used to examine the Dob electron density map provides reasonably precise data about the relative position and orientation of the domains in the different regions of the molecule, although it says nothing about the fine details of the individual domain structures. It is thus possible for the first time to obtain a three-dimensional picture of an Ig molecule in its entirety, in particular revealing the relationship of Fab to Fc. One unexpected result is the important role of the complex carbohydrate in forming part of the interface not only between the two C_H2 domains as described previously (13, 14) but also between C_H2 and C_H1. We cannot assess how important these interactions are in solution. The evidence for segmental flexibility of antibody molecules in solution (19, 20) and from electron microscopy (26) would imply that the conformation observed here in the crystal is only one of many possibilities. It would appear that the Dob molecule is not constrained by the 15-residue deletion in the hinge region, because examination of models reveals that considerable flexibility of the Fab relative to the Fc is still possible. A study at higher resolution should reveal the specific interactions between carbohydrate and protein and also clarify the nature and strength of the interactions between Fab and Fc.

It is interesting that the crystalline human intact Ig Mcg also has a 15-residue deletion in the hinge region (27). Should the Mcg quaternary structure turn out to be similar to that of Dob,

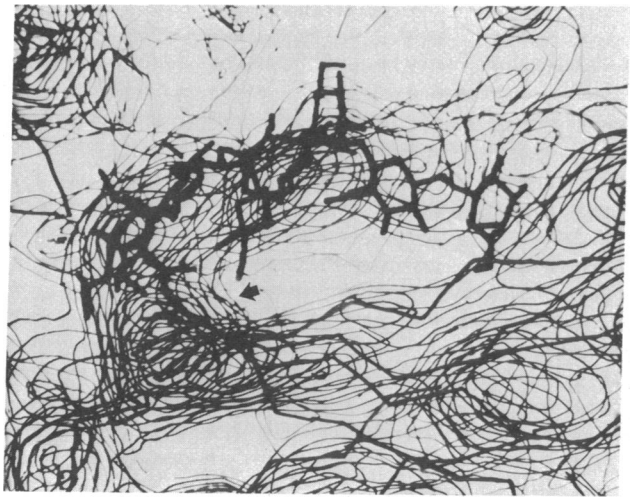
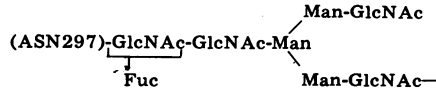


FIG. 4. Electron density map in the region of the C_H2 domain occupied by carbohydrate. Protein coordinates superimposed are as determined by the search procedure. The carbohydrate sequence (25)



has been fitted to the corresponding density by model building. The arrow indicates the position of ASN297.

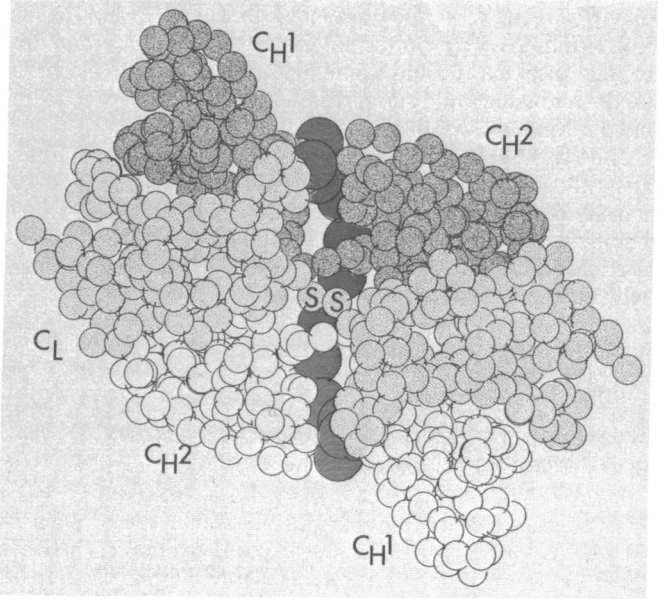


FIG. 5. Views down the 2-fold axis (toward C_H3) of the interface between the Fc and the two Fabs (shading is as described in Fig. 3). Carbohydrate provides the principal contacts between the C_H2 and C_H1 domains and is the only contact between the C_H2 domains. The two cysteine residues that form a disulfide bridge between the two light chains are labeled S.

Table 2. Elbow bends in Fabs

Fab	Bend, degrees
M603	134
Newm	131
Dob	147
Kol	170
Mcg	113

this might indicate a more central role for the T-shaped configuration, perhaps as an extreme in the range of flexibility between the Fab and Fc regions. At present, however, we cannot rule out the possibility that the T-shaped configuration of Dob is merely the result of crystal packing. The protein Kol, which apparently has a normal hinge, has a Y-shaped conformation in the crystal, and a model has been constructed in which there is very little interaction between Fc and Fab (21).

Another surprising result is the similarity of the Fc quaternary structure observed here to that of the isolated Fc (16, 17). Within the limits of error of the analysis, the two structures are identical in spite of the large hinge deletion in Dob that results in the absence of the heavy–heavy disulfide bridges. In Dob the two chains of the Fc are related by an exact crystallographic dyad axis, whereas in the isolated Fc crystal the structures of the two chains, although similar, are not identical (13, 14). The presence of the disulfide bridge between the two light chain COOH termini in Dob may help to stabilize this configuration.

On the basis of the differences between the structures of the Fab of Kol and of the isolated Fabs of McPC 603 and Newm (11), Huber *et al.* (21) proposed an allosteric Ig model in which antigen binding would cause a stiffening of the flexible antibody molecule by formation of longitudinal interdomain contacts in the heavy chain. They suggested that the hinge deletion in Dob would also result in rigid Fab arms. The observation here that the elbow bend of Dob (147°) is intermediate between that of the fragment Fab structures and that of Kol, together with a similar intermediate set of interdomain contact distances, would argue against a two-state allosteric model and would add support instead to the general concept of longitudinal flexibility in the Fab.

Finally, it is clear that, in order to obtain more information about the various interactions in this molecule, a better electron density map is needed at high resolution and some progress is being made in this direction (28). The model presented here can form a preliminary point for the investigation of such a map when it becomes available.

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