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Computer models of the human immunoglobulins

Shape and segmental flexibility

Richard Pumphrey

At present there is interest in the design and deployment of engineered biosensor molecules. Antibodies are the most versatile of the naturally occurring biosensors and it is important to understand their mechanical properties and the ways in which they can interact with their natural ligands. Two dimensional representations are clearly inadequate, and three dimensional representations are too complicated to manipulate except as numerical abstractions in computers. Recent improvements in computer graphics allow these coordinate matrices to be seen and more easily comprehended, and interactive programs permit the modification and reassembly of molecular fragments. The models which result have distinct advantages both over those of lower resolution[1], and those showing every atom, which are limited to the few fragments[2–5] or mutant molecules[6] for which the X-ray crystallographic coordinates are known.

In this review Richard Pumphrey describes the shape and flexibility of immunoglobulin molecules in relation to the three dimensional structure.

Computer generated models of the immunoglobulin classes and the T-cell antigen receptor are shown on the centre pages of this issue. The starting point for the computer graphics were sets of X-ray diffraction coordinates taken from the Brookhaven Data Base. The peptide sequences were taken from Kabat *et al.*[7]. A new homology table of all the human immunoglobulin domains was prepared using the coordinates of the paradigm domains and secondary structure prediction[8]. Each domain of each immunoglobulin was processed separately to generate new coordinates corresponding to the amino acid sequence: the starting coordinates were taken from the domain closest in beta-pleated sheet sequence and the bends then modified where necessary to accommodate the variations in sequence, taking the predicted secondary structure into account. These processed domains were then glycosylated and reassembled into complete molecules to give structures consistent with available structural data from biochemical (Refs 7, 9–13 and references therein), physical[14,15] and electron microscopic techniques[16,17]. I have also used new electron micrographs of human IgE and IgG subclasses in the assembly of these models.

A 5 angstrom (Å) diameter cylinder drawn between adjacent alpha-carbon atoms has been used as a simplified representation of the peptide chain. This emphasizes the 'hard' external surface of the molecules and has many advantages. The smaller amino acids lie within, or nearly within, the cylinder, and the larger side chains on the exterior are mostly polar, and will not have a rigidly

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For technical reasons we are unable to include the centre page diagram in this edition. See the June issue of *Immunology Today* for the centre pages.

fixed conformation when the protein is in solution. Sugars are represented as spheres which occupy roughly the same volume and are centred on the centre of mass of each of the sugars. Except for the few known from X-ray crystallography, the conformation of these is largely speculative, but any model of antibodies would be incomplete without some attempt to depict the oligosaccharides.

The new sets of coordinates were produced using an interactive computer graphic facility, IMMAM (Interactive Molecular Modelling At Manchester (P. Finn, A. Marsden, B. Robson). There are numerous atomic clashes, overstretched bonds, and unlikely dihedral angles after the initial side-chain substitutions. These can be relaxed by a few cycles of refinement, but in general the changes in alpha-carbon coordinates that result are not sufficiently great to warrant the effort for the low resolution modelling shown in the centre-page diagrams. A more serious defect is the possible change in the angle of twist of the beta-barrels when the effective number of strands is changed, either within domains or between them. I have no direct method of computing these changes, and have to rely on inspired guesswork, based on the general rules of protein architecture[18].

The building blocks

Domains

Immunoglobulin domains have been illustrated repeatedly in the literature (reviewed in Ref. 16) and are not shown separately here. They are typically 7 to 9 stranded beta-barrels[18], flattened by a disulphide bridge between invariant cysteines in two opposing strands to form two faces: that with the first cysteine called the X face, the other the Y. Domains may pair up by their X or Y faces to form further beta-barrels; some, such as the C_H2 of IgG, are not in close contact with each other.

Hinge

All human immunoglobulins have a flexible region between the Fab arms and the two C-terminal domains of the heavy chain. In IgE and IgM the middle part of this is formed from a pair of domains, with short flexible sequences at either end. In IgA the middle part is missing, leaving a single flexible region, which is long in IgA1 but very short in IgA2. The middle section of the hinge in IgG1 is reduced to a small but probably comparatively rigid structure which might be thought of as a body lying between the flexible arms extending to the Fab and the legs leading to the Fc. The body and arms can be identified in crystals of the IgG1 myeloma protein KOL, (Ref. 4), but electron microscope studies indicate that the arms unfold in solution, allowing independent movement of the Fab. To extend this analogy, in IgG4 the glycine in the hinge arm probably acts as an 'elbow', and the –Gly–Gly– sequence in the hinge legs of IgG1,3 and 4 may act as a 'knee', these being the most flexible sections. Nothing is known for certain about the organization of the long hinge of IgD, but it seems certain that at least the N-terminal half must be very flexible. These differences in the structure of the hinge between the classes and subclasses have profound effects on their properties[14,15,19–22]; this is discussed further below.

Membrane insertion

These sequences are probably largely alpha-helix, and in this form the hydrophobic section can just span the lipid bilayer of the membrane. They are coded for by two separate exons, which the B cell uses when membrane inserted immunoglobulins are to be made. The model of the immunoglobulin transmembrane helix-pair suggests that there are hydrophilic amino acids on the outward-pointing surfaces, which may well also be associated with another integral membrane protein, possibly (by argument of phylogenic homology) like the T3 complex of peptides[23,24].

Oligosaccharides

The structures of many of these are now known[25–29]. They are not homogeneous in all molecules of an immunoglobulin class, but an attempt has been made to illustrate a commonly occurring form in each case. They are thought to have a marked effect on the solubility of the immunoglobulins, and on their transport and catabolism, and it seems obvious looking at these models that they could also interfere with binding to potential ligands. A mannose-rich oligosaccharide is attached to any exposed (Asn)–(X, not Pro)–(Ser/Thr) sequence before the main assembly of the immunoglobulin[27]. After assembly, the accessible parts of this are reprocessed in the Golgi apparatus to give the complex bi-antennate form[30], which may or may not have a bisecting N-acetyl glucosamine, and which is sialated to a variable extent. The rules for the attachment of the O-linked oligosaccharides of the hinge of IgD and IgA1 are less clear, but seem to require a very free, accessible polypeptide strand; it has been suggested that the Ser/Thr has to take up a beta-turn configuration in the glycosylotransferase[25,31].

Immunoglobulin G

There are only slight differences between subclasses of human IgG apart from in the hinge region, though clearly even a single amino acid substitution can profoundly affect the properties of a protein, such as the substitution of Arg for His-435 in IgG3 preventing the binding of staphylococcal protein A.

The hinge on the other hand is totally different in each subclass and the effect of this can be seen in the models. In IgG1 it is long enough and freely flexible so that it allows the Fab to spin about their axis of quasi-2 symmetry and at the same time to flap about the mean position shown here, anywhere within the sphere centred on the first inter-chain disulphide bridge, limited by their impacts with each other and the Fc. This is reflected in our electron microscope pictures of human IgG1 – though most of the molecules are seen close to the illustrated position – and has also been seen with rabbit IgG[17]. (Both the low ionic strength buffers used in shadowed electron microscope preparations, and the heavy metal ions used for negative staining, may be expected to modify the average conformation of immunoglobulins, and differences in the supporting film can produce quite different appearances[19].)

It is difficult to measure accurately the time constants for all the different modes of rotational diffusion and intramolecular flexion and torsion[32]. Estimates from measurement of fluorescence anisotropy decay with fluorescent probes in the antigen binding cleft give time constants varying from 10 ns (presumably wobbling of the Fv on the rest of the Fab) to 80–150 ns (presumably a combination of flapping and spinning of the Fab). The

same parameter for rotation of the whole molecule may be calculated as 160 ns (Ref. 33).

Previous models of the hinge of IgG1 have placed the disulphide bridged body of the hinge either as shown[21] or deeper between the C_H2 domains[1]. Though it may be able to assume this deeper position, measurements from our electron microscope pictures of IgG1 show it most commonly to be as illustrated here. Two models are illustrated in the centre-page diagrams – the colour picture shows the body of the hinge in the configuration it takes in the crystal of the protein KOL (Ref 4); the stereo picture shows it twisted slightly about its axis, continuing the spiral set up in the lower part of the hinge.

IgG2 not only has a shorter hinge but the double disulphide bridge right at the base of the Fabs means that it is all body and has no arms; this will almost completely prevent any spinning of the Fabs and greatly restrict their flapping. The loss of a glycine in the 'knee' (between the body and Fc) makes it relatively stiff here as well.

IgG3 shows the other extreme, with the long body of the hinge holding the Fabs well clear of the Fc, and with long, flexible arms at the top allowing the Fabs very free movement. Our electron micrographs of IgG3 show that the length of the body is about 90 Å (R.S.H. Pumphrey *et al.*, unpublished); this is too short for a straight parallel arrangement of the two strands such as suggested by Marquart *et al.*[34], and too long for the model suggested by Kilár *et al.*[35], and so I have shown them as the double helix which was suggested by solid space-filling model building, though the double helix is almost certainly not so regular as shown. The time constants for spinning and flapping might be much as for IgG1, but the time spent by the Fab close to the Fc must be much lower, explaining why it is so much easier for Clq to bind to the Fc of IgG3 than that of IgG1 in solution[36]. The remoteness of Fc from Fab also means that, when IgG3 is bound to a solid phase antigen, the increase in binding of Clq must be due solely to the effective increase in local concentration of the Fc near the antigenic surface. The old concepts requiring some static structural change induced in the Fc by the Fab binding to antigen no longer seem tenable[4]. An elegant proof of this comes from the similarity of affinity between the isolated heads of Clq and complexed IgG, and between monomeric IgG and whole Clq[37].

The hinge arms in IgG4 are shorter than in IgG1; their flexibility is probably intermediate between those of IgG1 and IgG2, and they may allow some spinning of the Fabs, principally around the glycine. Their shortness will lead to the Fabs spending more time close to the Fc, hindering the access of the head of Clq.

A microcosm in motion

The models are presented to give an idea of their relative shapes and properties, but it may be difficult to imagine the significance of the time constants quoted above without a little more discussion of what life is like on the molecular level. The magnification of the models as reproduced in the centre-page diagram is 4×10^6. On this scale the molecular mass of IgG1 becomes 16g, and of IgM, 100g. To maintain a consistent mechanical system it is convenient to slow time down by 4×10^6 as well, and in this coordinate frame the models can be realistically 'animated'. The Brownian motion which affects bacteria seen down the microscope is familiar at 10^3 magnification but at 4×10^6 it becomes all-dominating: a molecule of IgG would be battered to and fro by the water around it; at each instant the speed of rotation and translation of the whole molecule and its components changes, but the average translation is about 3 ms^{-1} and the tumbling 7 s^{-1} in this slowed-down world. Because of the restriction imposed on the movement of the Fab by the hinge, it is seen to spin (on its axis when the hinge is taut, but in any direction when the hinge is slack) and flap erratically about the centre of gravity of the molecule. Some discussions of the flexibility of IgG have assumed that the hinge is relatively stiff[33]. Though it is possible to calculate the change in free energy for different configurations of the hinge peptide, there is no way to calculate the distribution of transferable energy provided by the fragments at either end of the hinge without making such great simplifications as to render the calculations almost meaningless. A crude simplified model indicates that the transferable diffusional kinetic energy exceeds the change in free energy on distortion of the hinge most of the time – in other words the hinge behaves more like a piece of string than a spring.

Immunoglobulin E

Barker *et al.* pointed out the close homology between IgE and IgG[38], and that it was possible to assemble the C_H3 and C_H4 of IgE in a very similar way to the C_H2 and C_H3 of IgG. The C_H2 of IgE is closest in its pleated sheet to that of C_λ, and so this was taken as the starting point for its coordinates. There were three possible ways in which these $C_\epsilon2$ domains might have paired (R.S.H. Pumphrey *et al.*, unpublished): the one considered most likely is shown in the diagram and is very similar to the way that the C_λ domains pair in lambda light chain dimers. This means that the inter-chain disulphide bridges are 'crossed', with the N-proximal cysteine on one chain paired with the C-proximal on the other. A model made with uncrossed cysteines produces an IgE molecule that is T-shaped: while this looks plausible, it is not compatible with our electron microscope pictures of human IgE (R.S.H. Pumphrey *et al.*, unpublished). Though there is no hinge in IgE, there seems to be scope for considerable flexibility, both between the $C_\epsilon1$ and $C_\epsilon2$, and between $C_\epsilon2$ and $C_\epsilon3$. The latter may allow torsion >50° and flexion >30° from the midpoint (though torsion is limited at maximum flexion, and vice versa). That this is important will become evident in the discussion of the interaction of IgE with its receptor in the second part of this review (which will be published next month). V.T. Oi *et al.* suggest that IgE is among the least flexible of the immunoglobulins[22]. If this is correct, then there must be some end-to-end interaction between the domains which is not apparent in the models.

Immunoglobulin M

The extra 18 C-terminal amino acids in IgM are predicted to contain a region of extended structure, which most probably forms an extra strand on the Y face of the $C_\mu4$ (though this could be on the same domain, on the $C_\mu4$ of the other chain of the dimer, or on another member of the pentamer). With this arrangement, the penultimate amino acid, cysteine, is brought close to the unpaired cysteine in the $C_\mu3$ and can conveniently be paired with it. This is supported by the simultaneous

disappearance of all four free sulphydryl groups on oxidation of intracellular monomeric IgM[39] and would agree with the data for IgA[10] which suggests a similar configuration. The most likely pairing of the $C_\mu4$ by their Y faces gives the same flattened beta-barrel-like structure as between the X faces: it is of interest that with the extra strand, the Y face of IgM has more hydrophobic amino acids pointing outward than any corresponding domain in the other classes and this may well be important in the formation of the pentamer.

For this review I modelled the J chain as a beta-barrel (though not exactly as in Zikan *et al.*[41]), and positioned it between two opposing C-terminal domains. The tail cysteines not captured by the J chain have been paired with the spare cysteines in the $C_\mu3$. Electron microscope pictures have a slight asymmetry suggestive of the irregularity caused by J chain in the model (E. Nunn and A. Feinstein, unpublished). The reasons for this departure from the accepted model and other possible configurations will be discussed in the second half of this review and an intermediate step in polymerization illustrated.

The C-terminal oligosaccharide is of an unusual type in the pentamer[28]: this might be explained by its partial inaccessibility due to the pentamerization. In the monomer it is shown here as the straightforward bi-antennate structure which would be expected for a freely accessible oligosaccharide.

The 'Fc' portion of IgM ($C_\mu3$ and $C_\mu4$) must be fairly rigid, but there is the same potential for flexibility between $C_\mu2$ and $C_\mu3$ as in IgE. This has been clearly seen in electron microscope pictures, in the 'staple' form of bound IgM[16]. Earlier models of IgM showed the Fab pairs in the same plane as the disc of the Fc[1]; their position here is based on electron microscope pictures which indicate that earlier models may not have been in the most relaxed configuration[22].

Immunoglobulin A

The two subclasses of IgA have dramatically different shapes. The long hinge of IgA1 is protected to a certain extent against proteolysis by the lack of charged amino acids and the O-linked oligosaccharides. Some bacteria have enzymes which specifically attack the IgA1 hinge[42]; the effectively hingeless IgA2 may be able to cope with these better than IgA1.

Like IgG1, IgA1 has a Fab which can spin and flap: IgA2 is even more restricted in flexibility than IgG2, and in the A2m2 allotype spinning may be impossible if there is a disulphide bridge between the two light chains.

There only seemed to be one possible way in which to assemble the two C_H2 domains of IgA: once the intra-chain disulphide bridges were linked[9], the inter-chain bridges had to be 'crossed', with the N-proximal cysteine of one chain linking with the C-proximal of the other; others have found the same[1]. J chain is shown interacting with the Y face of the $C_\alpha3$ domain of IgA, and one pair of tails has been exchanged between the IgA monomers. The argument leading to the conclusion that this is a reasonable model will be presented next month.

Immunoglobulin D

The most curious feature of IgD shape is its hinge. Its configuration is unknown, though the sequence suggests that there are alpha-helical sections. Like IgA1 the non-helical part is protected by O-linked oligosaccharides but the alpha-helical section is extremely sensitive to proteolysis[12].

The overall shape of the Fc is predicted to be about 3–4 Å longer than that of IgG but otherwise similar. In the C_H2 of IgD the decapeptide on the N-terminal side of the glycosylated asparagine is strongly predicted as alpha-helical: this is at the free edge of the domain and would not disturb the rest of the beta-pleated structure when modelled in this way, but the oligosaccharide would be rotated out of the space between the $C_\delta2$ domains. This seems unlikely, as it is in the unprocessed (high-mannose) form, and probably hidden between the $C_\delta2$ domains. (In IgG it must be able to 'float' out from between the C_H2 domains for the various glycosyl transferases to have access to it, at least in the intracellular environment in the Golgi apparatus.) An interesting possibility would be that the alpha-helical parts of the hinge, instead of interacting with each other, might interact with the $C_\delta2$, thus hiding the sugar. The overall conformation would then place the start of the hinge arms near the top of the $C_\delta3$ as they are in the mouse IgD which lacks both the alpha-helical part of the hinge and the $C_\delta2$.

T-cell antigen receptor

It seems likely that in their earliest form immunoglobulins evolved as a B-cell surface membrane receptor and only later did their heavy chain genes acquire the special exon coding for the tail of the secreted form of the molecule. For interest I have also shown the T-cell receptor, which, though it has clearly diverged from the immunoglobulins in several respects, must be more like the original lymphocyte antigen receptor than membrane IgM. The construction of the model of the T-cell receptor was more difficult than for the immunoglobulins, as there are several divergent features in the predicted secondary structure: for instance, the C_α domain[43] has shrunk by loss of amino acids from the bends, whereas the C_β has grown[44]. This makes it particularly difficult to be sure about the arrangement of the last beta strand of the C_β domain. A very interesting feature apparent in these models is that all the oligosaccharides are on one face of the molecule (this is true in the mouse as well as the human receptor). The model is obviously incomplete as the T3 complex of peptides has been omitted.

Conclusion

Lack of firm knowledge makes many of the details in these models speculative, and in time parts of them may be proved incorrect. They are intended as the low resolution forerunners to modelling in atomic detail with full energy calculations when more powerful computer facilities become available. However, this does not detract from their value in their present state. Guided by electron microscopy it has proved possible to assemble the reconstructed domains of each immunoglobulin into reasonable quaternary structures, and in doing so to prove that some of the configurations which seemed possible from the primary sequence data are most unlikely to be true. The interactive programs used to make these models allow twisting and flexing between component parts, which is very useful when investigating the flexibility of their parent molecules. They also allow surface features to be coloured in at will, so that binding sites or particular peptide sequences can be

highlighted; this will become apparent in the second half of this review which deals with binding sites and molecular interactions.

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