*A MODEL FOR THE 7S ANTIBODY MOLECULE**

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Recent studies have indicated that antibodies belong to a family of multichain proteins.[1–5] This fact has prompted efforts to determine the arrangement of the individual polypeptide chains in the whole antibody molecule and to assess their contribution to immunologic specificity.[2, 5–8] The several similar models[5, 7, 9] of 7S γ-globulins that have emerged from these investigations have been schematic and topological in character.

In the present communication, we incorporate information on the topological arrangement of the chains into a topographic model which includes the known size and shape of the molecular envelope, the location of the two combining regions, and the contribution of different polypeptide chains to these regions. This model appears to account for all of the known structural features of 7S γ-globulins, and has several implications for mechanisms of specific assembly of antibodies.

7S γ-globulin molecules consist of two types of polypeptide chains: L (light) chains with molecular weights of 20,000–24,000, and H (heavy) chains with molecular weights of 55,000–60,000.[10–13] All of the available information suggests that the whole molecule has a molecular weight of 150,000–160,000 and is composed of two L chains and two H chains.[10–14] The H chains of the three major classes of γ-globulin are sufficiently distinctive to be designated by separate nomenclature: Hγ, Hγ_{1A}, Hγ_{1M}.[3] L chains are common structural elements in all classes of γ-globulin, and are known to exist in at least two different forms, L_I and L_{II}.[3, 15, 16] Based on the above considerations, the molecular formulae for some representatives of the class of 7S γ-globulins would be: $(H\gamma)_2 (L_I)_2$ or $(H\gamma)_2 (L_{II})_2$.[17]

In previous communications[5, 18] the following general hypotheses were suggested: (1) The bivalent antibody molecule is likely to be symmetrical with respect to the chains contributing to the combining sites, i.e., the chains involved are duplicated.[5, 18] (2) The combining site results from chain interaction. There are three possibilities for chain interaction: L·H, L·L, H·H.[5]

The chain interaction hypothesis has been verified, and it has been shown that the correct pairing is L·H.[8, 19, 20] This fact and the requirement for symmetry exhausts the number of chains in the 7S molecule, which therefore must consist of two L·H pairs, each containing one active site. A similar conclusion has been incorporated in previous models,[5, 8, 9, 21] although that of Porter assumes that the site is wholly on the H chains.[9, 21] The demonstration that L·H interaction leads to the reconstitution of both activity[8, 19, 20] and whole molecules[22] suggests, however, that both chains are involved in the site. Moreover, efforts to label the active site have shown that amino acid residues of L chains[23] or of both L and H chains[24] are near the site.

With these requirements fulfilled, the problem becomes one of arranging the two identical L·H pairs in space to fit the known dimensions of the γ-globulin molecule. This arrangement must account for the position of the antibody-combining sites and also be consistent with the known ways in which the molecule may be degraded.

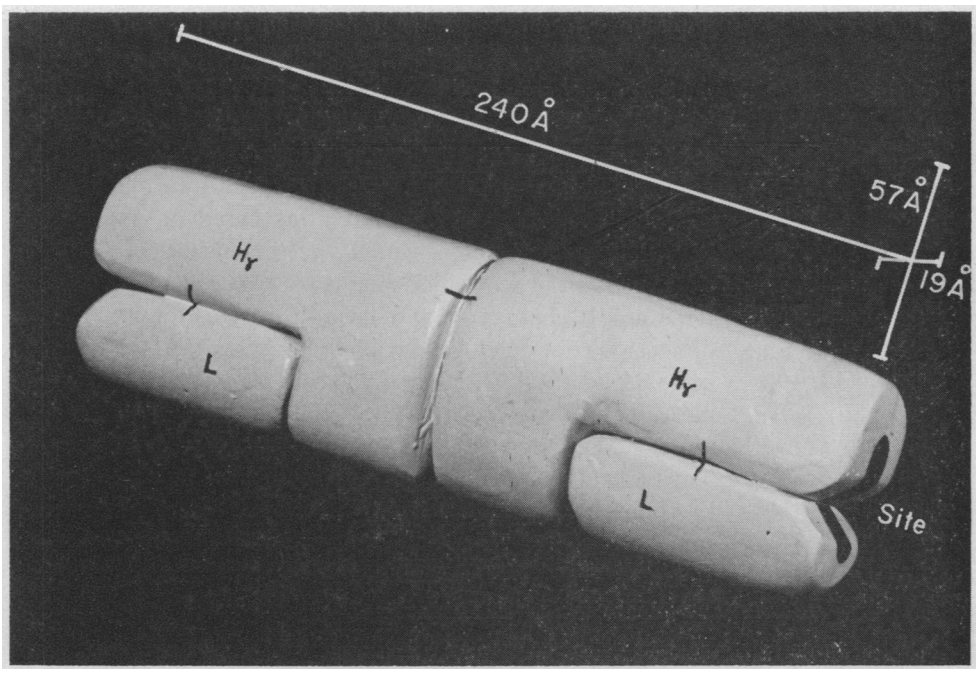

FIG. 1.—Model of the 7S antibody molecule. The molecular dimensions are taken from Kratky *et al.*[27] Hγ—heavy polypeptide chain; L—light polypeptide chain. The single lines drawn on the model represent interchain disulfide bonds.

The model depicted in Figure 1 meets the requirements for symmetry, arrangement in the known molecular shape and volume, chain interaction, site location and consistency with known degradative steps. It is based on composite information on 7S γ-globulins of several species since there is a general structural homology across species lines.[2-4]

Various measurements of the 7S γ-globulin molecule indicate that it is 230–250A long.[25-27] The cross-sectional dimensions and shape are less well defined, but the most detailed information comes from the low angle X-ray scattering experiments of Kratky *et al.*[27] Their measurements suggest that the molecule is a cylinder of elliptical cross section with dimensions of 240A × 57A × 19A. These values have been assumed in the present model; variations in the cross-sectional measurements would not change the over-all picture. It is assumed that each polypeptide chain is folded more or less compactly to fit within the cylinder of total volume 2.0×10^5 A^3.

The combining sites have been placed at the ends of the cylinder and may be visualized as shallow craters or crevices between the L and H chains. From the standpoint of combining without hindrance,[28, 29] particularly with large multivalent antigens, this position is most efficient. Almeida *et al.*[30] have recently obtained electron micrographs showing rod-like antibody molecules 250A long. At the ends of each rod were single polyoma virus particles to which the antibodies were specific.

The dimensions of the combining sites (Fig. 1) were chosen as those defined by Kabat[31] in his study of the antidextran antibodies. It is probable that the dimen-

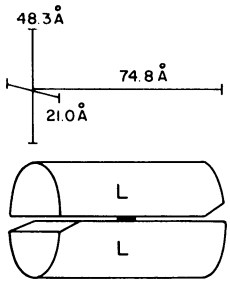

FIG. 2.—A representation of a Bence–Jones protein as a dimer of two L chains. The molecular dimensions are those of Holasek *et al.*[32] The black area between the chains indicates a disulfide bond which may be formed.

sions vary in different antibodies. On the present assumptions, the dimensions of the combining site cannot exceed those of the cross-sectional area of the molecule.

A certain amount of variation in the distribution of volumes between L and H chains can be accommodated in the model. In the absence of more detailed information, the assigned volume of the L chain was based on the data of Holasek *et al.*[32] who measured the dimensions of a Bence-Jones protein of molecular weight 43,000. Bence-Jones proteins are L chains[10, 33] and usually exist as dimers.[10] The dimensions obtained by low angle X-ray scattering are 74.8A × 48.3A × 21.0A. This volume is assumed to be filled symmetrically by two L chains disposed lengthwise (Fig. 2). Obviously, there are alternative distributions of this volume. The one chosen is consistent with the fact that each L chain has one —SH group for interchain disulfide bonding either to an Hγ chain or the other L chain in the dimer.[34, 35] The dimensions of a single L chain in the antibody model (Fig. 1) are taken to be the same as those in the L chain dimer (Fig. 2). Although a small conformational change may occur when L chains of the dimer dissociate and form L·H pairs,[34] contact of the two L chains in the antibody molecule is unlikely. The involvement in sites at separate ends of the antibody molecule precludes contact of the two L chains in any compact conformation resembling that of the dimer. This conclusion is compatible with the finding that L-chain dimers are unable to interact with Hγ chains to form 7S molecules.[34]

The L and H chains are linked to each other by weak interactions and a single interchain disulfide bond.[2, 9, 34, 35] The molecule does not dissociate after cleavage of interchain disulfide bonds, unless it is subsequently placed in denaturing solvents.[2] Evidence for the presence of a single interchain disulfide bond comes from the finding of a single free cysteine on dissociated L chains.[34, 35] Each L·H pair is linked to the other by weak interactions and a single disulfide bond connecting the H chains. This conclusion is derived from the data of Palmer and Nisonoff[36] who have shown that half-molecules may be obtained by cleavage of one disulfide bond per molecule, followed by exposure to acid pH. The regions between L and H chains and between L·H pairs are probably similarly arranged in all 7S γ-globulins since it has been shown that L and H chains of a variety of types and even of different species origin will interact to form 7S molecules.[22, 34] As depicted in Figure 1, there is a two fold rotation axis between the Hγ chains.

Details of folding of the chains within the molecular structure are not known and have not been depicted. It is fairly certain that the chains have no α-helical structure.[37, 38] The suggestion[39] that some of the structure is in interchain and intrachain extended β-conformation is reasonable. Serine, threonine, glutamine, and asparagine residues, each of which is present in relatively high amounts,[40, 41] all might participate through interaction with carbonyl groups of the peptide backbone to form a structure with occasional regions of β-conformation.

No indication of the localization of the carbohydrate moeity has been given in the picture of the model. The work of Fleischman *et al.*[9] would place the car-

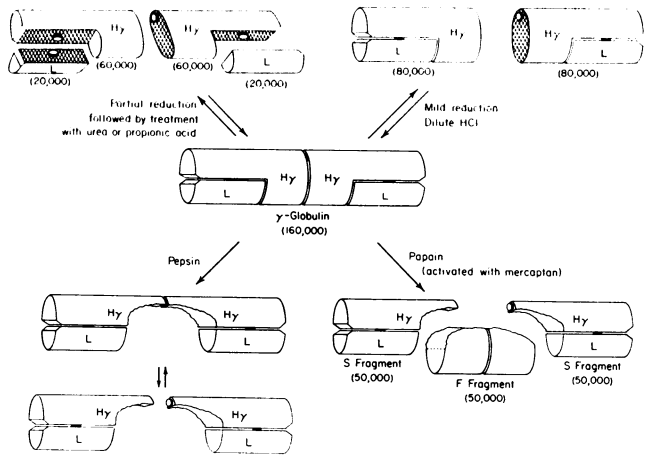

FIG. 3—Schematic diagram illustrating the ways in which the 7S antibody molecule may be degraded. Numbers in parentheses are approximate molecular weights. Hγ—heavy chain; L—light chain. Hatched areas indicate regions of noncovalent interchain bonding. Disulfide bonds or half-cystines are indicated in these regions.

bohydrate on the Hγ chain and in all likelihood near the center of the molecule as we have depicted it.

As pointed out above, one of the conditions to be met by the model is consistency with all known steps of degradation to subunits and fragments. This is fulfilled, as shown in Figure 3. Reduction of interchain disulfide bonds in the absence of urea followed by exposure to urea[2] or acid[7, 21] solutions leads to dissociation of the chains; neither treatment alone is sufficient. Separated L chains dimerize and may form an interchain disulfide bond[10, 34] (cf. Fig. 2). Mild reduction followed by treatment with HCl leads to separation of the L·H pairs as half-molecules.[42] Hydrolysis with papain[43] cleaves some peptide bonds of the H chains[44] and after reduction of the disulfide bond between these chains,[45] S and F fragments[46] are formed. Treatment with pepsin yields fragments closely resembling two S fragments held together by a disulfide bond.[45] Thus all of the known degradative steps are accounted for. Recent experiments[22, 34] indicate that separated L and H chains will reassociate to form 7S molecules with molecular weights of 160,000, and if the chains are in the partially reduced state, the interchain disulfide bonds can be reformed.[22] The arrangement of the chains in the reconstituted molecules is grossly similar to that of native γ-globulin since in both cases S and F fragments are produced after hydrolysis with papain.[22, 34]

Although the present model lacks a number of details, it appears to be sufficiently well grounded in experimental facts to warrant a brief discussion of its immunologic implications. We believe that it suggests how a sufficient variety of different combining sites specifically directed against different antigens might be built. It has been suggested[5] that chain interaction among a sufficiently large variety of different chains might provide an adequate mechanism.

The ensuing discussion will extend this notion in terms of the present model, after considering several pertinent observations. The first is that specifically purified antibodies to haptens have been shown to produce two to eight sharp L chain bands after starch gel electrophoresis in urea.[18] Band patterns of antibodies to the same

hapten but from different animals were similar, whereas antibodies of different specificities showed different band patterns. The multiplicity of bands has been shown to be related to the heterogeneity of the antibodies.[47]

It was suggested that the differences in the starch gel electrophoretic patterns resulted from differences in amino acid sequence among the various chains.[5, 18] Careful amino acid analyses[48] of purified antihapten antibodies have revealed differences in composition that were correlated with differences in specificity. Bence-Jones proteins, which are particularly homogeneous L chains, differ in amino acid sequence and so far no two Bence-Jones proteins have been found that are identical.[33, 49] Similar evidence for variation in the sequences of Hγ chains has not been found, but it is known that Hγ chains vary in allotypic specificity[50] and Gm character.[51, 52] Allotypic specificities of L and H chains are under genetic control.[53] Although no clear correlation between allotypic specificities and antibody specificities has yet been found, we will assume that all of the amino acid sequences in the chains of antibodies are under genetic control.

The above remarks suggest the possibility that antibodies of both similar and different specificities may be made of more than one combination of L and H chains. If antibody-producing cells contain information for the production of p L chains and q H chains, then there are pq different ways in which antibody combining sites might be constructed.[5, 54] In terms of the present model, this assumes that there is a single most stable conformation of the site for any particular L·H pair. If m different conformations were possible, then the number of different sites would be mpq. We are assuming that only one conformation is possible, since it has been found that each different Bence-Jones protein shows a different thermally induced molecular transition which is in some cases reversible.[10] Moreover, complete denaturation of active fragments of antibodies has been found to be reversible, suggesting that the final conformations of the sites are determined by the amino acid sequence.[55]

The number pq is an upper limit since several constraints may operate. More than one combination of different L and H chains may be capable of forming a site of given specificity, i.e., some combinations of chains may be degenerate with respect to specificity. The starch gel electrophoretic experiments cited above, the great heterogeneity of binding constants,[56] and the *in vitro* reconstitution of active hybrid molecules from chains of different antibodies[8, 22] all suggest that this is the case. Degeneracy would obviously reduce the number of different specificities that could be formed by interaction of a given number of L and H chains.

If there were 10^3 L chains and 10^3 H chains, and if on the average any given specificity were generated by 10 different combinations of L and H chains, then 10^5 different kinds of sites could be formed. It is not our purpose here to consider how the 2000 different chains could arise, but mutation and selection or variations in crossing over during mitoses[54] could possibly account for such numbers.

Since the combining sites are at the ends of the molecular model, and since certain relatively fixed regions of the chains are required for interchain bonding, it is probable that only those residues in certain regions of the H and L chains contribute directly to the site. The contributory residues would not necessarily be in the same linear stretch of the amino acid sequence. The assumption of such site regions would not limit prohibitively the number of possibilities. If 15 amino acids[56]

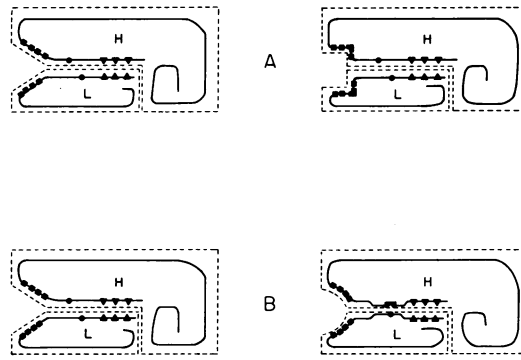

FIG. 4.—Schematic illustration of means by which the conformation of the active site may be influenced. (Only half-molecules are shown; the site is to the left.) (*A*) Differences in conformation of the site resulting from amino acid sequence differences in the site region. (*B*) Differences in conformation of the site resulting from differences in amino sequences not directly involved in the site (modulation). ■ or ⌐—amino acid residues in the site regions; ● or ⌐—amino acid residues in the modulating regions; ▲—amino acid residues in the interchain bonding regions; L—light chains; H—heavy chains.

contributed directly to the site, then genetic variations of the sequences of these amino acids could yield 20^{15} different combinations. In addition, the conformation of an active site may be influenced by modulating interactions.[8] Sequence variations in regions other than that of the site could influence the final conformation, even if the sequences of the site region remain unchanged. To illustrate these ideas, three regions of the antibody molecule are schematically indicated in Figure 4. The interchain bonding region contains amino acid residues required to hold the chains together. Changes of the amino acid residues in the site region can directly alter the specificity of the antibody, whereas changes in the modulating region alter the conformation of the binding site only indirectly.

Finally, it should be pointed out that the mechanism of chain interaction in the model proposed here requires that there be no restriction on the pairing or interaction of any L or H chains or L·H pairs. *In vitro* reconstitution experiments have shown that no restrictions appear to exist.[22, 23] Hybrid 7S molecules may be formed from a variety of L and H chains, and L and H chains each of different species origin will complement each other. As pointed out above, the bonding regions of the chains may not vary greatly in sequence. It has been found, for example, that different Bence-Jones proteins have many similar tryptic peptides, although they vary in over-all sequence.[49]

The formation *in vitro* of 7S molecules from a variety of L and H chains suggests that the *in vivo* synthesis of 7S antibody molecules from chains proceeds by self-assembly, without additional special mechanisms. Present evidence[57] indicates that the genes controlling L and H chains are not closely linked. This would suggest that the messenger RNA's for the two kinds of chains are formed and operate independently.

Although in the present proposal we have emphasized that genetic variation and differences in amino acid sequences among the polypeptide chains are consistent with the model of the 7S antibody molecule, we have not explicitly discussed any theories of antibody production. We feel, however, that the hypotheses used in

constructing this model may be of direct use in formulating an adequate description of antibody synthesis.

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POLYDEOXYADENYLIC ACID

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A reaction resulting in the addition of deoxynucleotidyl residues to the 3′-hydroxyl group of oligodeoxynucleotides has been observed in partially purified preparations of calf thymus DNA polymerase.[1, 2] The reaction was first thought to be a manifestation of the DNA polymerase, but subsequent investigation has demonstrated a *terminal*-deoxynucleotidyl transferase that is distinct and separable from DNA polymerase (*replicative*-deoxynucleotidyl transferase). This distinction was first recognized by Krakow *et al.*[3] in their work on polymerizing enzymes in extracts of calf thymus nuclei, and somewhat later by Keir and Smith[4] and by us.[5] We have now succeeded in preparing the terminal-addition enzyme[6] free of polynucleotides, deoxypolynucleotide degrading enzymes, and DNA polymerase, and find that it displays a marked, although not absolute, preference for d-ATP polymerization.

When the deoxyadenylate is grafted on to oligodeoxynucleotide initiators of known sequence and structure, sequence and structure is known throughout the entire length of the new graft copolymer. The fact that polymerization takes place on a fixed number of growing points allows us to predict that molecular weight distributions will be rather narrow, and it is possible to control the average degree of polymerization by limiting the amount of monomer. Thus, a large series of polydeoxynucleotides having definite sequence, structure, and chain length may be prepared. Some of the polymers available from the action of this enzyme are of special interest to current work on information transfer in biological systems, and they form the principal subject of this communication.

Materials and Methods.—Definitions: The term "initiator" is here defined as a polymer of deoxynucleotides having a free 3′-OH that will accept the deoxynucleotidyl residue from deoxy-