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Conformation of the Free and Antigen-bound IgM Antibody Molecules

THE two major classes of antibody molecule, IgG and IgM¹, have molecular weights of 150,000 and 900,000, respectively. They are composed of heavy and light polypeptide chains. In IgM each heavy (μ) chain, molecular weight 70,000, is linked by a disulphide bridge to a light (L) chain, molecular weight 23,000, and is probably linked by three further bridges to other μ chains²⁻¹⁰.

A model accounting for the flexible Y-shaped structure of IgG seen in the electron microscope has already been described^{11,12}, and we now propose a model for the IgM antibody consistent both with the appearance of free and antigen-bound molecules in electron micrographs and with the available chemical data. We have examined IgM preparations from a variety of mammals and from the chicken and dogfish, and complexes of *Salmonella* flagella with anti-flagella IgM from several of these species^{13,14}. All specimens were negatively stained with cold 2 per cent sodium phosphotungstate (pH 7.3) on holey carbon films¹⁵ and dried at 4°C.

The stellate appearance of the free IgM molecule (Fig. 1*a*) is somewhat variable. Sometimes we find molecules displaying the simple symmetry of that shown in Fig. 1*b*. Svehaug and his colleagues^{16,17} have published good pictures of this type, which they interpret as arising from five cyclically linked subunits. We agree with this interpretation, but believe that the IgM molecule consists of two similar halves which in this view are superimposed. The arms are about 25 Å wide and 100 Å long; the less well defined central region of the molecule is approximately 100 Å across. Fig. 1*c* shows examples displaying a more commonly encountered feature: in each, one or more of the radial units appears branched into two, and in one case all five arms are branched. The length of the branched sections of the arms is 55–70 Å. We suggest that the points at which the arms branch are flexible regions of the molecule where two heavy polypeptide chains in each subunit are joined. This feature is embodied in the model shown in Fig. 3. Because the details of the material present at the centre of the mole-

cule are not revealed in the electron micrographs, this part has been omitted from the model.

Fig. 3*a* represents a single $\mu + L$ chain protomeric unit, with three numbered cysteine residues available for disulphide bridge formation. The numbering, which runs from the N-terminus, is that used previously⁹, but, because the model represents the folded polypeptide chains, the linear sequence of bridges shown here need not correspond to that in the extended chain. In Fig. 3*b* a pair of these units have been assembled through bridges 2 and 4 to form a dimeric subunit, leaving two cysteine residues 3 available for bridging to identical neighbouring units, generating the cyclic structure of the IgM molecule, Fig. 3*c*. It is this latter view of the molecule which we believe corresponds to images of the type seen in Fig. 1*b*. The branching referred to in Fig. 1*c* occurs at a flexible "hinge" region associated with bridge 2, as represented in the model in Fig. 3*d*.

Each of the ten $\mu + L$ chain protomer units carries a single antigen-binding site, situated at the outermost tip of the units arranged radially in the IgM molecule. This positioning follows from electron micrographs of IgM antibody molecules cross-linking two bacterial flagella, as seen, for example, in Fig. 2*a*. When, however, IgM molecules are seen in profile attached along a single bacterial flagellum, they are "staple"-like in appearance (Fig. 2*b*). Similar forms of IgM antibodies have been seen attached to fragments of erythrocyte membrane¹⁸.

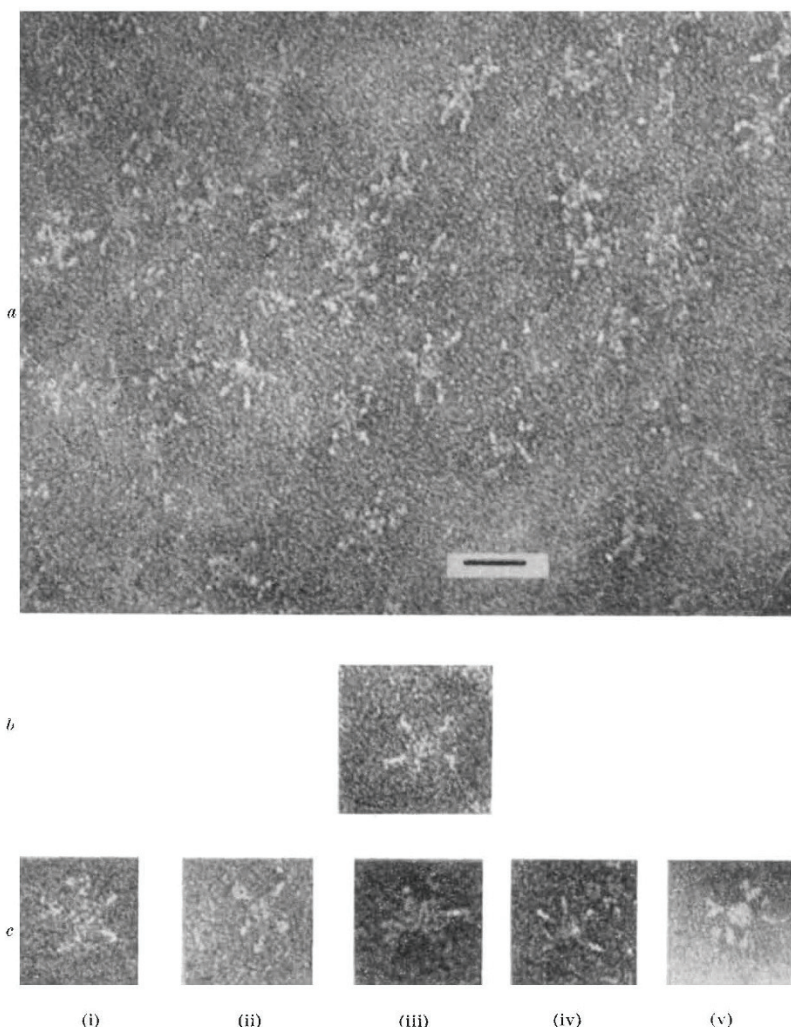


Fig. 1. Electron micrographs of free IgM molecules. *a*, IgM preparation isolated from the serum of mice carrying plasma cell tumour MOPC 104E (generously provided by Dr A. J. Munro). *b* and *c*, Selected individual IgM molecules; (i)–(iv) as *a*; (v) isolated from dogfish serum. The bar represents 250 Å.

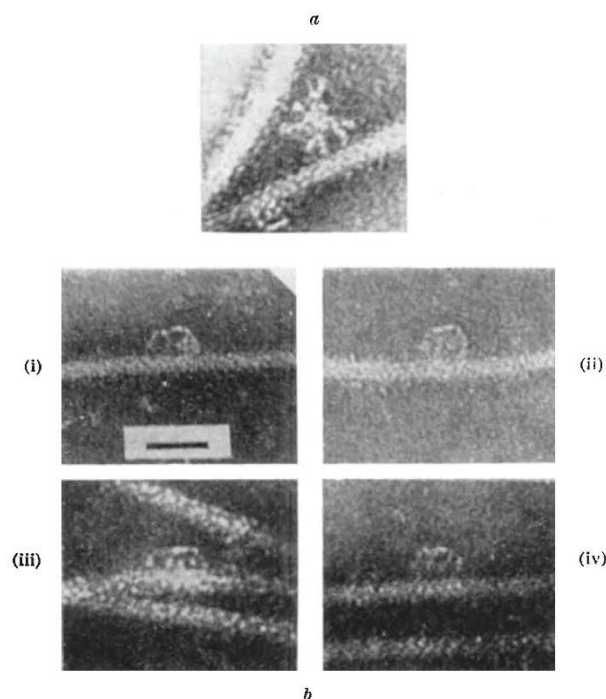


Fig. 2. Electron micrographs of IgM antibody molecules bound to *Salmonella* flagella. *a*, Dogfish antibody cross-linking two flagella; *b*: (i) and (ii) dogfish, (iii) sheep and (iv) rabbit antibodies, each attached to a single flagellum. The bar represents 250 Å.

The reduced maximum dimension of molecules in the staple form is evident when Figs. 2*a* and *b* are compared. This appearance can be explained if several of the radial units have folded at a flexible hinge region to resemble the legs of a table, thus making possible the attachment of all, or most of, the terminal binding sites to antigenic determinants on the surface of a single flagellum. This folded conformation, represented by the model in Fig. 3*d*, is confirmed by the fact that the perimeters of the staples have the approximate length, 300 Å, of the extended IgM molecule. In Fig. 3*d* the legs are folded only at the hinge region near bridge 2 already postulated as their branch point. In some staples, however, the cross-bar is only 100 Å long, with uprights of comparable length. These uprights would be formed from entire 100 Å arms, with the central 100 Å disk in profile forming the cross-bar. Thus, in addition to the flexible regions at the branched points of the arms, hinging can also occur in those regions of the heavy chains linked to neighbours by bridge 3.

In addition to those already mentioned, the following known features of the IgM molecule are consistent with our model: (1) The interheavy-chain disulphide bridges are symmetrical⁹. (2) Reductive cleavage of a single bridge to each heavy chain yields dimeric subunits with a structure corresponding to that seen in Fig. 3*b*^{9,19}. (3) Enzymatic cleavage of IgM preparations yields²⁰⁻²² (a) an Fab fragment, with a molecular weight of 45,000, having a single antigen-binding site, and composed of an L chain and part of a heavy chain carrying no inter-heavy-chain bridge; (b) an (Fab')₂ fragment, of molecular weight 120,000, carrying two antigen-binding sites; bridge 2 links the halves of this dimeric fragment, which arises from cleavage between bridges 2 and 3; (c) a fragment Fc, of molecular weight 300,000, carrying no L chains or antigen-binding sites; this is the central disk of the IgM molecule, composed of the ten C terminal ends of the μ chains linked cyclically by bridges 3 and 4.

An alternative symmetrical IgM model fulfilling the chemical requirements but not immediately reconcilable with the structure seen in the electron microscope can be

constructed by linking each dimeric subunit (Fig. 3*b*) to the next but one on either side.

The Y-shaped appearance and variable conformation of the IgM subunit seen in the electron microscope

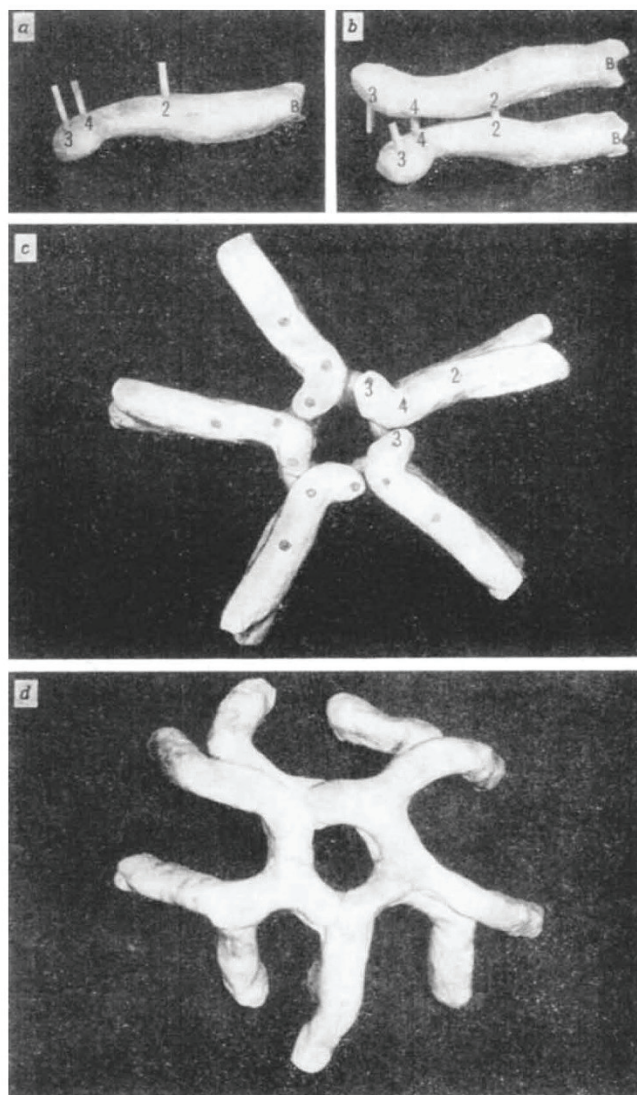


Fig. 3. Schematic models of the IgM molecule and its subunits. *a*, A single μ +L chain protomeric unit; 2, 3 and 4 are cysteine residues available for interchain disulphide bridge formation: the L chain and disulphide bridge 1 linking it to the heavy (μ) chain lie between the antigen binding site (B) and cysteine residue 2. *b*, A dimer formed by linking two protomers through bridges 2 and 4. *c*, Model showing how five of the dimeric units shown in *b* linked cyclically through disulphide bridges 3 are assembled in the IgM molecule. *d*, Model incorporating the features shown in *c* but with the subunits opened to demonstrate the proposed flexibility about a "hinge" region associated with bridge 2. The resulting branched appearance matches that in certain electron micrographs.



Fig. 4. Model of IgM antibody bound to a particulate antigen, photographed in profile so as to simulate the "staple" appearance in micrographs of the type seen in Fig. 2*b*.

resemble those of IgG^{11,12}. Such flexibility in antibodies must facilitate multipoint attachment. The altered stabilized conformation in the bound IgM molecule may be that which is recognized by, and activates, the first component of complement, leading to phagocytosis and membrane lysis.

Finally, the similarity of mammalian and dogfish IgM antibodies is of interest, suggesting that the features described were present in the common ancestor of the dogfish and ourselves.

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Multiple Forms of Monoamine Oxidase in the Developing Brain

MUCH attention has recently been centred on the biosynthesis and metabolism of the catecholamines and indolealkylamines. Because the enzyme monoamine oxidase (monoamine : O₂ oxidoreductase (deaminating), EC 1.4.3.4) is involved in the metabolism of both these classes of biogenic amines, and because of our interest in development^{1,2} we were prompted to investigate the nature of this enzyme and especially its activity in developing brain and other tissues of the maturing organism.

Several investigators have suggested that there might be multiple forms (isoenzymes) of monoamine oxidase (MAO), based on indirect evidence derived from multiple substrate and inhibitor studies³⁻⁷. There are even some reports of attempts to isolate the pure enzyme which also suggest the possibility of the existence of one or more multiple forms of MAO (refs. 8-13). As a means of approaching this problem, we attempted to look at any differences in MAO(s) there might be between embryonic and adult brain.

We used 16 day old embryo and adult New Hampshire red hens. After the appropriate tissue had been homogenized, the monoamine oxidase was solubilized by sonic oscillation¹⁴ for 30, 60 or 120 min, partially purified by ammonium sulphate precipitation and then subjected to polyacrylamide gel electrophoresis. In some cases, the enzyme was solubilized by homogenization of the tissue with 15 per cent 'Lubrol'. The general methods and techniques of gel electrophoresis used were those described

by Davis¹⁵. The gel concentration was 7.25 per cent and a Tris glycine buffer at pH 8.3 was used. The running time of the electrophoresis was 2 h at 4°C with current at 4.5 mA per tube. Monoamine oxidase activity was revealed by the appearance of purple coloured bands after incubation with a nitroblue tetrazolium substrate staining solution. Tyramine, kynuramine, benzylamine⁸ or tryptamine¹⁶ was used as substrate. All substrates were used in excess. Monoamine oxidase activity was also determined by the spectrophotometric method of Weissbach *et al.* with kynuramine as substrate¹⁷.

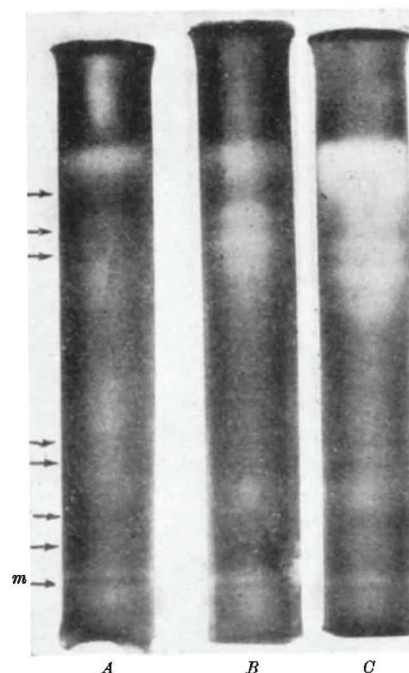


Fig. 1. Gel electrophoretic patterns of chick brain monoamine oxidase. A, 16 day embryo; B, 1 day old chick; C, adult. Arrows point to bands discussed in text. Arrow labelled *m* indicates the dye marker. The anode is at the bottom of the tube while the cathode is at the top.

The results of acrylamide gel electrophoresis of embryonic, newborn and adult chick brains stained for monoamine oxidase activity, with benzylamine as substrate, are shown in Fig. 1. The slow moving MAO band which is always obtained from embryonic brain is completely absent from the pattern derived from the adult brain. It is also apparent that the newborn chick brain has a pattern similar to that of the embryonic brain. At least five bands with monoamine oxidase activity are common to both adult and infant brain. Our preliminary results indicate that these same MAO band patterns are obtained whether the tissue is disrupted by homogenization followed by sonication or disrupted by homogenization with a non-ionic detergent. The data obtained after sonication for 120 min were the same as those after 30 min. These results are in accord with the statement by Kim and D'Iorio that either method of solubilizing the enzyme results in similar patterns of MAO active bands.

Similar patterns of the multiple forms of MAO are obtained if tyramine or kynuramine is used as substrate of the enzyme, although there seem to be quantitative differences in the relative intensities of the various bands. It should be pointed out, however, that when chick brain MAO patterns, from embryo as well as adult, were determined on acrylamide gel using tryptamine as substrate, no bands could be observed. This is in contrast to rat brain, where we and others¹⁸ have observed that tryptamine as well as the other substrates mentioned earlier can readily serve as substrate for the multiple forms of the enzyme.