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Übersichten

Spatial Structure of Immunoglobulin Molecules

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Die räumliche Struktur der Immunglobulin-Moleküle

Zusammenfassung. Immunglobulin Moleküle der Klasse G (Antikörper-Moleküle) bestehen aus zwei schweren Ketten (50000 dalton Molekulargewicht) und zwei leichten Ketten (25000 dalton Molekulargewicht). Ihre Gestalt ist Y-förmig, wobei die Arme von je einer leichten Kette und der N-terminalen Hälfte einer schweren Kette in enger Assoziation gebildet werden. Der Stamm wird von den C-terminalen Hälften der schweren Ketten aufgebaut.

Die schweren und die leichten Ketten sind in globuläre Domänen mit einem Molekulargewicht von 12000 dalton gefaltet. Die schweren Ketten bestehen aus vier, die leichten Ketten aus zwei Domänen. Diese Domänen zeigen eine ähnliche Grundstruktur aus zwei β-Faltblättern, aber erhebliche Unterschiede im Detail.

Die N-terminalen, variablen Domänen der schweren und leichten Ketten, spezifisch die hypervariablen Polypeptidsegmente der Domänen, die an den Spitzen des Y liegen, bauen die Antigen- und Hapten-Bindungsstelle auf. Die Art der Aminosäuren in den hypervariablen Schleifen bestimmt die Form und die Spezifität des Antikörpers. Alle Domänen mit Ausnahme der C_H2 Domäne der schweren Kette aggregieren eng lateral. Die C_H2 Domäne hat Kohlehydrat gebunden, das die laterale Assoziation verhindert.

Longitudinale Wechselwirkungen zwischen den Domänen sind locker und erlauben Flexibilität in der relativen Anordnung der Domänen. Diese Flexibilität ist wahrscheinlich für die Funktion der Antikörper von Bedeutung.

Arm (Fab) und Stamm (Fc) Teile sind durch ein Scharnierpeptide verbunden, das zwei parallelen Polyproline Helizes enthält.

Antigenbindung initialisiert die Effektorfunktionen der Antikörper. Antigen bindet an die Spitzen des Y-förmigen Moleküls, die Effektorfunktionen sind im Stammteil lokalisiert. Es ist eine offene Frage, ob Konformationsänderungen im Antikörpermolekül bei der Initialisierung eine Rolle spielen.

Schlüsselwörter: Immunglobulin – Antikörper – Proteinstruktur – Glykoprotein

Summary. Immunoglobulin molecules of the class G (antibody molecules) consist of two heavy chains (50,000 dalton molecular weight) and two light chains (25,000 dalton). The overall shape is a Y with the arms formed by the light chains and the N-terminal half of the heavy chains in tight association. The stem is formed by the C-terminal halfs of the heavy chains.

The heavy and the light chains fold into globular domains of molecular weights of 12,000 dalton. There are four domains of the heavy chain and two of the light chain. All these domains show a similar fold, consisting of two β-sheets but display considerable differences in detail.

The N-terminal variable domains of heavy and light chains and specifically the hypervariable polypeptide segments of the domains, located at the tips of the Y, constitute the antigen and hapten binding site. The nature of the amino acid residues of the hypervariable loops determines the shape and the specificity of the antibody.

All domains pair tightly laterally, except the C_H2 domains of the heavy chain. This domain has carbohydrate bound which prevents lateral association.

Longitudinal interaction between the domains is loose and allows flexibility in the arrangement. Flexibility is probably of significance for antibody function.

Arm (Fab) and stem (Fc) parts are linked by the hinge peptide which contains a segment with a unique conformation of two parallel poly-proline helices.

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Antigen binding triggers effector functions of antibodies. Antigen binding is at the tips of the Y-shaped antibody, but effector functions are displayed by the stem part. It is an open question whether conformational changes of the antibody molecule play a significant role in the trigger mechanism.

Key words: Immunoglobulin – Antibody – Protein structure – Glycoprotein

Antibody molecules (immunoglobulins) form the basis of the humoral immune defence reactions in probably all vertebrate species. They recognize foreign macromolecules or cells (better: antigens on cell surfaces) and, by binding these antigens, initiate their elimination. One route of elimination utilizes complement components in a complicated cascade of reactions, which is intensively studied (for reviews see: [1, 2]). Immunoglobulin-like molecules also occur as membrane-bound receptors on the surface of bone-marrow derived B-lymphocytes. Recognition of the corresponding antigen leads to proliferation and antibody secretion (for reviews see: [3–5]).

Our present understanding of the molecular basis of antigen antibody recognition and complement activation began with the elucidation of the chemical nature of immunoglobulins, their covalent structure [6–8] and culminated in the analysis of their spatial structure.

These studies were almost exclusively based on myeloma and Bence-Jones proteins, which are found in large quantities and homogenous form in patients with multiple myeloma or Waldenstroem's macroglobulinemia. In most cases the corresponding antigens are unknown. Recently large amounts of homogenous antibodies elicited against streptococcal or pneumococcal polysaccharides became available from certain rabbit and mouse strains [9, 10]. These antibodies and the use of hybrids obtained from myeloma and spleen cells have offered the possibility to obtain homogenous antibodies of predefined specificity [11, 12]. Biochemical studies with these materials fully confirm the notion that there is no basic difference between the structures of myeloma proteins and induced antibodies [13].

In this article I shall describe our present understanding of the spatial structure of immunoglobulins and its functional implications. The detailed picture which we have today is based on crystal structure analyses of a number of immunoglobulin molecules and their fragments performed during the last seven years (for recent reviews see [14, 15]).

Immunoglobulins are divided in a number of classes and sub-classes according to differences in heavy chains: IgG, IgM, IgA, IgD, IgE. There are two light chain classes: kappa and lambda (κ, λ), which are shared by all Ig classes.

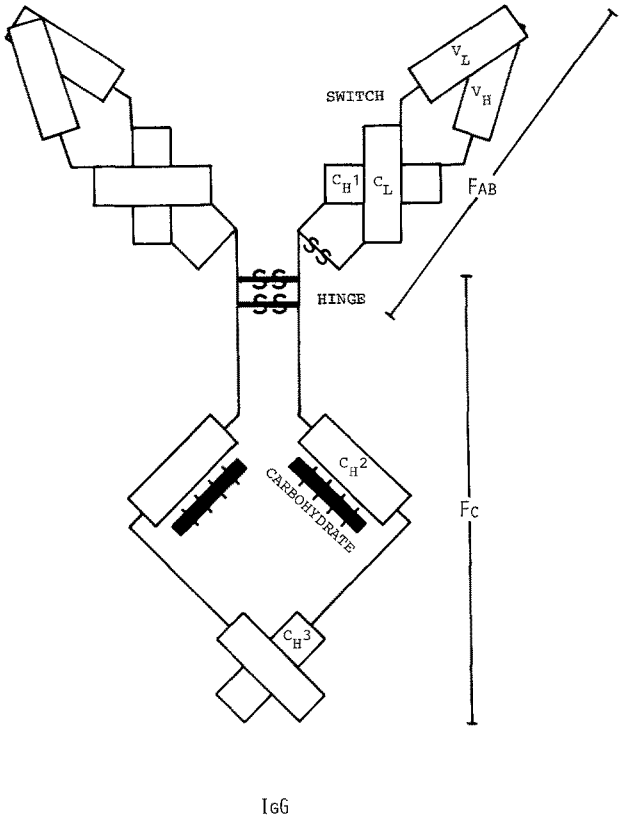

Fig. 1. Schematic drawing of an IgG1 immunoglobulin molecule. The arms and the stem of the Y-shaped molecule are formed by the Fab parts and the Fc part, respectively. The light chains are linked to the heavy chains by a disulfide bond close to the C-terminus. The two heavy chains are covalently connected by two disulphide linkages located in the hinge region

The schematic drawing in Fig. 1 represents an immunoglobulin molecule of the most abundant class G (IgG) as it was obtained from the structural studies described below. It is composed of two identical heavy and light chains with molecular weights of about 50,000 and 25,000 daltons, respectively. These are held together by non-covalent forces and disulphide linkages. Limited proteolytic digestion of IgG yields stable and functional fragments. The Fab fragment comprises the light chain and the N-terminal half of the heavy chain. It binds antigen. The C-terminal Fc part of the heavy chain is involved in effector functions such as complement activation and binding to Fc receptors on certain cell types [6, 7].

The polypeptide chains are folded into compact domains: four domains of the heavy chains and two of the light chains. These domains are designated V_H, C_H1, C_H2, C_H3 in the heavy chain V_L and C_L in the light chain. V stands for variable and C for

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constant amino acid sequence. Amino acid sequence analysis has shown that the N-terminal domains, with a molecular weight of about 12,000 daltons, are highly variable, while the constant domains show identical amino acid sequences in a given sub-class and species except for a few allotypic variations due to allelic genes [8]. The V domains bind antigen while C domains exhibit other functions. The view, that these domains are under separate genetic control, was experimentally confirmed for the light chains by chemical analysis of the corresponding genes of embryonic and mature antibody forming cells. In addition, it was found that part of the third hypervariable segment and the switch peptide connecting V and C domains is controlled by a third gene [16, 17].

Amino acid sequence analyses has shown that there is homology between all domains suggesting a similar chain folding [6]. There are also close relations between amino acid sequences of the various Ig classes. The differences between the Ig classes and sub-classes reside predominantly in the hinge segment, in the interchain disulphide linkages, in the bound carbohydrate, and in the state of aggregation. A close relationship in amino acid sequence is also found when immunoglobulins from different species are compared.

There is no doubt therefore that the basic structural principles found for IgG are valid for other classes. Class specific structural variations are of course important; they alter functional properties of the molecule considerably and certainly need to be analysed in detail in the future.

Domain Structure

The folding pattern is very similar in all immunoglobulin domains. It is shown schematically in Fig. 2 for a V domain, looking along the polypeptide strands. The folding is characterized by two pleated sheets connected by an internal disulphide bridge linking strands B and G. The two sheets cover a large number of hydrophobic amino acid sidechains.

Figure 3 compares V and C domains seen in the intact IgG1 (λ) molecule Kol and the V (κ) chain of Rei [18–26]. The domain structures are represented by the positions of the C^{α} atoms of the amino acids.

It is clear that the topology of the strands is identical in all domains. There are only minor differences between members of the V family and the C family with one another but substantial differences when we compare V and C domains: The number of strands and the length of the loop regions is different, changing the overall shape considerably.

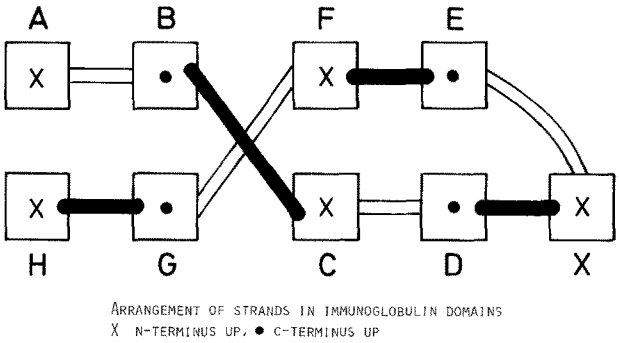

Fig. 2. Topology of strands in a V domain looking along the strands. (x) and (o) indicate N- and C-termini pointing towards the observer

V_H, $V_{L,\kappa}$ and $V_{L,\lambda}$ form a family of closely related structures as do C_L, C_H1, and C_H3.

C_H2 represents yet a third type, differentiated from the other C domains mainly by the branched carbohydrate chain linked to it. It will be discussed in more detail below.

Domain Domain Interactions

Lateral Interactions

Immunoglobulin domains other than C_H2 interact strongly in a lateral fashion to form modules V_H-V_L, C_L-C_H1, C_H3-C_H3. Large parts of the domain surfaces are in contact. In V modules V_H may be replaced by V_L to form a light chain V dimer as seen in the Bence Jones protein fragments Rei or Au [18–20]. In Bence Jones proteins, which are light chain dimers, one of the light chains simulates the heavy chain in Fab parts, as described for Mcg [27].

Figure 4 shows the Fab parts of Kol [21, 26]. It is obvious that V and C pairings are entirely different. In a V pairing the HGCD faces of the domains and in a C pairing the opposite ABFE sides are in contact. C_H3 exhibits C pairing, as shown below for the Fc part (Fig. 6).

The basis of the different aggregation characteristics of V and C domains resides in the amino acid sequence. Residues important for lateral contact formation are conserved in all Ig classes and subclasses. The lateral pairing buries hydrophobic residues which would be exposed in isolated domains. The distribution of these residues is different in V and C domains. There are hydrophobic patches on the HGCD face of V domains and the ABFE face of C domains.

C_H2 is an exception, as it forms a single unit without lateral domain domain interaction. Instead it in-

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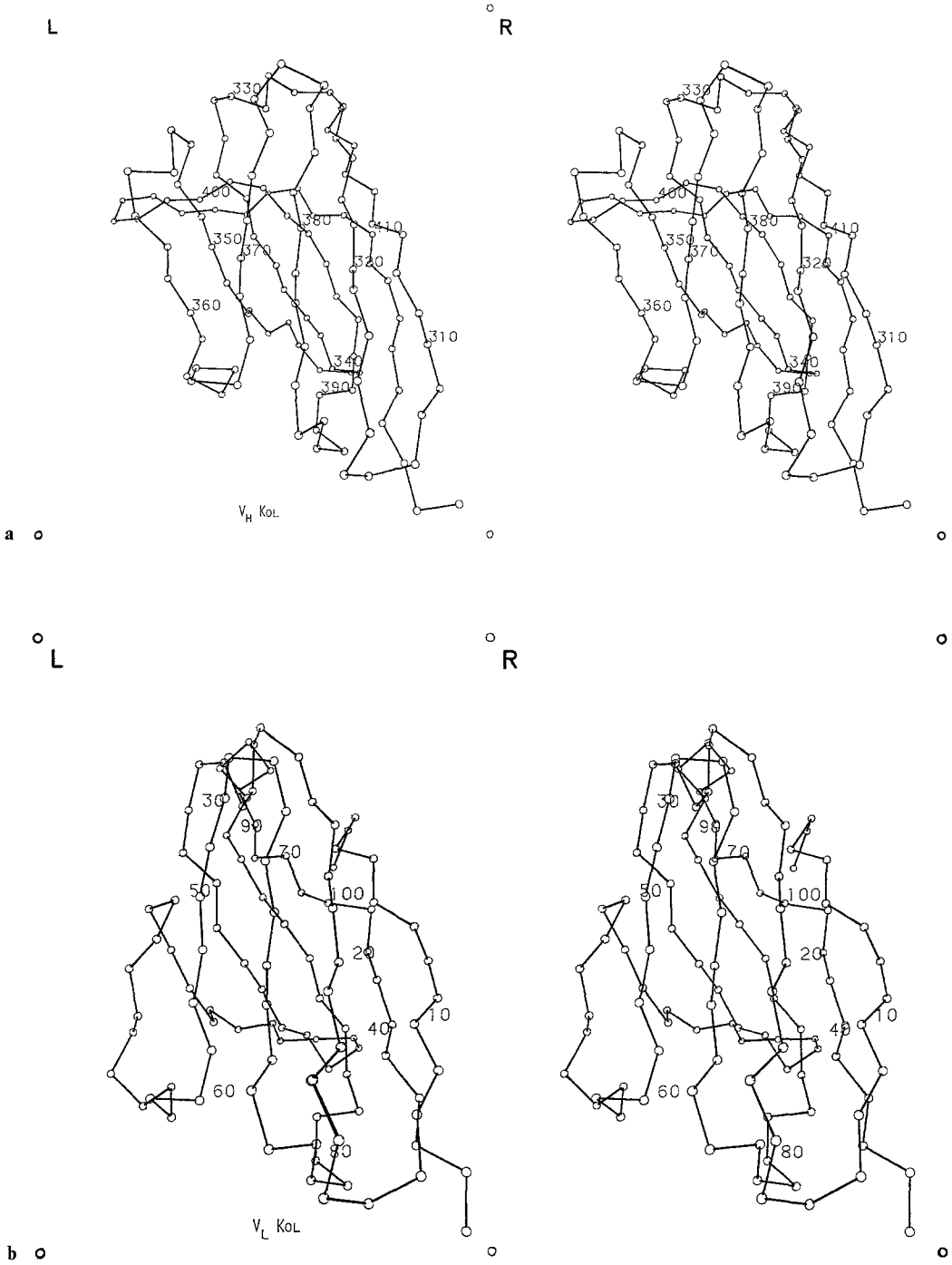

Fig. 3a–g. Polypeptide chain folding of V and C domains oriented approximately in the same way. V_H of Kol (**a**), $V_{L,\lambda}$ of Kol (**b**), $V_{L,\kappa}$ of Rei (**c**), C_H1 of Kol (**d**), C_L of Kol (**e**), C_H2 (**f**) and C_H3 (**g**) of IgG from pooled serum. In C_H2 the carbohydrate has been omitted. Light chains are numbered from 1 to 214 and heavy chains from 300 to make differentiation easier; the Fc fragment is numbered in the usual way with the unique hinge sequence Cys 226 – Pro 227 – Pro 228 – Cys 229 [18–26]

teracts with bound carbohydrate, which covers a large proportion of the ABFE face normally involved in a C type interaction, and there are amino acid exchanges within the ABFE face not compatible with a C type aggregation (Fig. 5) [22, 25].

The complex, branched carbohydrate chain bound to C_H2 forms a few hydrogen bonds with the protein moiety, but the dominant interactions are of hydrophobic nature. The carbohydrate covers a hydrophobic patch of the protein made up of Phe 241,

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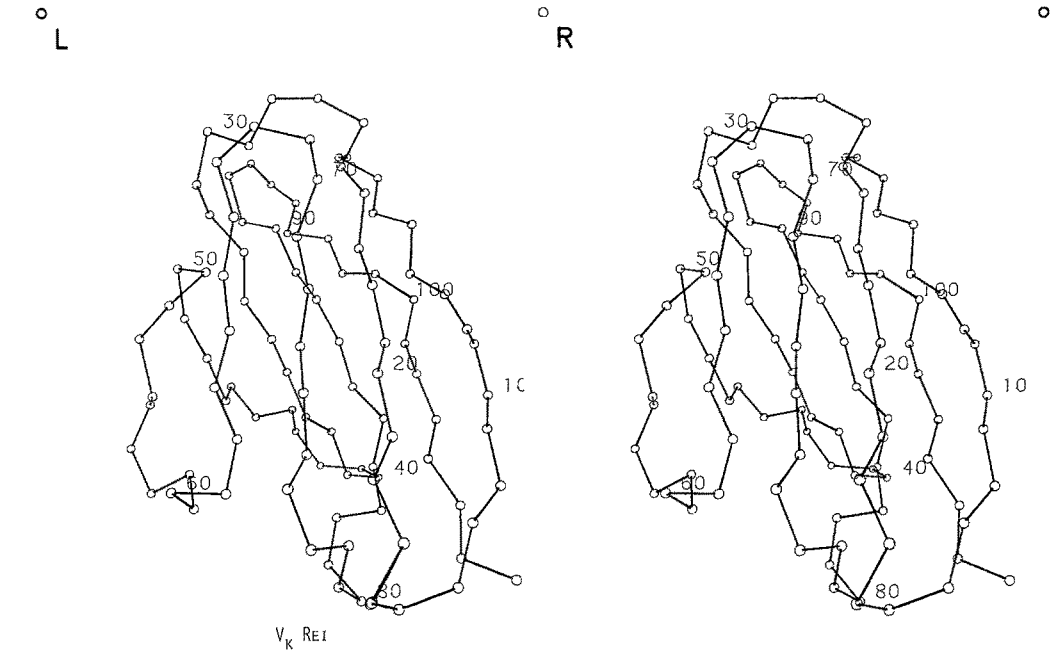

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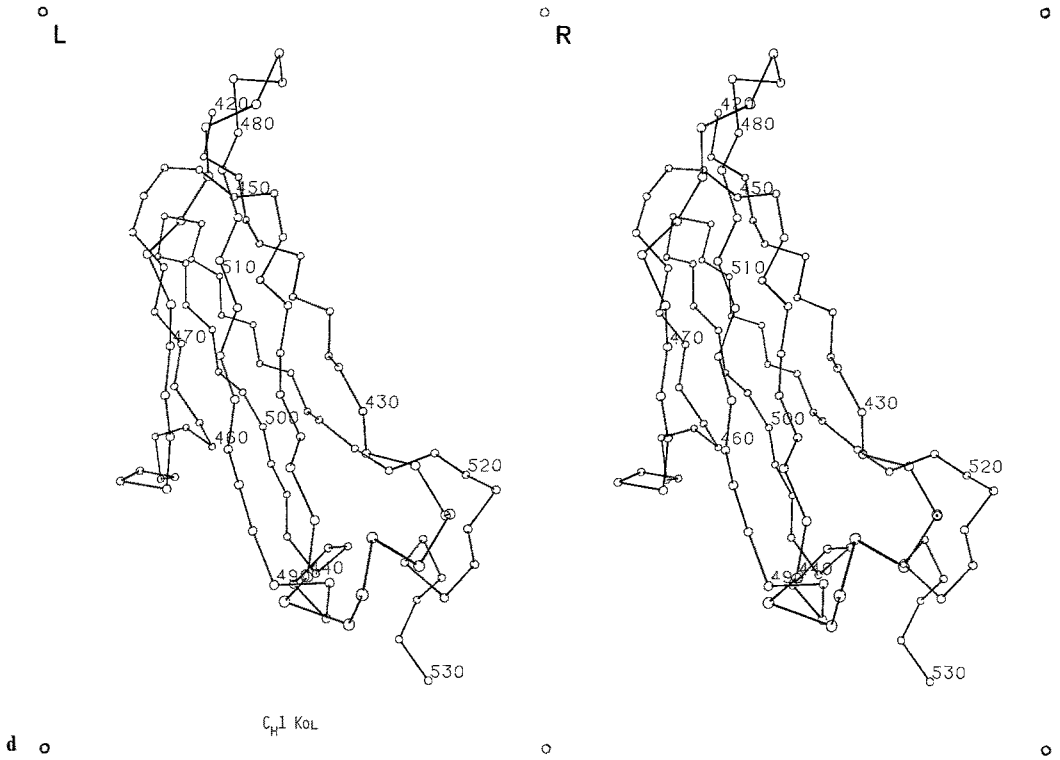

d

Fig. 3c–d.

243 Val 262, 264 Tyr 296 Thr 260 Arg 301. Removal of the carbohydrate would probably destabilize the compact three-dimensional conformation of the C 2 domain, since these residues would then be exposed. The functional relevance of carbohydrate in antibodies is unclear. It might be involved in intracellular movements of the glycoproteins and in secretion [28–30]. It may well be that the origin of the altered functional properties of carbohydrate-free antibody variants is structural destabilization.

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