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Novel Arrangement of Immunoglobulin Variable Domains: X-ray Crystallographic Analysis of the λ-Chain Dimer Bence-Jones Protein Loc†

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ABSTRACT: We have characterized and crystallized a human λI light-chain dimer, Bence-Jones protein Loc, which has variable (V) region antigenic determinants characteristic for the λI subgroup and constant (C) region determinants of the $C_{\lambda I}$ gene Mcg. The crystal structure was determined to 3-Å resolution; the *R* factor is 0.27. The angle formed by the twofold axes of the V and C domains, the "elbow bend", is 97°, the smallest found so far for an antibody fragment. The antigen-binding site formed by the two V domains of the Loc light chain differs significantly from those of other immunoglobulin molecules (light-chain dimers and Fab fragments) for which X-ray crystallographic data are available. Whereas, in other antibody fragments, the V domains are related by a local twofold axis, a local twofold screw axis with a translational component of 3.5 Å relates the V domains in protein Loc. In contrast to the classic antigen binding "pocket" formed by V domain interactions in the previously characterized antibody structures, the V region associations in protein Loc result in a central protrusion in the binding site, with grooves on two sides of the protrusion. The structure of protein Loc indicates that immunoglobulins are physically capable of forming a more diverse spectrum of antigen-binding sites than has been heretofore apparent. Moreover, the unusual protruding nature of the binding site may be analogous to structures required for some anti-idiotypic antibodies. Further, the complementarity-determining residues form parts of two independent grooves. Therefore, the Loc structure might be viewed as a possible model for the T-cell receptor, which is composed of two light-chain-like polypeptides and can simultaneously bind two different proteins.

In recent years it has been determined that antibodies constitute only one element of the immunoglobulin superfamily, which includes the T-cell receptors, the polymeric immunoglobulin receptor, major histocompatibility complex antigens, and perhaps others. Immunoglobulin-type domains form the basic building blocks for all the above molecules. While it was clear from the earlier studies that Bence-Jones proteins (antibody light chains) were an effective model system for the antigen-binding fragment of an antibody molecule, it is now apparent that broader perspective is appropriate. Structures formed by antibody light chains may reveal attributes of any of the members of the immunoglobulin superfamily.

Antibody molecules consist of two heavy and two light chains; the light chains and the amino-terminal half of the heavy chains form the antigen-binding (Fab)[1] fragments.

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Much of our knowledge about the tertiary structural features of the antigen-binding site has come through X-ray crystallographic analyses of homogeneous (monoclonal) antibody light chains and Fab fragments. The light chain is characterized by a compact amino-terminal variable (V) domain and carboxyl-terminal constant (C) domain linked by an extended "switch" peptide. Bence-Jones proteins, especially λ type, exist as covalent dimers.[2] The two light-chain monomers have identical primary structures; however, the quaternary structure of the covalent dimer closely resembles that of an Fab fragment where one monomer has the structural conformation of the light chain and the second monomer has that of the heavy chain (Schiffer et al., 1973). The light-chain V domain contains four segments of limited variability, termed framework regions (FR), interspersed between three regions of extensive variability, termed hypervariable or complementarity-determining regions (CDR) (Wu & Kabat, 1970). Multiple V region subgroups (isotypes) of light and heavy chains have been defined on the basis of sequence homologies in FRs (Kabat et al., 1979). The CDRs define the antigen-binding site formed by V–V domain interactions between the two monomer constituents in light-chain dimers or by those of the light and heavy chain V domains in Fab molecules.

The structure at atomic resolution has been solved for only one complete light chain, the λ_V Bence-Jones protein covalent dimer Mcg (Schiffer et al., 1973; Edmundson et al., 1975) and for three Fab fragments (Poljak et al., 1973; Segal et al., 1974; Marquart et al., 1980). The primary associative interactions in protein Mcg and in the Fab fragments occur laterally through like domains; the longitudinal interactions between the V and C domains are less extensive. Local twofold axes relate the two V and the two C domains and define the "elbow bend" of the molecule.

The crystal structure of these proteins and of three κI V domain dimeric fragments (Epp et al., 1975; Felhammer et al., 1975; Colman et al., 1977) revealed a common conformation for the antigen-binding site resulting from similar V–V domain interactions. The variations in the size and configuration of the binding site for the above proteins reflect differences in the CDRs only. However, the binding site in another V_L dimer, λ protein Rhe (Wang et al., 1979; Furey et al., 1983), did not conform to this pattern. The unusual Rhe structure resulted from differences in the V–V domain associations.

To investigate the influence of CDRs and FRs on the three-dimensional structure of light chains, we have been engaged in a systematic effort to crystallize human κ and λ Bence-Jones proteins of chemically and serologically defined V region subgroups (Schiffer et al., 1978; Stevens et al., 1981a,b). We report here the crystallization and structural determination of the λ_I Bence-Jones protein dimer, protein Loc,[3] and compare its structure with that of the only other intact light chain dimer, protein Mcg. The two proteins share common V region cross-idiotypic antigenic determinants and have an identical C region sequence (Zhu et al., 1983). Despite their closely related primary structures, we have found that the V region of λ-chain Loc has a quaternary structure markedly different from protein Mcg and from all other human and murine light chains or Fab fragments thus far described. The unusual V–V domain interactions in protein Loc result in a unique conformation of the antigen-binding site.

Materials and Methods

Isolation and Characterization of Bence-Jones Protein Loc. Bence-Jones protein Loc was isolated by Pevikon block (zone) electrophoresis (Solomon & McLaughlin, 1969) from urine specimens of a patient who had multiple myeloma. The dimeric character of the protein was established by gel filtration through a calibrated Sephadex G-75 superfine column with 0.1 M NaCl and 0.05 M Tris buffer (pH 7.2) and by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (Laemmli, 1970) in both the presence and absence of 0.1 M β-mercaptoethanol. The G-75 column was calibrated with bovine serum albumin, chymotrypsinogen A, ovalbumin, and ribonuclease. The SDS gels were calibrated with bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and ribonuclease. Antisera rendered monospecific for the λ-chain V region isotypic subgroups and for the C region isotypes "Mcg" and "non-Mcg" were used in immunodiffusion analyses as previously described (Solomon, 1977, 1981).

Crystallization of Bence-Jones Protein Loc: Characterization of the Crystals. For crystallization, protein Loc was precipitated from urine specimens by the addition of solid ammonium sulfate to 60% saturation. The ammonium sulfate paste was dissolved by dialysis against 0.05 M Tris and 0.10 M NaCl buffer, pH 7.2. Crystals were obtained by three different methods, namely, by precipitation with ammonium sulfate, by precipitation with polyethylene glycol (PEG), and by dialysis against distilled water. Crystals from ammonium sulfate were grown by batch procedures. Sufficient 3 M ammonium sulfate (adjusted to pH 6.5 with ammonium hydroxide) was added to a solution containing 15 mg/mL protein [assuming A_{280}(1 mg/mL) = 1] to attain a final ammonium sulfate concentration of 1.3–1.5 M; crystals grew in 1–2 months. For crystallization from PEG 6000, vapor diffusion was used; 25-μL droplets of solution containing ~10 mg/mL protein were placed on depression slides to which 10-μL droplets of 8% PEG 6000 were added. The slides were enclosed in a plastic box having a reservoir of 8% PEG, and the crystals formed 1 month later. Crystals were also obtained within several weeks by dialyzing a solution containing 30 mg/mL protein against distilled water. The unit cell dimensions and space group of the crystals were determined from precession photographs taken with a rotating anode X-ray source.

Data Collection and Processing. Diffraction data were collected with a Picker diffractometer with a Krisel Control system. The Krisel Control software was modified by Fred J. Stevens (unpublished results). For data collection, a modification of the Wyckoff method (Wyckoff et al., 1967) was used; ω-step scans with five to eight steps of 0.04° were used, and the largest sum of three adjacent measurements was taken as the observed count. The backgrounds for each reflection were measured at an ω offset of ±0.4°. The above procedure was adopted because the background was φ dependent due to the asymmetrical cross section of the crystals. The average 2θ-dependent background used previously (Edmundson et al., 1972) did not give adequate results in this case. Absorption correction was based on a φ scan of an axial reflection at χ = 90°. Linear decay correction was applied by using the averaged intensity changes of three reflections

[1] Abbreviations: Fab, antigen binding fragment; V, variable domain; C, constant domain; FR, framework region; CDR, complementarity-determining region; SDS, sodium dodecyl sulfate; PEG, polyethylene glycol; OCMP, *o*-(chloromercuri)phenol; MIR, multiple isomorphous replacement; SIR, single isomorphous replacement; ISIR, iterative single isomorphous replacement; Tris, tris(hydroxymethyl)aminomethane.

[2] A light-chain covalent dimer is formed by a disulfide bridge between the penultimate cysteine residues of the two chains. This cysteine residue forms the disulfide bond with the heavy chain in an antibody molecule.

[3] Preliminary reports of this work were given in two abstracts (Short et al., 1982; Schiffer et al., 1983).

Table I: Parameters of Heavy-Atom Derivatives,[a] Used to Calculate the MIR Map at 5-Å Resolution

derivative	site	x	y	z	A	R_K (all)	R_C (centric)	R_F (centric)
o-(chloromercuri)phenol (2 mM)	1	0.122	0.070	0.640	97	0.136	0.78	0.21
	2	0.458	0.308	0.322	30			
	3	0.363	0.348	0.692	16			
$K_2Pt(CNS)_6$ I (0.14 mM)	1	0.111	0.099	0.004	77	0.101	0.62	0.19
	2	0.366	0.387	0.770	63			
	3	0.130	0.067	0.647	49			
$K_2Pt(CNS)_6$ II (0.14 mM)	1	0.111	0.101	0.000	58	0.069	0.60	0.13
	2	0.366	0.383	0.769	45			
	3	0.124	0.055	0.631	9			

[a] A = occupancy on a relative scale only; average protein F = 975 on this scale. Temperature factor = 5. $R_K = (\sum|F_{PH,obsd} - F_{PH,calcd}|)/\sum F_{PH,obsd}$, $R_C = (\sum|F_{H,obsd} - F_{H,calcd}|)/\sum F_{H,obsd}$, and $R_F = (\sum|F_{PH} - F_P|)/\sum F_P$. F = structure factor amplitude; P = protein; H = heavy atom; PH = protein plus heavy atom. Figure of merit = 0.62.

measured every 3 h. The absorption of the crystals varied from 1 to 1.8, and the decay correction was typically 1.15 for the 5-Å data set collected in 70 h.

For subsequent calculations, only reflections of the native protein that had the intensity higher than 3σ (based on counting statistics) were used.

Preparation of Isomorphous Derivatives. The derivatives were prepared by soaking the crystals for 4–6 weeks in 1.7 M ammonium sulfate solutions (pH 6.5) to which one of the following heavy atom compounds was added in 0.1–10 mM concentrations: mersalyl, *o*-(chloromercuri)phenol (OCMP), $KHgI_3$, $K_2Pt(CNS)_6$, K_2PtCl_6, K_2PtBr_4, $KAuI_4$, $TmCl_3$, mercuric acetate, *p*-(chloromercuri)benzoic acid, (ethylmercuri)thiosalicylic acid, mercury chloranilate, and *p*-(chloromercuri)benzenesulfonic acid. A covalent Hg derivative of the interchain disulfide bond was prepared by diffusing the reactants into the crystals according to the procedure described by Ely et al. (1973).

Determination of Heavy Atom Positions. The position of the main site of the $K_2Pt(CNS)_6$ derivative was determined from the three Harker sections; the position and occupancy were refined by using the method of alternate cycles of phase determination and least-squares refinement (Dickerson et al., 1968). For the OCMP derivative, the position of the main site, as derived from the Harker sections (see Figure 1), was in agreement with the largest peak from the difference Fourier calculated with the phases derived from the Pt derivative. For the determination of the protein phases to 5-Å resolution, an OCMP and two $K_2Pt(CNS)_6$ derivatives with different relative occupancies of the three sites were used. The results of the refinement are shown in Table I. The figure of merit for all reflections is 0.62. The covalent interchain Hg derivative (Ely et al., 1973) was not useful because the main site had relatively low occupancy and other secondary sites were present. To eliminate the secondary sites, the crystals of the covalent Hg derivative were washed with ammonium sulfate solution. The washing of the crystals with ammonium sulfate not only reduced the secondary sites but also lowered the occupancy of the primary site.[4]

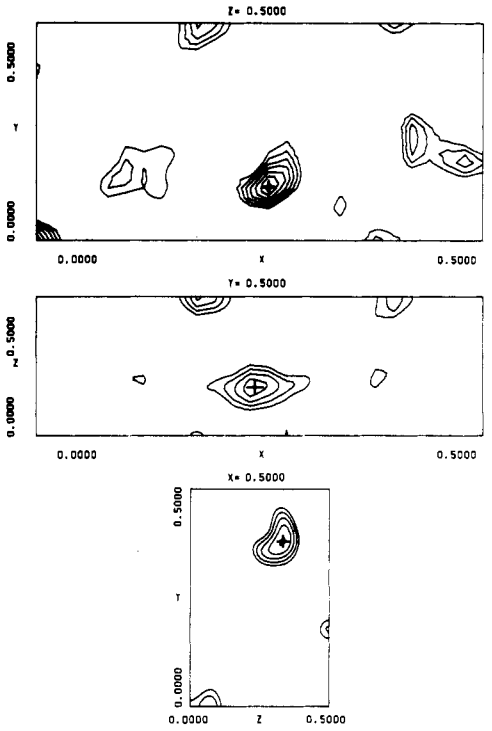

FIGURE 1: Difference Patterson map of the *o*-(chloromercuri)phenol derivative. The three Harker sections are shown; the Harker peaks are marked by (+). The height of the origin peak of the Patterson map is 1000; the contours are drawn at intervals of 40, with the first contour at 40.

Only the $K_2Pt(CNS)_6$ derivative was usable up to 3.5 Å. The data to 3.5 Å were collected on three crystals each with different relative occupancies of the sites. The Cullis R factor for this derivative was 0.49 for the 10–5-Å shell, 0.59 for 5–4-Å shell, and 0.62 for the 4–3.5-Å shell.

Determination of Phases by Iterative Single Isomorphous Replacement Method. The method of iterative single isomorphous replacement (ISIR) (Wang, 1985) was applied with both 5.0- and 3.5-Å data for the $K_2Pt(CNS)_6$ derivative. At each resolution, 16 cycles were run with three different solvent masks to define the molecular boundaries (Wang, 1985). The first mask in direct space was based on phases derived by single isomorphous replacement (SIR), the second on the combination of SIR and refined phases after four cycles, and the third on the combination of refined phases with SIR phases after four more cycles of refinement. The final phases were obtained by combining the SIR phases with the phases obtained after eight additional cycles of refinement with the third mask. For the 3.5-Å resolution data, the R value of the inversion of the last map was 0.25. The combination of SIR phases with the ones obtained in the last cycle had an average figure of merit

[4] Steiner & Blumberg (1971) found that Hg substitution into the interchain disulfide bond of an Fab fragment was not reversible in a 0.1 M Tris buffer, pH 8. Further, Sperling et al. (1969) showed that addition of mercury to the disulfide bond in ribonuclease resulted in the addition of one or two Hg atoms. They found that [RNase·2Hg] is in the $-S-Hg^+$ form; it loses one Hg^{2+} to form the $-S-Hg-S-$ compound [RNase·1Hg] when dialyzed against water (adjusted to pH 7). They further showed that all the mercury could be washed out from the [RNase·2Hg] by 0.1 M Tris buffer, pH 8. In the Loc crystal, the Hg position is asymmetric relative to the local twofold axis between the two C domains. Since the Hg can be washed out by 1.7 M ammonium sulfate, pH 6.5, we postulate that the mono-$S-Hg^+$ derivative of the disulfide bond in Loc was formed instead of the $-S-Hg-S-$ derivative.

Table II: Results of the Restrained Least-Squares Refinement[a]

resolution (Å)	no. of reflections	observed[b] (%)	R
8.0–6.5	407	83	0.33
6.5–5.0	865	66	0.27
5.0–4.0	1165	53	0.24
4.0–3.5	1387	64	0.28
3.5–3.2	738	34	0.30
3.2–3.0	335	23	0.31
3.0–2.65	275	6	0.30

[a] 2913 atoms of total of 3200 used. Temperature factor = 14. Root mean square deviation of bond length = 0.15 Å and plane = 0.01 Å. [b] With intensities greater than 3σ.

of 0.79. The average phase shift in the last cycle was 1.7°. The mean phase difference between the final phases and the SIR phases was 46.4°.

Electron Density Map. The electron density map to 5 Å was calculated by using "best" phases (Blow & Crick, 1959); the observed structure factors were weighted by the figure of merit. The 3.5-Å map was calculated with phases and figures of merit determined by the ISIR method.

Interpretation of 3.5-Å Electron Density Map. In the 3.5-Å ISIR map the boundaries of the domains were very clear. To help us interpret the 3.5-Å map, we used the known structure of the Mcg Bence-Jones protein. The backbone structures of the Mcg domains were fit into the density, and their positions were improved visually through examination of a minimap. The fit was improved by maximizing the overlap of α-carbon positions of the individual domains with the electron density map using a method similar to that of Navia et al. (1979). Each domain was rotated around the x, y, z axes; for each rotation angle the domain was translated along three directions parallel to the axes. The rotation searches were performed in successive 10°, 5°, 2°, and finally 1° intervals; the translation grid was ~1 Å. The maximum overlap was 74 and 68 out of 109 α-carbons for the V domains, and 77 and 65 out of 104 α-carbons for the C domains. Through the use of a rigid body refinement, with all atoms of the domains, the positions and orientations of the four individual domains were further refined. The starting R factor, after adjustment of a scale factor, was 0.39 for 4300 reflections between 15 and 3.5 Å, which refined in six cycles to an R factor of 0.35.

An electron density map was calculated by using $2F_o - F_c$ structure factors with calculated phases. For this structure factor calculation, 2600 out of 3200 atoms of Loc were used. Chain segments that appeared to have a different conformation in Loc than in Mcg were removed. Side-chain atoms belonging to residues that differed in the two proteins were removed after the β-carbon. The map was examined, and the structure was adjusted by using the GRIP-75 interactive computer graphics system of the Computer Science Department of the University of North Carolina at Chapel Hill.[5] Positions of 300 additional atoms were identified, including 20 of the 42 residues that were not used for the structure factor calculations.

Restrained Least-Squares Refinement. Restrained least-squares refinement (Hendrickson & Konnert, 1981) was carried out with 5172 reflections between 8- and 2.65-Å resolution. In five cycles the R factor decreased from 0.33 to 0.27. The refinement statistics are shown in Table II.

[5] GRIP-75 developers included E. G. Britton, F. P. Brooks, Jr., J. Hermans, J. S. Lipscomb, J. E. McQueen, M. E. Pique, and W. V. Wright. GRIP-75 development has been supported by the National Institutes of Health Division of Research Resources, the National Science Foundation, the Atomic Energy Commission, and the International Business Machines Corp.

RESULTS

Crystallographic Analysis

Characterization of the Loc Crystals. Protein Loc was crystallized from ammonium sulfate, PEG, and distilled water. The crystals grown from ammonium sulfate are rectangular plates, with dimensions of 0.1 × 0.3 × 0.4 mm and belong in the orthorhombic space group $P2_12_12_1$ with a = 149.3 ± 0.9 Å, b = 72.4 ± 0.5 Å, and c = 46.5 ± 0.3 Å. Assuming that one dimer with M_r 46 000 is in the asymmetric unit, we determined the fractional volume occupied by solvent to be 0.55. This value is within the range observed for globular proteins (Matthews, 1968) and is comparable to the value of 0.6 observed for the trigonal crystal form of the Mcg Bence-Jones protein (Edmundson et al., 1972), which was also crystallized from an ammonium sulfate solution. The crystals grown from both PEG and distilled water are prisms elongated along the b axis and bounded by {101} faces with dimensions of 0.1 × 0.5 × 0.05 mm. Their space group is $P2_12_12_1$, and the unit cell dimensions are a = 119.2 ± 0.8 Å, b = 74.3 ± 0.5 Å, and c = 50.7 ± 0.3 Å. Assuming that one dimer with M_r 46 000 is the asymmetric unit, we determined the fractional volume occupied by solvent to be 0.50.

We are determining the structure of the Loc protein with the crystals grown from ammonium sulfate because these crystals are easier to handle than the crystals grown from PEG or water. The crystals that were obtained from the first sample of protein diffracted to 2.3-Å resolution. Crystals grown from subsequent preparations did not diffract as well. Although the diffraction pattern did not seem to be affected to 3.5-Å resolution, it is much weaker below that, extending only to 2.6-Å resolution. In Table II the percentages of observed reflections are listed as a function of resolution.

Evaluation of the ISIR Method. The Loc structure was first solved at 5-Å resolution by the MIR method; subsequently ISIR phases based on the $K_2Pt(CNS)_6$ derivative were also calculated. Electron density maps calculated by the two methods were then compared. The ISIR map appeared less noisy in the solvent region, and the boundary of the molecule was better defined in some parts of the ISIR map than in the MIR map. For example, in Figure 2, the true boundaries of the molecule (indicated by the dashed lines) are less clear in the MIR map than in the ISIR map. This comparison gave us confidence to use the ISIR phases to calculate the electron density map at 3.5-Å resolution.

Description of the Loc Molecule

Characterization of λ Light-Chain Loc. Bence-Jones protein Loc represents a complete λ light chain as shown by serological and chemical analyses. The protein was classified immunochemically as a λ chain with V_λ antigenic determinants associated with proteins of the λI subgroup (Solomon, 1976a,b, 1981) and C_λ determinants of λ chains possessing the Mcg isotype (Solomon, 1977), i.e., a product of the $C_{\lambda 1}$ gene (Heiter et al., 1981). The complete amino acid sequence (Zhu et al., 1983) confirms these results. Protein Loc exists as a covalent dimer as shown by gel filtration and SDS electrophoresis. Upon filtration through Sephadex G-75 in aqueous buffer, pH 7.2, the protein eluted as a symmetrical peak with a molecular weight of ~46 000. On SDS–polyacrylamide gel electrophoresis in the absence of a reducing agent, the molecular weight was also determined to be 46 000. In the presence of β-mercaptoethanol, the molecular weight was 23 000, as can be expected for the monomer of a complete light chain.

Relationship of the V Domains. The relative positions of the two domains in the V–V dimer are completely different

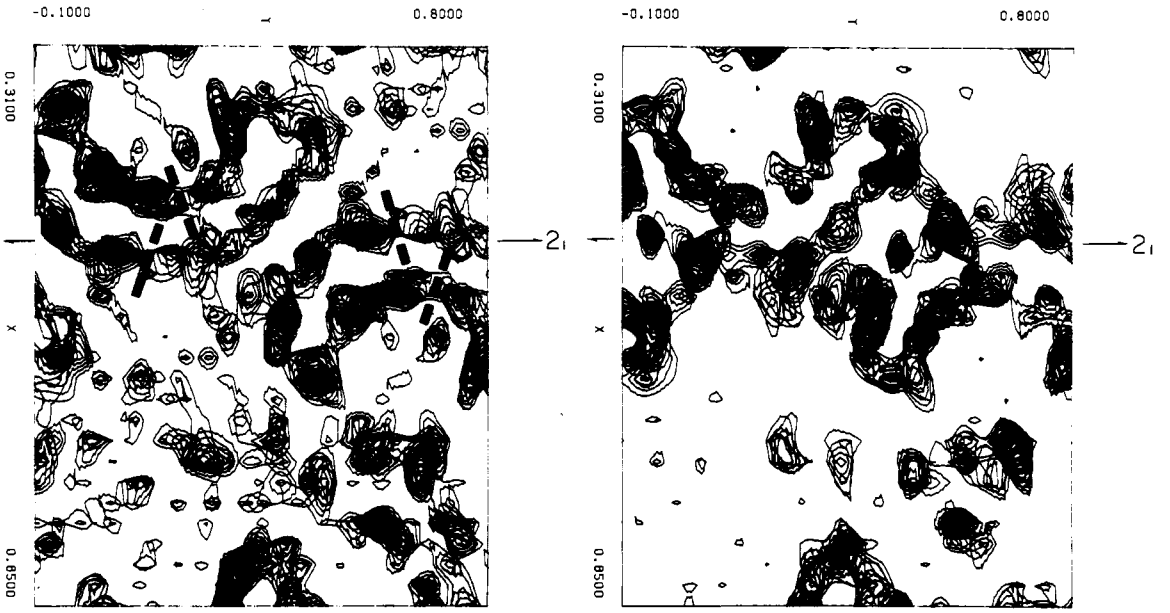

FIGURE 2: A comparison of the MIR (left) and ISIR (right) maps at 5-Å resolution. The dashed lines represent the boundary between C domains along the 2_1 axis.

FIGURE 3: Stereo drawings of the C_α backbone of the Loc variable domains (upper) and Mcg variable domains (lower). The hypervariable segments are indicated by heavy lines. The view is along the local twofold axis between the domains. In the Loc structure, the twofold is a twofold screw axis with a 3.5-Å translation along the axis.

than have been observed in the Mcg protein. The two V domains in the Loc structure are related by a local twofold axis and an additional 3.5-Å translation along this axis. The local twofold screw axis is approximately in the *xy* plane and

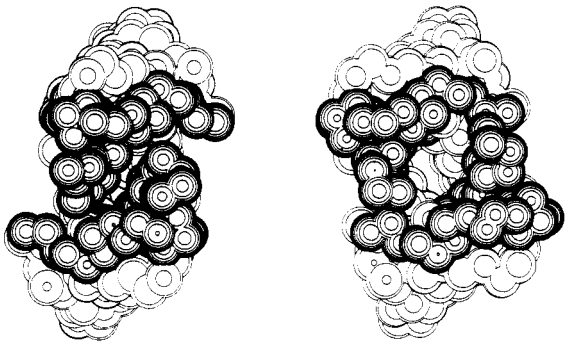

FIGURE 4: Computer-generated hard sphere representation of the antigen-binding site of Loc (left) and Mcg (right) proteins, in the same orientation as in Figure 3. Each α-carbon is represented by a sphere of 3.3 Å. The hypervariable residues are shown darker (with more contours). The relative orientation of the V domains in the Mcg protein is virtually identical with the known structures of Fab fragments; the Mcg molecule forms a classic antigen-binding "pocket". In contrast, the association of the V domains in the Loc protein results in a central protrusion in the binding site, with grooves on two sides of the protrusion.

makes an angle of 21.5° with the positive x axis. (This twofold was observed in the self-rotation function with both the 6- and the 5-Å resolution data.) As an apparent consequence, the obvious cavity between the two V domains seen in the Mcg protein does not exist (see Figures 3 and 4). In contrast, the two CDR3 segments form a well-defined protrusion. The CDR3 regions of each V domain are located close to each other and to the CDR1 segments; the CDR2 segments are removed from the local twofold screw axis. The two Loc V domains form a "keylike" structure with a pointed tip and two grooves, reversing the classical lock and key model of antibody function. As a result of the twofold screw axis relating the two V domains, the two grooves formed by the V domains have different conformations. Residues from CDR3 and CDR2 contribute to the grooves. The bottom of the concave surface is formed by FR2 and FR4 chain segments in both grooves.

The most likely explanation for the specific V–V interaction observed in the Loc protein is that the residues in the CDR3 region influence the arrangement of the V domains. Specifically, in protein Loc, tryptophan-91 residues in CDR3 of both monomers interact across the local twofold screw axis. The interaction of the tryptophan side chains probably leads to the unusual twofold screw axis between the V domains. The equivalent tyrosine residues in Mcg are removed from each other and thus do not participate in the V–V interactions.

Relationship of C Domains. The C domain interactions in the light-chain dimers Loc and Mcg are very similar. Further, the packing arrangement of the C domains in the Loc crystal, which crystallized in the space group $P2_12_12_1$, is also similar to that found in the trigonal crystal form of the protein Mcg (Ely et al., 1978), which has the space group $P3_121$. The local twofold axis relating the C domains is approximately parallel to the z axis (and is in the xz plane making an angle of $\sim$15° with the z axis). For both protein Loc and Mcg, the C domains of neighboring molecules are related by a 2_1 axis of the unit cell, which passes through the centers of the domains. The local twofold axis between the C domains is approximately perpendicular to the 2_1 axis. Therefore, another local twofold axis is generated between C domains of neighboring molecules. The outside three-stranded sheets from the neighboring C domains interact across this local twofold axis to form a six-stranded β pleated sheet structure. The similar packing arrangement of the C domains in the Loc and Mcg crystals explains the close similarity between the cell dimensions of 72.4 Å for Loc and 72.3 Å for Mcg, along the 2_1 axis described above.

V–C Junction. The Loc molecule is shown in Figure 5; its elbow bend is 97°, the smallest observed so far for a light-chain dimer or Fab. By comparison, the elbow bend of the Mcg light-chain dimer in the trigonal crystal form (crystallized from ammonium sulfate) is 113° (Schiffer et al., 1973), whereas for four different Fabs the elbow bend ranges between 131 and 166° (Poljak et al., 1973; Segal et al., 1974; Matsushima et al., 1978; Navia et al., 1979).

DISCUSSION

Implications of the Structure of Protein Loc. The crystal structure of protein Loc is of special interest because it represents a novel type of V domain interaction. With the exception of the λ-chain V_L dimer Rhe (Wang et al., 1979; Furey et al., 1983), the relatively similar arrangement of V domains in protein Mcg, the V_L dimers, and the Fab fragments has led to the assumption that, in forming the antigen-binding site, the relative positions of the V domain components are fixed (Davies et al., 1975; Padlan, 1977) and that the conformation of the site is changed only by differences in the CDRs. The fact that both proteins Loc and Rhe have binding site conformations formed by novel and different V domain interactions suggests that variability of the antigen-binding sites may be significantly enhanced by changes in the relative positions of the V domains.

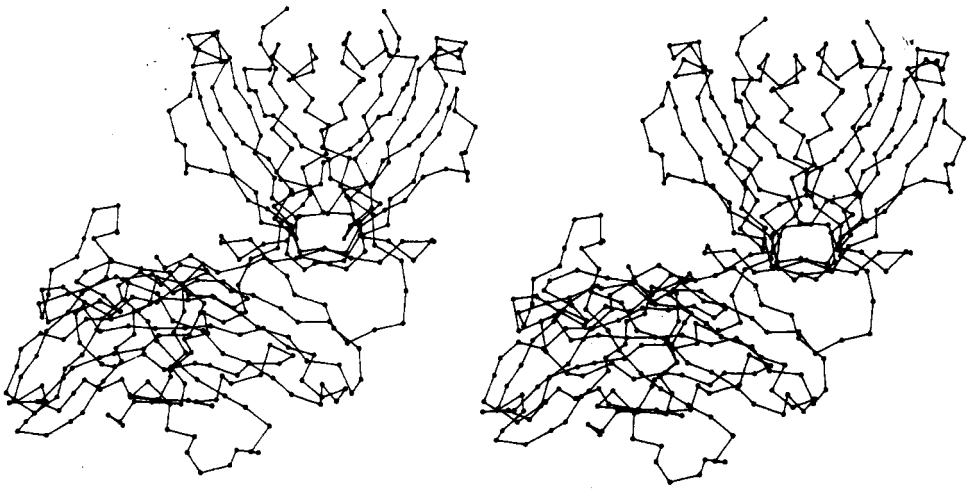

FIGURE 5: Stereo drawing of the C_α backbone of the Loc protein. The constant domains are on top; the variable domains are on the bottom of the figure. The small (97°) elbow bend brings the variable and constant domains close together.

The configuration of the antigen-binding site in protein Loc is unique in that, instead of the characteristic concave "pocket" or ligand-binding groove, the manner of V–V domain association results in the site assuming a convex or protruded structure (see Figures 3 and 4). The structural relevance and significance of the conformation obtained by the Loc protein is, of course, not known. The possibility may exist that the structure is in some way the result of the fact the dimer is composed of identical subunits, and as a result, the Fab composed of heavy and light chain components cannot attain the Loc conformation. This assertion is weakened, however, by the observation that all but one of the light-chain dimers, including the only other intact light-chain dimer, Mcg, are also composed of identical chains and have structures that do emulate the four known structures of Fabs. Therefore, Loc can represent a possible Fab conformation but does not prove the existence of a Fab with this conformation. Similarly, the statistically insignificant sample of four Fab structures out of a population of greater than one million does not prove that they represent the only possible structures.

The Fab structure represented by Loc may be an essential element of the immune system. The network theory (Jerne, 1974) of immune regulation requires selection of binding sites against binding sites. Experimentally, it has been established that anti-idiotypic antibodies are capable of mimicking the antigenicity (McNamara et al., 1984) and in some cases the receptor specificity (Sege & Peterson, 1978) of the original antigen. It is difficult to rationalize this finding with the concept of a series of antibodies in which the binding sites are composed of interacting residues recessed in a pocket. However, if at least one of the antibodies in this series is characterized by an extruding arrangement of the complementarity-determining residues, the experimental observations can be directly explained.

One additional observation may be of interest. While Loc can be described as exhibiting a protruding binding site, one can consider the arrangement of CDRs as constituting two concave binding surfaces on opposite sides of the dimer. Analogously, the binding pockets of the known Fabs can be partitioned arbitrarily as two or more binding sites, but in contrast to the structure illustrated by Loc, multiple binding sites in a pocket are mutually exclusive in that binding of a protein antigen to one site will block all others in the pocket. A Loc-type structure could simultaneously accommodate two bound molecules. Such an immunoglobulin function has not been experimentally observed. However, antibodies are only one representative of a class of homologous molecules—the immunoglobulin superfamily—which includes histocompatibility antigens and T-cell receptors. The T-cell receptor is composed of polypeptides that have sequence homology to immunoglobulin light chains (Hedrick et al., 1984; Saito et al., 1984; Chien et al., 1984). The T-cell receptor has been experimentally characterized as binding two molecules simultaneously (Zinkernagel & Doherty, 1979; Kappler et al., 1981)

In conclusion, the structure of Loc represents a stable conformation physically attainable to antibodies or other molecules of the immunoglobulin superfamily. The unanticipated features of the structure of this molecule may have implications in the understanding of immune diversity and regulation.

Added in Proof

We have recently noted that E. A. Kabat has anticipated the possible existence of convex antigen-binding sites (Kadat, 1984).

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Cooperativity among Calmodulin's Drug Binding Sites†

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ABSTRACT: The binding of felodipine, a dihydropyridine Ca^{2+} antagonist, to calmodulin has been studied by equilibrium dialysis and fluorescence techniques. Analysis using the Hill equation gives a Hill coefficient of 2. A plot of bound [felodipine] vs. free [felodipine]^2 gives a B_{max} of 1.9 mol/mol and a $K_{0.5}$ of 22 μM. Two calmodulin antagonists, prenylamine and R24571, which have previously been shown to potentiate the fluorescent enhancement observed when felodipine binds to calmodulin [Johnson, J. D. (1983) *Biochem. Biophys. Res. Commun. 112*, 787], produce a reduction in Hill coefficient to 0.7 and 1.0, respectively, and account for the observed potentiation of felodipine binding. Titrations of felodipine with calmodulin in the absence and presence of prenylamine and R24571 suggest that these drugs decrease the $K_{0.5}$ of calmodulin for felodipine by 25-fold. Thus, potentiating drugs (prenylamine and R24571) bind to either of the two felodipine binding sites and, through an allosteric mechanism, result in felodipine binding to the remaining site with greatly enhanced affinity. Two types of potentiating drugs are observed. Prenylamine exhibits a Hill coefficient of 0.8 whereas felodipine, R24571, and diltiazem exhibit Hill coefficients of 2 in their potentiation of felodipine binding. Titrations of felodipine and calmodulin with Ca^{2+} exhibit cooperativity with a Hill coefficient of 4. Half-maximal binding occurs near pCa 6.0. In the presence of R24571, the calcium dependence of felodipine binding is biphasic, now exhibiting a much higher affinity (pCa 7.6) component. A model is presented to explain the relationship of these various allosterically regulated conformers of calmodulin and their interactions and activation with its target proteins.

Calmodulin is a ubiquitous calcium binding protein that binds 4 mol of calcium/mol of protein and undergoes large calcium-dependent changes in structure. These structural changes form or expose hydrophobic binding sites on its surface (Laporte et al., 1980; Tanaka & Hidaka, 1980) where calmodulin binds and activates as many as 30 different proteins in a calcium-dependent manner [see Klee et al. (1980) for review]. Certain drugs, including trifluoperazine (TFP), W-7, and R24571, can bind to these sites with high to moderate affinity and inhibit calmodulin's interaction and activation of many of the proteins that it modulates. Presently, it is uncertain how calmodulin can bind and selectively activate so many different proteins with any degree of specificity.

Several models have been proposed to explain how calmodulin might exhibit selectivity as a calcium-dependent modulator. For example, some target proteins appear to exhibit

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