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Dual conformations of an immunoglobulin light-chain dimer: Heterogeneity of antigen specificity and idiotope profile may result from multiple variable-domain interaction mechanisms

(Bence Jones protein/antibody structure/antibody diversity/idiotope diversity)

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ABSTRACT **The structure of an immunoglobulin antigen-binding fragment (Fab) has been thought to be invariantly defined by well-conserved amino acid residues in the variable domains of the heavy and light chains. These conserved residues enable folding of the polypeptide segments into the characteristic immunoglobulin fold domains and are the major controllers of interactions between domains. However, crystallographic studies of some immunoglobulin light-chain dimers have suggested and the crystallographic structure of the Fab in an Fab–neuraminidase complex may have proven that antibodies are not restricted to a single, invariant relative positioning of the two variable domains. We propose that in some cases the detailed quaternary structural relationships between the variable domains of heavy and light chains are not restricted to those of the canonical Fab. It is unclear whether alterations of these relationships occur only after complex formation with antigen or, if in ligand-free solution, Fab conformers might coexist in relative concentrations determined by isomerization rates. In the latter case, antibody-presenting lymphocytes may be polyspecific, and the specificity of lymphocytes might be modulated by anti-idiotopic antibodies complexed to cell surface receptors. In either case, the idiotopic repertoire displayed by an antibody or lymphocyte surface receptor might be changed by the presence or absence of antigen.**

Crystallographic studies of antigen-binding fragments (Fabs) have indicated that the principal determinants of quaternary structure are highly conserved domain–domain interactions (1–5) and that functional diversity originates from residue alternations, insertions, and deletions in segments of the heavy (H)- and light (L)-chain variable (V) domains designated hypervariable or complementarity determining regions (6). Several analogous studies with light-chain dimers have also supported the monomorphic conformation concept (7–10). However, in two instances (11, 12), the observed Bence Jones protein conformations were anomalous; V–V domain interactions were found that had not been observed in Fabs. Although the relationships between these V domains were of significant interest in terms of the physical chemistry of antibody domain interactions, the physiological implications of the structures of the light-chain dimers formed by proteins Rhe (11) and Loc (12) were obscured by the absence of corresponding structures among the known conformations of Fabs.

The possibility of additional antibody-binding site and Fab configurations has been proposed on a theoretical basis (13). Several recent experimental observations suggest that antibody "flexibility" includes variation in the spatial relationship between V_L and V_H domains. Multiplicity of allowable V-domain interactions available to a single Fab provides a basis for (*i*) antibody multispecificity in which the contacts between paratope and epitope can be maximized, (*ii*) the stabilization of a single Fab conformation by binding to antigen may significantly alter the apparent idiotope spectrum displayed by the Fab, and (*iii*) the stabilization of a single Fab conformation by binding of an anti-idiotope may alter the apparent antigen specificity of the Fab.

Evidence for Fab Heterogeneity?

Antibody Flexibility. Flexibility of immunoglobulins had been considered as permitting conformational changes that might serve as signals to trigger an immune response. Bending and rotation at the hinge regions between Fc and Fab segments and at the elbow bend (switch region) between V and constant (C) domains of the Fab are well appreciated (14, 15) and may result in antigen-dependent changes in the steric relationships or time-averaged relationships of these subunits accompanying complex formation with multivalent antigen. In some studies (16, 17), no conformational changes were observed upon binding of a small ligand. In others, conformational alterations correlated with hapten or antigen binding have been inferred on the basis of increased conformational stability of the Fab (18, 19), decreased solubility (20), changes in the accessibility of bound hydrogens for solvent exchange (21), and results of numerous spectroscopic studies (22–26). In crystallographically based studies of conformational changes in the light-chain dimer Mcg, it was concluded that ligand binding caused alterations in both V_L–V_L and C_L–C_L interactions (27, 28). No correlation has been found between antigen-induced conformational changes and triggering of an immune response (for reviews, see refs. 29–31) although some support for a conformational signal remains (32).

Effect of Anti-Idiotope or Antigen Binding on Idiotope Expression

Antibody 7-22. Antibody 7-22 is a murine monoclonal antibody that binds phosphatidylcholine (33). Antibody 7-22 differs from another well-characterized phosphatidylcholine-binding antibody, TEPC-15, by a single amino acid insertion at the diversity (D)–joining (J) junction. This heavy-chain residue is positioned at or near the V_H–V_L interface. Four of five monoclonal anti-idiotopic antibodies specific for TEPC-15 also react with 7-22. The fifth anti-idiotope, B36-82, recognized 7-22 only when 7-22 complexed first with the anti-idiotopic antibody B24-44. Strickland *et al.* (33) suggested that binding of B24-44 led to conformational changes in 7-22 that resulted in the reacquisition of the B36-82 idiotope evidently lost due to the insertion of a tyrosine residue at the

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Abbreviations: V, variable; C, constant; D, diversity; J, joining; L, light; H, heavy.

D–J heavy-chain junction. If the tyrosine insertion directly caused spatial displacement of residues comprising the B36-82 idiotope, it is difficult to visualize the process by which binding of a second antibody would negate the physical presence of an extra residue in the epitope. However, if the B36-82 idiotope requires precise positioning of residues from both light and heavy chains and the insertion of the tyrosine elsewhere established free energy minima for V–V domain interactions different than that of TEPC-15, then binding of a second antibody such as B24-44 could restabilize the prototypical TEPC-15 conformation and, as a consequence, reestablish the B36-82 idiotope.

Other Examples. Epitopes or idiotopes were also observed to be lost in a manner that indicates conformational changes have taken place upon binding of hapten or second antibody. Kabat (34) concluded that binding of $\alpha(1\rightarrow 6)$ glucoses by the myeloma protein W3129 resulted in conformational changes outside the binding cavity that inhibited association with idiotype-specific antiserum. Campbell *et al.* (35) observed nonreciprocal inhibition patterns in competition experiments among anti-idiotopic antibodies but were unable to account for the phenomenon. Roux *et al.* (36) used immunoelectron-microscopy to determine that two anti-idiotopic monoclonal antibodies previously characterized by competition studies as recognizing overlapping sites actually bound to idiotopes on opposite poles of the V domain. Since the two anti-idiotopic antibodies do not physically compete for the same site, binding inhibition is probably explained by correlated conformational changes. The extensive separation of the two idiotopes suggests that the conformational changes are not limited to alterations of amino acid side chain positions or localized movements of polypeptide segments. However, the necessary spatially extended conformational adjustments could be explained by translation or rotation of the V_L and V_H domains relative to each other. Instances have also been documented in which binding of anti-idiotopic antibodies increased affinity for hapten or binding of hapten increased affinity for anti-idiotopic antibody (37, 38).

Crystallographic Studies of Fabs and Light-Chain Dimers

Antibody NC41. Direct demonstration of conformational heterogeneity is dependent upon crystallographic study. Colman *et al.* (39, 40) described the crystal structure of neuraminidase complexed with the Fab prepared from the monoclonal antibody NC41. When compared to the structures exhibited by the crystallographically analyzed free Fabs or by the lysozyme–Fab complexes described by Amit *et al.* (41) and Sheriff *et al.* (42), a shift in the relative positions of the NC41 V_L and V_H domains is apparent although determination of the precise magnitude of the shift may have to await further refinement of the structure. Since the free Fab has not yielded structurally suitable crystals, it is not yet known if the shift occurs following binding of the antigen, if it represents the native conformation of the NC41 Fab, or if in fact NC41 is characterized by multiple isomerizing conformations in solution.

Protein Loc. The λ light-chain dimer Loc represented the second intact Bence Jones protein to be successfully analyzed crystallographically (12). The protein, when crystallized from ammonium sulfate, exhibited a conformation distinct from that of the previously studied light-chain dimer, Mcg (7), and several Fabs (16, 41–45). In Loc, a translation and rotation of domains resulted in the formation of what was described as a protruding binding site (12), rather than the classic (canonical) concave binding pocket. Because of the unexpected nature of this conformation, additional efforts were made to obtain crystals of Loc. Chang *et al.* (46) have recently described the structure of Loc in crystals obtained at low ionic strength in which the crystallized structure is that typically observed for light-chain dimers and Fabs.

The two conformations obtained by the light-chain dimer Loc are compared in Fig. 1. The tertiary structures of the individual V domains are very similar in the ammonium sulfate and water crystal forms. The arrangement of the two V domains in the crystal grown from distilled water is similar to that found in the other light-chain dimers; they are related by a two-fold axis with no translation and form a hapten-binding cavity. When one of the V domains from the salt-crystallized form of Loc is superimposed on one of the V domains of the water-crystallized form, a rotation of 35° and a translation of 6.3 Å are required to superimpose the other V domains. Comparative studies of the structure of the Mcg light-chain dimer have previously shown solvent-dependent conformation characteristics (47) that appear to affect the positioning of hypervariable loops but do not cause the binding-pocket characteristic to be lost.

Structural Basis of Fab Heterogeneity

V-Domain Interactions. The absence of a covalent bond between V domains is a prerequisite for the generation of Fab heterogeneity resulting from alterations in the relative positions of V_L and V_H domains. In contrast to the presence of disulfide bonds between various C domains, the absence of a disulfide linkage between V domains may suggest an evolutionarily significant selection preserving the opportunity of conformational variance of V–V interaction. The interaction between heavy- and light-chain domains in the Fab is characterized by affinities as high as $10^{12}\ M^{-1}$ (48, 49) achieved through changes in free energy contributed by both V–V and C–C domain interactions. However, the high affinity of interaction does not directly imply a singular allowed mode of interaction. In many cases, the free energy change is predominantly the result of the formation of many hydrogen bonds and the exclusion of water from interfaces of large surface area rather than formation of a few high-energy ion or salt bridges that depend critically on the precise location and the orientation of complementary residues. It is reasonable to expect that several different modes of domain interaction may result in similar free energy changes. Since the magnitude of the free energy change is sensitive to the presence or absence of specific energetically favorable V_L–V_H interactions, light- and heavy-chain sequences govern the range of heterogeneity available to a given Fab. Therefore, different heterogeneic characteristics might be associated with substitutions of residues encoded at V–J and V–D–J junctions (50, 51). Similarly, if polypeptide segments associated with a given isotype contribute to the V_L–V_H interface, then heterogeneity class might be correlated with isotype.

Mechanisms of Fab Heterogeneity. We consider three nonexclusionary classes of heterogeneity (Fig. 2). Stable heterogeneity describes an antibody population consisting of individually monomorphic immunoglobulins that exhibit isotype- or sequence-specific stable conformations. In this case, the Fab found in the complex with neuraminidase (39, 40) might represent one conformation exhibited by a subset of antibodies, whereas the Fabs characterized in complexes with lysozyme (41, 42) and other monoclonal or myeloma-related Fabs are archetypical of perhaps a prevalent and physiologically dominant conformation. It is assumed that the fundamental V–V domain interactions are invariant and unaltered upon binding of antigen although localized adjustments of residue positions or polypeptide segments are not precluded. A chemically homogeneous sample of Fab is assumed to be conformationally homogeneous.

There can be two classes of a dynamic heterogeneity. Active heterogeneity functionally describes cases in which

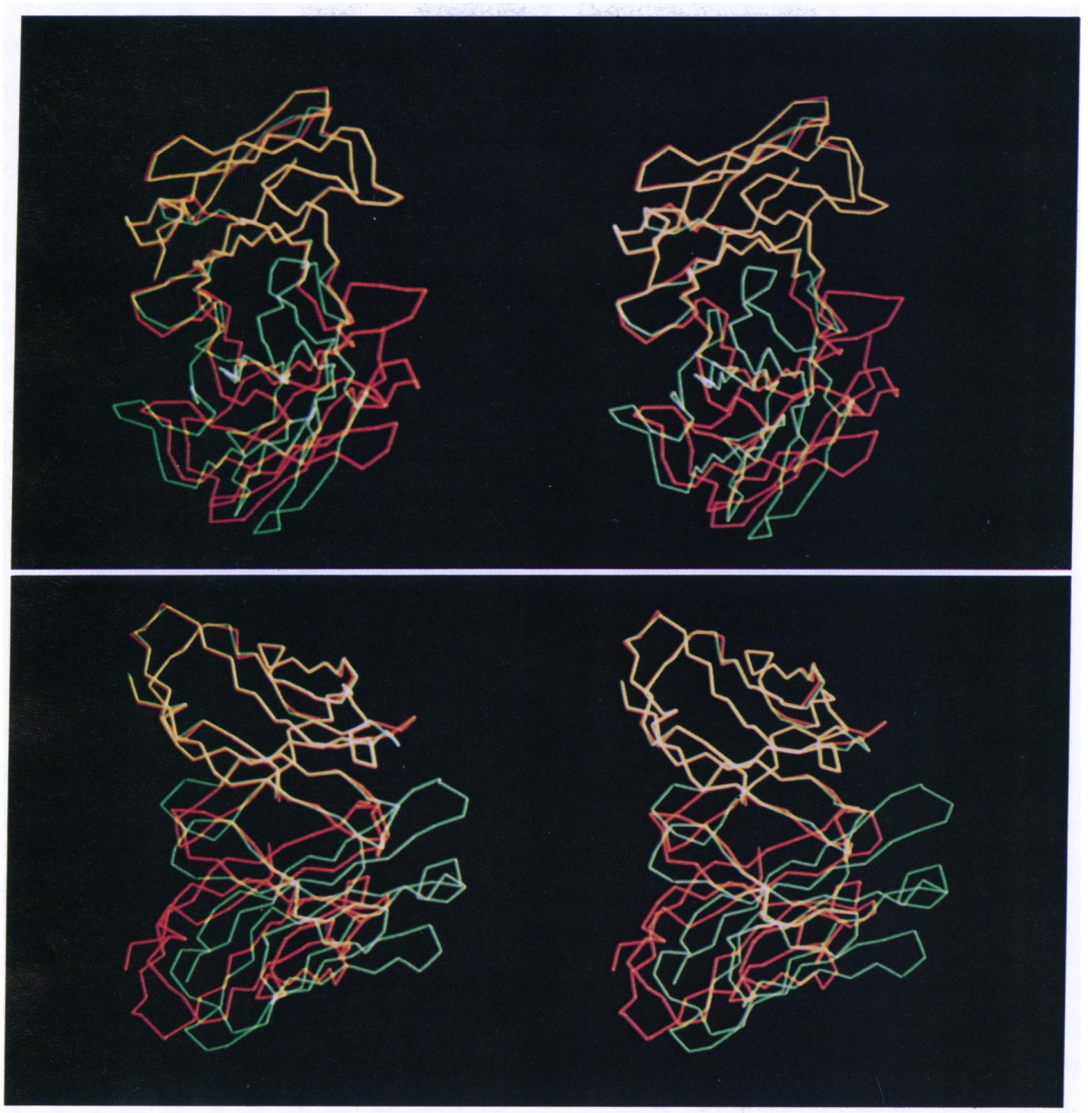

FIG. 1. Comparison of the arrangement of the variable domains in the Loc ammonium sulfate and water forms. To illustrate the difference in packing, in these stereo pairs one variable domain from each structure is superimposed. The view is along the local dyad (*Upper*) of the water form and in a direction perpendicular to the local dyad (*Lower*).

continual transitions between modes of V–V association occur. The Fab population would then be a mixture of isomers representing a continuum of relationships or multiple discrete V–V relationships that are in equilibrium. Regardless of spatial location of idiotope on the Fab surface, apparent competition between antigen and anti-idiotope or between two different anti-idiotopes would occur if each were associated with a Fab with different V_H–V_L interactions.

The number of available quaternary structures would be a function of the relative free energy minima associated with the set of allowed V_L–V_H relationships. Rates of isomerization would likewise be a function of the energy barriers involved in transitions between them. Multiple conformational energy minima separated by high energy barriers relative to the temperature-dependent kinetic energy would lead to a mixture of effectively static Fabs, relative to the time frame of a given experiment. Thus, a chemically homogeneous antibody sample may be structurally and functionally heterogeneous in that only one subpopulation exhibits a characteristic antigen specificity and associated idiotope repertoire. This subpopulation aspect would not necessarily be evident. In contrast, small energy barriers between conformational energy minima would permit apparent functional homogeneity in which the isomerization rates between conformers are part of the apparent association rate of antigen binding or binding by anti-idiotopic antibody.

Finally, an extreme limit of the above occurs if the kinetics of isomerization of free Fabs are small but the isomerization rates of Fab–antigen or Fab–anti-idiotope complexes are significant. In this case, a free Fab sample that is chemically homogeneous may be conformationally homogeneous but functionally heterogeneous by a mechanism that may be termed induced heterogeneity.

Implications of Fab Structural Heterogeneities

Antibody Diversity. Stable heterogeneity, in which multiple Fab conformations are possible but individual lymphocyte clones are limited to producing antibodies with a discrete Fab conformer, would not contribute to immune system diversity since the number of paratopes is still a function of $n\mathrm{V_L} \times n\mathrm{V_H}$, the product of the number of different V_L and V_H genes. Dynamic processes of heterogeneity clearly amplify the number of different paratope conformations available to a given V_L–V_H pair.

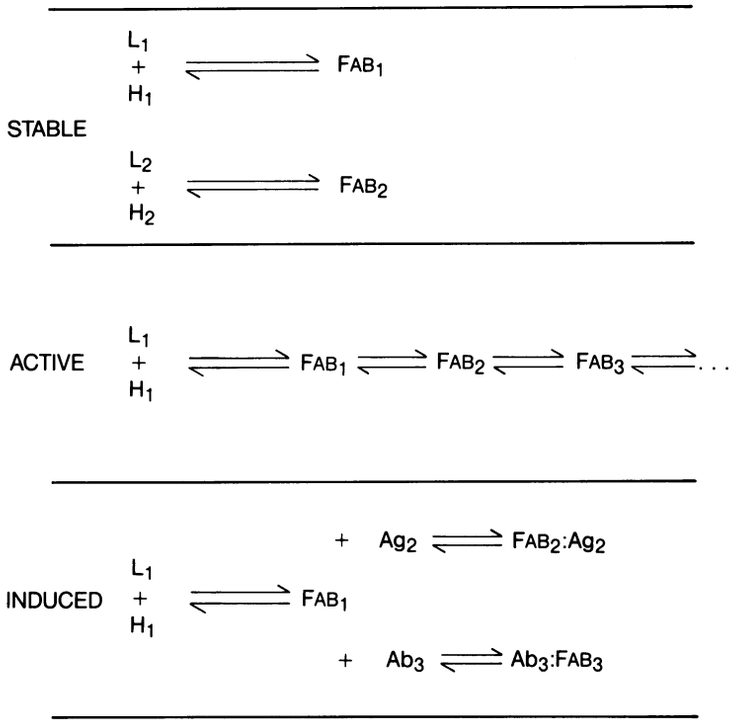

FIG. 2. Schematic representation of three mechanisms of Fab heterogeneity. Fab_j symbolize different hypothetical conformation classes of Fab characterized by different V_L–V_H interactions. (*Top*) Stable heterogeneity. Different light and heavy chains combine to form Fabs of different conformation class, but no transitions between conformation classes are permitted. (*Middle*) Active heterogeneity. Multiple conformation classes coexist for a given light- and heavy-chain pair. If isomerization half-lives are long relative to the time frame of a given experiment, the Fab sample will be functionally heterogeneous. If isomerization half-lives are short relative to experimental duration, the sample will appear homogeneous, but the isomerization rates may contribute to the apparent forward rate constant of antibody–antigen association. Each conformation class is associated with individual antigenic specificity and a set of characteristic idiotopes. (*Bottom*) Induced heterogeneity. Conformation of free Fab may be homogeneous; conformational changes are associated with the formation of a complex with either antigen (Ag) or other antibodies (Ab). From top to bottom, the characteristics of a chemically homogeneous sample of free Fab of each of the three heterogeneity classes may be summarized as conformationally and functionally homogeneous (*Top*), conformationally and functionally heterogeneous (*Middle*), and conformationally homogeneous but functionally heterogeneous (*Bottom*).

Idiotype Expression. Discontinuous idiotopes may consist of residues and polypeptide segments contributed by both light and heavy chains (52–54). In other cases, even an idiotope consisting solely of residues from a single chain may be blocked by the unfavorable location of residues from the other chain. The expression of discontinuous idiotopes is highly sensitive to the relationship of the two V domains. Therefore, the presence or absence of these idiotopes can be controlled by the binding of antigen if antigen association is correlated with altered V–V interactions in the Fab. Conversely, binding of some anti-idiotopes may be expected to control the effective antigenic specificity of the antibody and lymphocyte surface receptors through stabilization of a specific V–V interaction. In these cases, the receptor specificity of a B lymphocyte might be directly modulated by the concentrations and specificities of anti-idiotopic antibodies.

Regulatory Considerations. Multispecificity of antibody would appear to create complications for immune system regulation by possibly decreasing the correlation between antibody specificity and idiotope profile. However, the property of antibody cross-reactivity does not depend upon heterogeneity of V_L–V_H interaction (55, 56) since even a structurally invariant binding site may recognize multiple epitopes through interactions involving different subsets of residues. Accordingly, interactions of lymphocyte with monoclonal anti-idiotope may result in pleiotropic alterations in antigen responsiveness. Similar regulatory complications are, therefore, already intrinsic to monomorphic immunoglobulin models. A linkage between antigen specificity and idiotopes recognized by antibodies evoked by an unrelated challenge might be expected to lead to problems of immune regulation, perhaps as seen in some cases of autoimmunity.

Experimental Considerations. It is probable that, in at least some cases, observed immunoglobulin characteristics may be more sensitive to experimental conditions than previously understood. Predominant conformations may be dependent upon specific conditions of solvent composition, the presence or absence of antigen and other antibodies, and the rate of transition between conformers irrespective of whether transition occurs prior to or following formation of a complex with antigen or second antibody. These considerations may be particularly relevant in studies of antibody idiotopes (34, 57). The possibility of conformational changes is perhaps a more important aspect of competitive epitope mapping assays (58) than has been assumed. If ligand-free Fabs are conformationally heterogeneous in solution, it will be necessary to be more cautious in our application of the canonical Fab structure as an invariant model on which to base a detailed understanding of the functional characteristics of antibody–antigen interactions.

The possibility of heterogeneity created by different V–V domain interactions of an individual Fab is dependent upon the detailed residue interactions between the V_H and V_L domains. Therefore, among the millions of Fabs, ample examples of each of the three described categories of heterogeneity may exist. If, for instance, alteration of domain relationships is associated with loss of an energetically favorable ion bridge, then it may be expected that the Fab is effectively stable and homogeneous conformationally and functionally. However, in other cases it may be useful to consider the interaction between antibody and macromolecular antigen as a ternary reaction in which the final free energy change upon binding is a combination of free energy changes associated with antigen–light chain, antigen–heavy chain, and light chain–heavy chain interactions.

Conclusions

The crystallographic studies of the light-chain dimer Loc and the NC41–neuraminidase complex reveal a previously undetected mode of generating antibody diversity mediated by alterations in the interaction between V_L and V_H domains. It remains to be determined whether the NC41 conformation illustrates an example of static or dynamic heterogeneity. The NC41 conformation may represent an induced change in conformation that is precipitated by formation of the antigen–antibody complex. Alternatively, as suggested by the dual conformations determined for the Bence Jones protein Loc, an active isomerization process may be occurring. In either case, it may be hypothesized that the presence or absence of antigen modulates the expression of idiotopes at or near the V_L–V_H interface. Conversely, the presence or absence of

appropriate anti-idiotopes may alter the antigenic specificity of the antibody and lymphocyte surface receptors.

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