X-RAY STUDIES ON ANTIBODY FRAGMENTS

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1. Introduction

The three-dimensional structure of antibody and its fragments has been the subject of several recent crystallographic studies. The determination of 6 Å resolution of the structure of a myeloma protein [1] was followed by high resolution studies of a Bence-Jones dimer [2] and Fab fragments [3,4]. These latter studies [2–4] have demonstrated the independent folding of domains along the polypeptide chains [5] at least for the Fab fragment. We have recently reported the structure of a crystalline variable domain from a Bence-Jones protein REI [6] for which the complete amino acid sequence is known [7]. In section 2 of this communication we outline some of the features of this structure.

It seems likely that the observed structural homology between variable (V) and constant (C) domains in the Fab fragment [2,3] is extended to the Fc fragment. Studies of such homology can be made using the Rotation Function [8]. The results given in section 3 indicate a molecular symmetry for the Fc fragment which is consistent with self rotation functions of Fc crystal data and Immunoglobulin G crystal data.

2. The three-dimensional structure of a dimer of variable domains from a light chain

The domain structure of the N-terminal half of the Bence-Jones κ-type protein REI [6] is shown in fig. 1. Approximately 50% of the residues contribute to the two levels of β-sheet, and between these sheets one finds conserved hydrophobic residues (Try 35, Cys 23–88, Tyr 86, Gly 99, Gly 101). The structure is consistent with the notion of minimal structural perturbation in the face of extensive sequence variability. The proximity of α-carbons 8 and 10 (about 5 Å) accomodates the deletion of Ser 9 in all λ-type chains. The hypervariable segments (24–34, 50–66, 89–97), where insertions and deletions often occur, are found in external loops at the right hand end of fig. 1. Gly 16 (conserved in 18 out of 18 human V sequences [9]), Gly 41 (17 out of 18) and Gly 57 (17 out of 18) and Gly 57 (17 out of 18) lie in type II β-bends [10] where glycine must occupy the third position in the turn.

Antigen binding capacity has been shown to be localised in the N-terminal domains of heavy and light chains (i.e. V_H and V_L) in the antibody molecule

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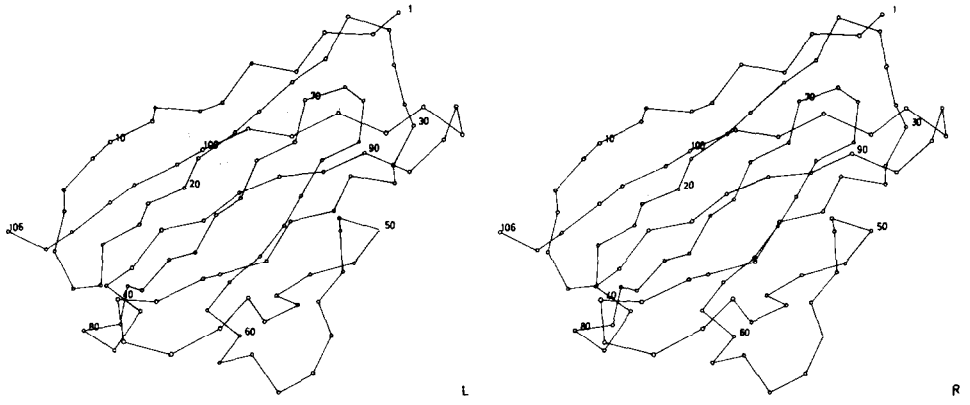

Fig. 1. Alpha-carbon positions in the N-terminal domain of the Bence-Jones protein REI.

[11]. Two criteria maintain the identity of the antigen binding site, viz (i) the monomer configurations of V_L and V_H, and (ii) the aggregation configuration of the V_L–V_H dimer.

Bence-Jones dimers have also been observed to exhibit some of the antigenic reactivity of the native antibody [12], and in crystals of a Bence-Jones dimer [2] the observation of two structurally different, but chemically equivalent, chains has led to the speculation that one of the light chains is simulating a heavy chain [2]. The difference in the two chains here resides in the nature of the V_L–C_L contact on each chain and not so much within the domains. In this Bence-Jones dimer one local diad axis passes between the two C domains and another, 120° from the first, between the two V domains [2]. A local diad relationship is also found between the monomers in the crystals of the variable domain of REI [6] and in fig. 2 is shown a view along the dimer axis of the α-carbon positions in the dimer. Comparison with fig. 9 of ref. [2] indicates that V_L domains dimerise independently of the covalent attachment of C_L

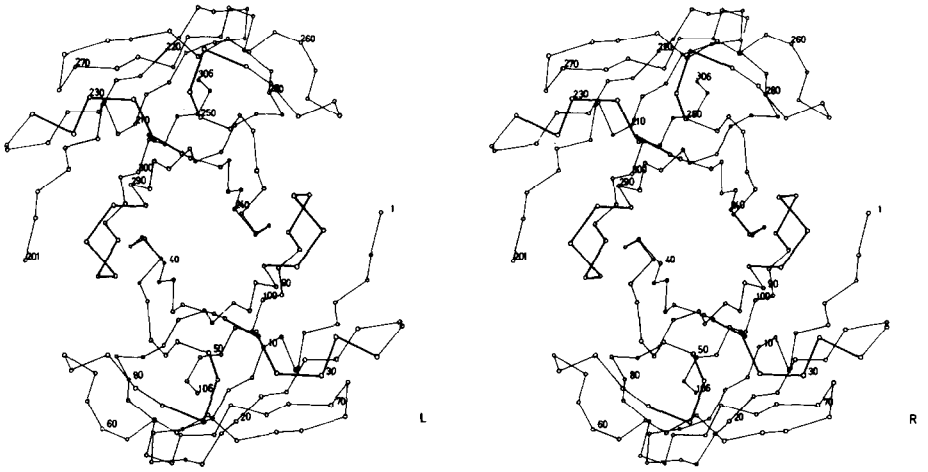

Fig. 2. View along the local diad axis of the α-carbon positions in the dimer.

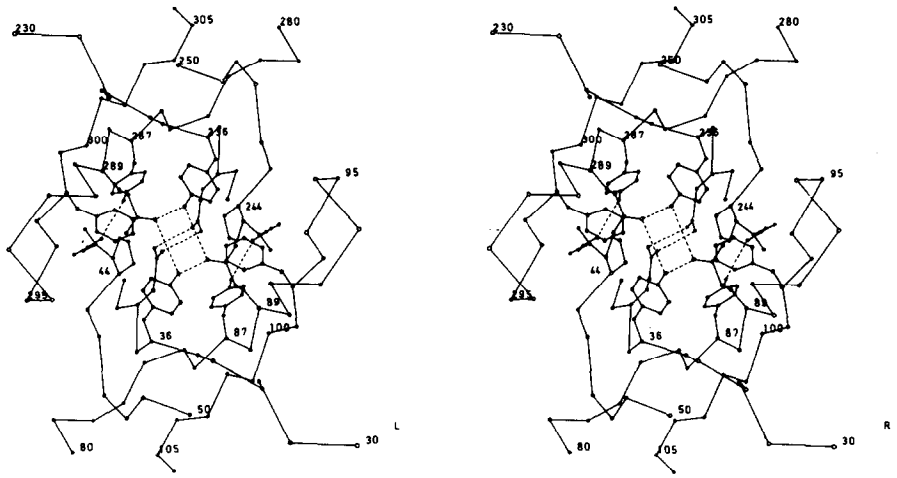

Fig. 3. Residues important in securing the dimer interface, viewed down the dimer axis.

domains. This is apparently the structural basis for the observed localisation of antigen binding capacity in V_L-V_H [11]. A view down the diad axis of the residues in this contact is shown in fig. 3. Gln 38, Tyr 36 and Gln 89 make hydrophilic interactions around a core of hydrophobic contacts including Phe 98, Tyr 36, Pro 44, Tyr 87 and Ala 43. Phe 98, Tyr 87 and Pro 44 are found in all of the 18 sequences referred to above and Gln 38 is consistent with 17 of them.

Fig. 4 is also a view down the two-fold axis and shows those residues contributing to the surface of the molecule in the vicinity of this symmetry axis. A pocket flanked by six tyrosine residues and ending with the hydrophilic interaction between Tyr 36 and Gln 89 with their symmetry equivalents is large enough to accomodate only a single ring system with small polar substituents. This group would be oriented in the pocket by tyrosines 49, 91, 96 and their symmetry equivalents. The three hyper-variable loops lie within a radius of 10 Å from this pocket. Experiments in progress are aimed at studying the binding

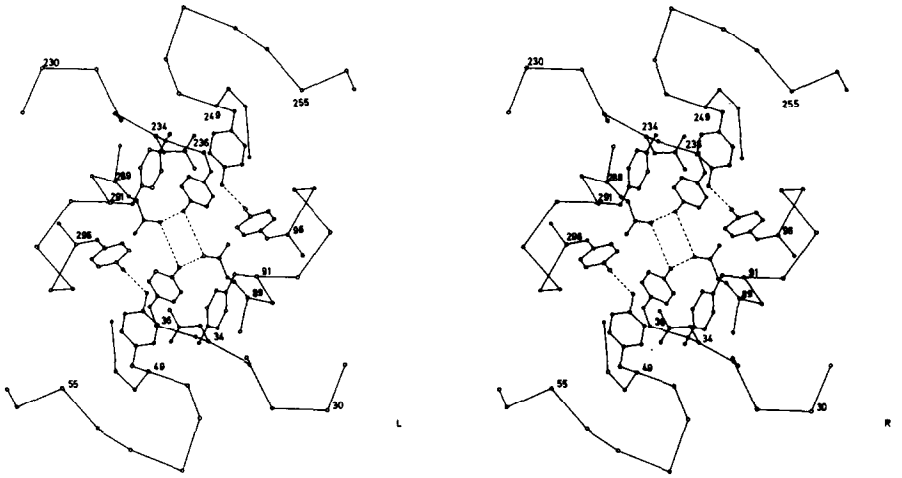

Fig. 4. The potential hapten binding pocket, viewed down the dimer axis.

properties of this pocket.

A preliminary list of α-carbon coordinates has been given elsewhere [6].

3. Molecular symmetry in the Fc fragment

3.1. *Crystalline materials*

Crystalline human Fc fragment was kindly provided by Dr H. G. Schwick, Behring Werke, Marburg, Germany.

The space group is $P2_12_12_1$ with cell dimensions 80.4 Å, 146.4 Å and 50.4 Å, i.e. essentially identical with the crystal form proposed by Humphrey [13].

Heavy metal binding studies with these latter crystals [14] suggested a local diad axis between the two polypeptide chains in the asymmetric unit. Indeed we note that the native low angle diffraction pattern shows evidence of $A2_122$ space group symmetry and thereby implies the addition of diad axes parallel to Y and Z in the $P2_12_12_1$ unit cell. The length of the Fc fragment as determined from the low resolution study of a myeloma protein [1] is 85 Å, and it is therefore not possible for the Fc particle to lie around the Z-diad. The highest peak in the 5 Å native Fc Patterson function is 0.08 times the height of the origin peak at fractional coordinates (0.03, 0.50, 0.50) and provides further evidence of the pseudo A-face-centre. The next highest peaks are 0.05 times the origin peak.

Crystals of a human myeloma protein suitable for high resolution study have recently been reported [16]. They belong to the trigonal space group $P3_121$ with one half molecule in the asymmetric unit, the other being related to it by a crystallographic diad.

4. Results

The Rotation Function [8] is an angular correlation function and has been used to study three-dimensional structural homologies within the Fc fragment. In the Fc crystals one is seeking evidence firstly for the 'main' diad between the two chains and secondly for structural similarity between the C_{H2} and C_{H3} domains. In the immunoglobulin crystals this 'main' diad is crystallographic and one

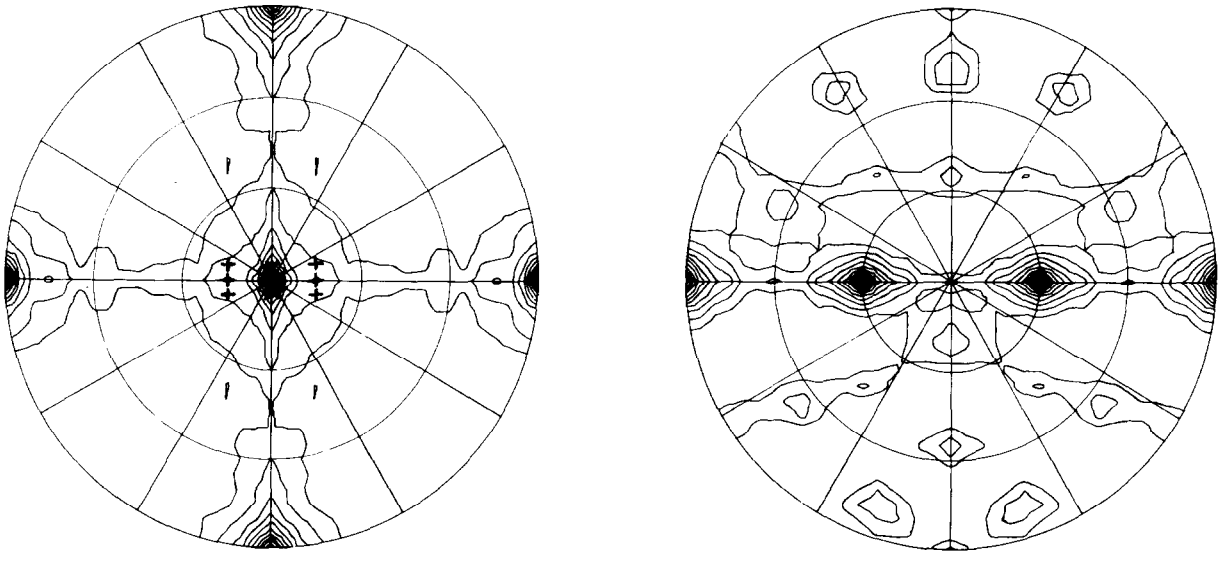

Fig. 5. Rotation function sections, $\chi = 180°$. These figures are stereograms with the OX axis horizontal, OZ vertical and OY normal to the plane and on this section indicate the presence of two fold axes. The polar coordinates (θ, ψ) are measured around from the OX direction for θ and down from the OY direction for ψ. a) Fc crystal data. The main diads are indicated by x and the secondary diads by +. b) IgG crystal data.

seeks evidence only for the latter pseudosymmetry.

For the Fc self Rotation Function seventeen hundred independent intensities in the shell of reciprocal space from 10 to 4 Å were used to calculate the coefficients of the spherical harmonics used in the Fast Rotation Function program [15]. The radius of Patterson space around the origin which was investigated was 23 Å. All of the significant features in the map correspond to diad axes and this section of the rotation function is shown in fig. 5a. The most prominent features are the three crystallographic diad axes in the Patterson function parallel to OX, OY and OZ. A diad axis with polar coordinates (0, 15) is just resolved from the peak corresponding to the crystal screw axis parallel to OY (in the centre of figure 5a) and evidence for another diad axis nearly parallel to OY lies in the further distortion of this origin peak into the OYZ plane.

The immunoglobulin self rotation function was generated from two thousand independent intensities in the 10 Å to 4 Å shell of reciprocal space and again a sphere of radius 23 Å around the Patterson function origin was investigated. The $\chi = 180^\circ$ section is shown in figure 5b were the most prominent features are the crystal diads parallel to the *a*, *b* and (110) crystal directions. Around these crystal symmetry peaks is found a similar distortion to that observed in the Fc map and if the data between 10 and 6 Å (i.e. 600 intensities) are omitted from the rotation function, side peaks, 11° in ψ from the crystal peaks, emerge. Other peaks in this section possibly derive from local symmetries within the Fab fragment.

Finally, a cross rotation function between the two sets of crystal data has been calculated. Six hundred and fifty independent intensities from the IgG crystal and 800 independent intensities from the Fc crystal were used. The resolution limits were 10 and 5 Å and the radius around the Patterson function origin which was studied was 28 Å. Maximum overlap occurs when the Fc data are rotated 90° about their OZ axis and then 15° about the new position of their OY axis. Such an operation aligns the OY axis in the Fc crystals along the crystallographic diad OX in the IgG crystals. A number of subsidiary peaks of approximately equal height are distributed around this point. All of these features are 50% higher than other features in the map.

5. Discussion

The cross rotation function study supports the result deduced from packing considerations that the diad axis between the two identical halves of the Fc fragment in the Fc crystals is nearly parallel to the OY crystal axis. The location of this diad axis at the polar coordinates ~(90, 5) (fig. 5a) is consistent with such a notion and with the observed strong distortion of the origin peak in this direction. Pseudosymmetries on each Fc chain would have to satisfy this local diad relationship and a secondary diad, i.e. between the C_{H2} and C_{H3} domains, at ~(15, 15) is sufficient to explain this weaker but broader distortion of the origin peak around the equator of fig. 5a. It is not possible to decide whether such a secondary diad relates C_{H2} and C_{H3} on the same or different chains.

Further ambiguity is introduced through the crystal symmetry which allows one to argue that the secondary diads could lie not near the OY crystal axis but rather near the OX crystal axis. The former possibility with a translation element to satisfy the known dimensions [1] is shown in fig. 6, a model which seems not unlike the structure of a Bence-Jones protein [2] where an approximate diad, with translation, might relate two domains on one chain.

The proposed model is clearly consistent with the IgG self rotation function (fig. 5b) where again a distortion of the peak due to the crystal diads is seen.

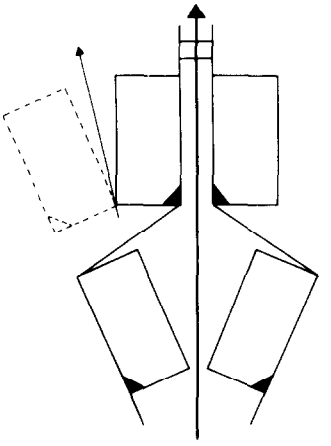

Fig. 6. A model for the Fc fragment. Unresolvable ambiguities are explained in the text.

Again the crystal symmetry generates some ambiguities in the real location of the secondary diad with respect to the main diad, i.e. it may lie 11°, 49°, 71° etc. in ψ from the crystal diad. Only some of these possibilities are eliminated when the rotation function on the Fc crystal data is jointly considered.

The finding here of a local diad relating the C_{H2} and C_{H3} domains, together with the possible orientations of this diad with respect to the main Fc diad, leads to the result that the $C_{H2}-C_{H2}$ contact across the main diad is different from the $C_{H3}-C_{H3}$ contact across this diad. Such a model is consistent with studies on the properties of free C_{H2} and C_{H3} domains [17,18] which imply a stronger contact for $C_{H3}-C_{H3}$ than for $C_{H2}-C_{H2}$.

Acknowledgements

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