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Domain Association in Immunoglobulin Molecules

The Packing of Variable Domains

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We have analyzed the structure of the interface between VL and VH domains in three immunoglobulin fragments: Fab KOL, Fab NEW and Fab MCPC 603. About 1800 Å^2 of protein surface is buried between the domains. Approximately three quarters of this interface is formed by the packing of the VL and VH β-sheets in the conserved "framework" and one quarter from contacts between the hypervariable regions. The β-sheets that form the interface have edge strands that are strongly twisted (coiled) by β-bulges. As a result, the edge strands fold back over their own β-sheet at two diagonally opposite corners. When the VL and VH domains pack together, residues from these edge strands form the central part of the interface and give what we call a three-layer packing; i.e. there is a third layer composed of side-chains inserted between the two backbone side-chain layers that are usually in contact. This three-layer packing is different from previously described β-sheet packings. The 12 residues that form the central part of the three observed VL–VH packings are absolutely or very strongly conserved in all immunoglobulin sequences. This strongly suggests that the structure described here is a general model for the association of VL and VH domains and that the three-layer packing plays a central role in forming the antibody combining site.

1. Introduction

Immunoglobulins are the best-studied examples of a large and ancient family of proteins, which also [illegible] (i.e. class I) and minor (i.e. class II) histocompatibility antigens and cell surface receptors. Functionally, all these structures are involved in cell recognition processes (Jensenius & Williams, 1982), either actively as vehicles endowed with

recognition specificity (antigen-combing antibodies) or passively as surface structures that are being recognized (histocompatibility antigens). Only the immunoglobulin tertiary structures are known to date (Schiffer *et al.*, 1983; Epp *et al.*, 1974; Saul *et al.*, 1978; Segal *et al.*, 1974; Marquart *et al.*, 1980; Deisenhofer, 1981; Phizackerley *et al.*, 1979). However, the homology among primary structures of immunoglobulin, β-microglobulin, *Thy-1* antigen, some of the histocompatibility antigen domains, T-cell receptor β chain and the transepithelial "secretory component" has been interpreted as evidence for a common fold (Cunningham *et al.*, 1973; Orr *et al.*, 1979; Feinstein, 1979; Cohen *et al.*, 1980, 1981*a*; Novotný & Auffray, 1984; Yangai *et al.*, 1984; Hedrick *et al.*, 1984; Mostov *et al.*, 1984).

A typical antibody molecule (IgG1) consists of two pairs of light chains (M_r 25,000) and two pairs of heavy chains (M_r 50,000), each of the chains being composed of domains made up of approximately 100 amino acid residues. The domains are autonomous folding units; it has been demonstrated experimentally (Hochman *et al.*, 1973; Goto & Hamaguchi, 1982) that a polypeptide chain segment corresponding to a single domain can be refolded independently of the rest of the polypeptide chain. All the immunoglobulin domains are formed by two β-sheets packed face-to-face and covalently connected together by a disulfide bridge. The topology of the N-terminal, variable domains in both the light and heavy chains differs from that of the C-proximal constant domains. While the two variable domain sheets consist of five and four strands, respectively, the constant domain sheets are three- and four-stranded (Fig. 1). The four-stranded β-sheets of the two domain types are homologous; the five- or four- stranded β-sheet of the variable domains derives from the three-strand sheet of the constant domains by the addition, at one side, of a two-stranded β-hairpin or a single β-strand, respectively.

In a complete immunoglobulin molecule, domains that correspond to different polypeptide chains associate to form domain dimers VL–VH, CL–CH1 and CH3–CH3. Edmundson *et al.* (1975) were the first to note the phenomenon of rotational allomerism between the variable and constant domain dimers, that is, whereas the C–C dimers interact *via* a close packing of their four-strand sheets, the V–V dimers pack "inside out", with the five-stranded sheets oriented face-to-face. The reversal of domain–domain interaction is reflected in the amino acid sequence homology between, and among, the constant and variable domains (Novotný & Franěk, 1975; Beale & Feinstein, 1976; Novotný *et al.*, 1977).

Different antibody molecules in the same organism bind different antigenic structures. The variation in specificity is produced by several mechanisms: mutations, deletions and insertions in the binding regions of the VL and VH domains; and the association of different light and heavy chains. Aspects of the second mechanism are analyzed in this paper. In particular, the nature of the interface between VL and VH domains is examined by comparing the Fab fragments of KOL, NEW and MCPC 603 myeloma proteins whose X-ray structures are known. The relative contributions to the buried surface between the domains from the conserved framework residues and the hypervariable regions are determined. Attention is focused on the unique packing of the interfaces and the reasons for this packing are examined.

2. Materials and Methods

(a) *Fab fragment co-ordinates*

Cartesian co-ordinates for Fab fragments KOL, NEW and MCPC 603 were obtained from the Brookhaven Data Bank (Bernstein *et al.*, 1977). Table 1 lists the domain classification, the nominal resolutions and the crystallographic residuals (R factors) for the 3 Fab fragments. To facilitate comparisons of the 3 structures, their residue numbering was changed from that used in the original descriptions to that used by Kabat *et al.* (1983). Thus, in this paper residues that are structurally homologous have the same sequence number.

To obtain consistent sets of atomic co-ordinates, the original co-ordinates were dissected into individual VL–

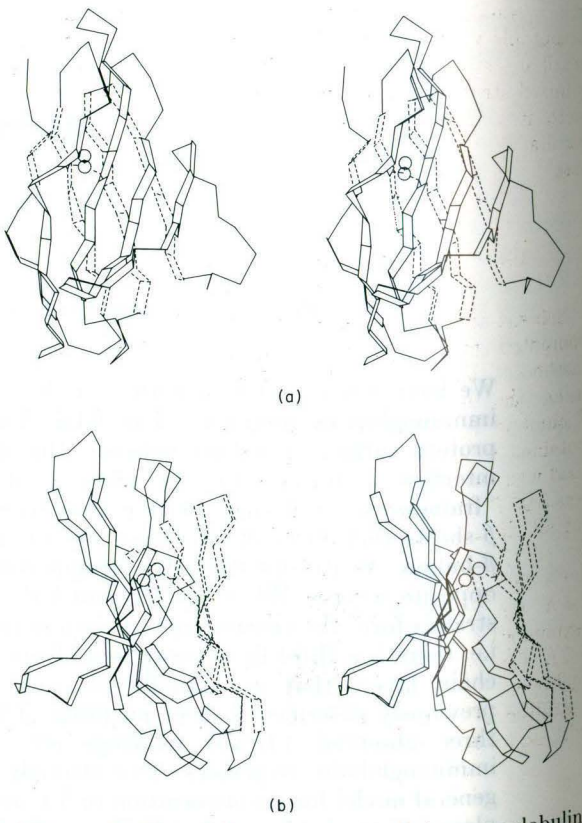

Figure 1. The β-sheets in typical immunoglobulin domains. Vertices represent the position of Cα atoms: those in β-sheets are linked by ribbons; and those between strands by lines. (a) The VL domain of KOL: the β-sheet involved in VL–VH contacts is closer to the viewer (unbroken line). (b) The same VL domain rotated by approximately 90°. Note that the interface-forming β-sheet is strongly twisted at diagonally opposite corners (drawing by A. M. Lesk).

Table 1
Summary of X-ray crystallographic data

Protein	L and H chain types	X-ray data		Minimized		Reference
		Resolution (Å)	*R* factor (%)	Energy (kJ)	r.m.s. shift (Å)	
Fab KOL human	λI, γIII	1·9	26	−3010	—	Marquart *et al.* (1980)
Fab NEW human	λI, γII	2·0	19	−2592	0·21	Saul *et al.* (1978)
Fab MCPC 603 mouse	κ, γI	2·7	24	−3703	0·26	Segal *et al.* (1974)

The energy given for Fab KOL is that of the unminimized crystallographic data.

VH domain dimers. The structures were subjected to 100 cycles of constrained energy minimization with the program CHARMM version 16 using the adopted-basis Newton-Raphson procedure (Brooks *et al.*, 1983) with constraints of 41·8 kJ (10 kcal) present on all the atoms (Bruccoleri & Karplus, unpublished results). Typically, the constrained minimization converged from original positive values of potential energy to values of about −2·1 kJ/atom (−0·50 kcal/atom) with an average root-mean-square co-ordinate different from the original X-ray structure of 0·3 Å (see Table 1). The results indicate that the crystallographic structures were satisfactory and that acceptable values of potential energy can be achieved by small adjustments of the co-ordinates. Thus, both energy minized structures and the crystallographic co-ordinates were used in the present study; essentially identical results were obtained from the 2 types of co-ordinates sets.

(b) *Computation of solvent-accessible surfaces and contact areas*

Solvent-accessible surfaces (Lee & Richards, 1971) were computed with programs written by A. M. Lesk using the method of Shrake & Rupley (1973) and by T. Richmond using the methods of Lee & Richards (1971) and Richmond & Richards (1978). The latter program was obtained from Yale University. The water probe radius used was 1·4 Å and the section interval along the *Z* axis was 0·05 Å; the atom van der Waals' radii used were 2 Å for all the (extended) tetrahedral carbon atoms, 1·85 Å for all the planar (*sp2* hybridized) carbons, 1·4 Å and 1·6 Å for carbonyl and hydroxyl oxygens, respectively, 1·5 Å for a carbonyl OH group, 2·0 Å for all the (extended) tetrahedral nitrogen atoms, 1·5 Å, 1·7 Å and 1·8 Å for *sp2*-hybridized nitrogen atoms carrying no hydrogen, 1 and 2 hydrogen atoms, respectively, 2·0 Å for a sulfhydryl group and 1·85 Å for a divalent sulfur atom with no hydrogens.

(c) *β-Strands and β-sheets*

Protein structures were analyzed using the CHARMM program (Brooks *et al.*, 1983) in the so-called explicit hydrogen atom representation: aliphatic hydrogens were combined together with their heavy atoms into "extended atoms" whereas hydrogens bound to polar atoms and possibly involved in hydrogen bonds were explicitly present. The β-strands and β-sheets were defined by their inter-strand backbone (C=O···H–N) hydrogen bonding pattern. A hydrogen bond list was generated in CHARMM for all the polypeptide chain segments under consideration and amino acids with hydrogen bonds of nearly optimal geometry (energy of −4·18 kJ/bond or less) were taken to be parts of the β-sheets (cf. Fig. 3 of Novotný *et al.*, 1983). This method of defining β-strand boundaries gives results essentially identical to those obtained by visual inspection of crystallographic models, although it tends to be somewhat more restrictive (the 2 methods sometimes differ in inclusion of the N- or C-terminal β-strand residues). Ambiguities arise in cases of edge β-strands that start and end with irregular conformations (β-bulges); such cases are discussed in more detail below.

(d) *β-Strand conformation*

In a typical extended polypeptide chain segment, the dihedral angle between the 2 consecutive side-chains is not 180° as in the ideal β-sheet (Pauling *et al.*, 1951) but closer to −160°; that is, the β-strands are twisted (Chothia, 1973). The out-of-planarity angle (180° − 160°) = 20° can be obtained explicitly from the values of the principal backbone torsion angles φ, ψ and ω (see, e.g. Chou *et al.*, 1982). We define the local backbone twist for 2 consecutive residues as:

$$\vartheta = \left(-\frac{\tau}{|\tau|}\right)(180-|\tau|),$$

where τ is the torsion angle Cβ–Cα–C′α–C′β and |τ| denotes its magnitude. When glycine residues that lack Cβ atoms are encountered, the torsion angle ϑ is measured with respect to the C′β atom following the glycine. Thus, glycine residues contribute to the local backbone twist indirectly, by being included in the virtual bond Cα–Cα that spans from the residue preceding the glycine to that which follows it.

Backbone twist profiles (plots of ϑ as a function of the amino acid residue) serve to characterize polypeptide chain conformations. Certain conformational characteristics of polypeptides are more clearly seen using ϑ values instead of the φψ values for individual residues. In our plots, the value of the torsion angle Cα–Cβ–C′α–C′β is assigned to the second (C′) residue. The angle ϑ is related to "the amount of twist per 2 residues", defined as δ by Chou *et al.* (1982); in fact, $\vartheta = \frac{1}{2}\delta$. It thus follows that ϑ can be obtained from the helical parameters *n* (number of residues per turn), *h* (the rise per residue) and *T* ($T = 360°/n$) in a corresponding way to that described for δ by Chou *et al.* (1982).

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