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Structure of a Second Crystal Form of Bence-Jones Protein Loc: Strikingly Different Domain Associations in Two Crystal Forms of a Single Protein†

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ABSTRACT: We have determined the structure of the immunoglobulin light-chain dimer Loc in a second crystal form that was grown from distilled water. The crystal structure was determined to 2.8-Å resolution; the *R* factor is 0.22. The two variable domains are related by local 2-fold axes and form an antigen binding "pocket". The variable domain–variable domain interaction observed in this crystal form differs from the one exhibited by the protein when crystallized from ammonium sulfate in which the two variable domains formed a protrusion (Chang et al., 1985). The structure attained in the distilled water crystals is similar to, but not identical with, the one observed for the Mcg light-chain dimer in crystals grown from ammonium sulfate. Thus, two strikingly different structures were attained by this multisubunit protein in crystals grown under two different, commonly used, crystallization techniques. The quaternary interactions exhibited by the protein in the two crystal forms are sufficiently different to suggest fundamentally different interpretations of the structural basis for the function of this protein. This observation may have general implications regarding the use of single crystallographic determinations for detailed identification of structural and functional relationships. On the other hand, proteins whose structures can be altered by manipulation of crystallization conditions may provide useful systems for study of fundamental structural chemistry.

Immunoglobulin light chains have an N-terminal variable (V) and a C-terminal constant (C) domain of about 100 residues each. These two domains are linked by a flexible "switch" peptide that makes movement of the domains possible. In the crystal structures of the Mcg (λ_V) light-chain dimer (Schiffer et al., 1973) and structurally analogous antigen binding fragments (Fab) [for a review, see Davies and Metzger (1983)], local 2-fold axes of symmetry, one relating the variable domains and one relating the constant domains, were identified. The angle between these dyads was called the "elbow" bend.

The Loc protein is a Bence-Jones protein consisting of two covalently linked λ_I immunoglobulin light chains (Zhu et al., 1983), which are disulfide bonded at their penultimate residues. In previous work, we determined the structure of the Bence-Jones protein Loc as crystallized from ammonium sulfate (Chang et al., 1985). In this structure, an unusual association of the V domains was found. Instead of the concave hapten binding cavity found in other light-chain dimers and Fabs, we observed a protrusion resulting from V domains that are related to each other by a 2-fold axis and a translation of 3.5 Å. Because of this unusual conformation of the antigen binding site, we again determined the structure of the same protein, but this time used a crystal form grown from distilled water. The Loc structure we are reporting here is more similar to the "usual" conformation with a local 2-fold and a hapten binding cavity between the V domains. Implications for structural studies of finding two structures for a molecule when it is crystallized from different solvents will also be discussed.

Materials and Methods

Preparation of Crystals. To prepare crystals, previous procedures (Chang et al., 1985) were slightly modified as follows: Crystals were grown at 4 °C by dialyzing 50–100-μL samples in dialysis buttons, at a concentration of 40 mg/mL protein [assuming A_{280}(1 mg/mL) = 1] in 0.3 M NaCl and 0.05 M Tris (pH 7.4) against distilled water; the pH was adjusted with NaOH or $Ca(OH)_2$ to 6.5. The crystals were then transferred into 16% poly(ethylene glycol) (PEG) 8000 in 0.05 M cacodylate, pH 6.5.

Heavy-Atom Derivatives. The exchange of the distilled water mother liquor for 16% PEG made the preparation of several heavy-atom derivatives possible; crystals in distilled water dissolved when salt solutions were added. Five heavy-atom derivatives were used to determine the phases to 5-Å resolution; these were $K_2Pt(CNS)_6$, [*p*-(hydroxymercuri)-phenyl]sulfuric acid, methylmercury chloride (MeHgCl), a covalent mercury derivative with the mercury inserted into the interchain disulfide between the two monomers (S–Hg–S), and the MeHgCl, S–Hg–S double derivative.

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$K_2Pt(CNS)_6$ and [*p*-(hydroxymercuri)phenyl]sulfuric acid derivatives were obtained by soaking native crystals in 2 mM solutions prepared in 16% PEG 8000 in 0.05 M cacodylate, pH 6.5, for 30 and 5 days, respectively. To prepare the MeHgCl derivative, a native crystal was mounted in a capillary, and solid MeHgCl was introduced into one end of the capillary. The capillary was then fused closed. Data were collected the same or the following day. The interchain mercury derivative was prepared in solution (Ely et al., 1973) and then crystallized by the same procedure used for the native protein.

Heavy-atom positions were located by using difference-Patterson and difference-Fourier techniques and refined by using the method of alternate cycles of phase determination and least-squares refinement (Dickerson et al., 1968). The figure of merit was 0.77 for the 6.5-Å shell and 0.72 for the 5-Å data set.

Data Collection and Processing. A Picker diffractometer with a Krisel Control operating system was used for the collection of the diffraction data. The Krisel Control software was modified by Fred J. Stevens and Clint Ainsworth (unpublished results). A modification of the Wyckoff method (Wyckoff et al., 1967) was used for data collection; floating step scans in ω were carried out in steps of 0.03°; the number of steps was adjusted so that it always included the three highest consecutive counts, starting at the expected peak position. The largest sum of three adjacent measurements was taken as the observed count. The average 2θ-dependent background was used (Edmundson et al., 1972). The absorption correction was based on a ϕ scan of an axial reflection at $\chi = 90°$; it varied from 1 to 1.2. A 2θ-dependent decay correction was determined by monitoring the intensities of reflections in a broad 2θ range. To minimize the consequence of crystal deterioration, high-resolution data were collected first. Data up to 20% decay correction were used.

Crystals were cooled to near 0 °C by a stream of cold N_2 gas. Cooling was found to increase the useful lifetime of all crystals. This effect was most significant for the heavy-atom-derivative crystals.

Interpretation of the Electron Density Map. The molecular boundaries and the locations of the four domains of the Loc light-chain dimer were identified in a 6.5-Å MIR map calculated with best phases (Blow & Crick, 1959); the observed structure factors were weighted by the figure of merit. The two constant domains were easily identified in the electron density map because their interactions forming the dimer, their locations, and the neighbor–neighbor interactions within the unit cell are like those observed previously in the ammonium sulfate form.

The initial positions of the domains, determined visually, were refined individually by maximizing the overlap between the α-carbons in the domain and the electron density in the 6.5- and 5-Å maps. The rotational searches (Navia et al., 1979; Chang et al., 1985) were carried out in 5°, 2°, and finally 1° intervals, translation searches in 2- and 1-Å steps. After this refinement of the 112 α-carbon atoms of each V domain, 82 and 85 fell in electron density. The C_1 and C_2 domains were moved as a unit; of the 204 α-carbon atoms of the C domains, 157 fell in electron density. The positions of the domains were further refined with a rigid-body refinement that used most of the atoms of the domains. The *R* factor for 15–3.5-Å data decreased from 45 to 35%.

The correctness of the solution was also shown by the presence of electron density for three residues at the switch regions between the V and C domains in the electron density map. These residues were not included in the structure factor calculation. The electron density map was calculated with observed structure factors weighted by the figure of merit and MIR phases for 15–6.5-Å data and $2F_{obsd} - F_{calcd}$ as coefficients with phases from the model calculated for 6.5–3.5-Å data. After several manual adjustments were made by using FRODO (Jones, 1978) on an Evans and Sutherland PS300 terminal, the *R* factor decreased to 33% for 15–3.5-Å data and to 31% for 6.5–3.5-Å data.

Table I: Summary of Restrained Least-Squares Refinement Parameters

	σ values	
	model	target
diffraction data		$0.45\langle\|\|F_o\| - \|F_c\|\|\rangle$
bonding distances (Å)		
bond length	0.01	0.02
angle-related distance	0.03	0.03
intraplanar (1–4) distance	0.04	0.05
deviation from plane (Å)	0.01	0.02
chiral volume (Å^3)	0.16	0.15
nonbonded contacts (Å)		
single torsion contact	0.24	0.50
multiple torsion contact	0.37	0.50
possible hydrogen bond	0.35	0.50
conformational torsion angle, ω (deg)	1.8	3.0
thermal factors (Å^2)		
main-chain bond	0.35	1.0
main-chain angle	0.63	1.0
side-chain bond	0.65	1.5
side-chain angle	1.05	2.0

Table II: Results of Restrained Least-Squares Refinement

resolution (Å)	no. of reflections	obsd[a] (%)	*R*
6.5–5.0	976	86	0.24
5.0–4.0	1788	91	0.18
4.0–3.5	1629	84	0.21
3.5–3.2	1332	73	0.24
3.2–3.0	1042	65	0.25
3.0–2.8	900	43	0.28

[a] With intensities greater than 3σ.

Refinement. Restrained least-squares refinement (Hendrickson & Konnert, 1981) was carried out by using data collected on cooled crystals in which the mother liquor was replaced by PEG. The refinement was started by using 10–2.8-Å data and an overall temperature factor. In the last 11 cycles, 6.5–2.8-Å data were used, and individual temperature factors were allowed to refine. The final *R* factor equals 22% for 7673 reflections between 6.5- and 2.8-Å resolution, with the tight stereochemical restraints suggested by Hendrickson (1985). Restrained least-squares refinement parameters are summarized in Table I. The *R* factor as a function of resolution is shown in Table II.

Results and Discussion

Description of the Structure. The tertiary structures of the individual V and C domains are very similar in the ammonium sulfate and water crystal forms; detailed comparisons, however, will have to wait until both are refined with high-resolution data. The arrangement of the two V domains in the crystal grown from distilled water is similar to but not identical with that found in other light-chain dimers (Schiffer et al., 1973; Epp et al., 1975); they are related by a 2-fold axis (with no translation) and form a hapten binding cavity. (Di-DNP-lysine, a hapten that bound to Mcg, when diffused into the crystal, bound in this cavity.) The difference between the interactions of Loc (water) and Mcg (ammonium sulfate) variable domains apparently results from the replacement of

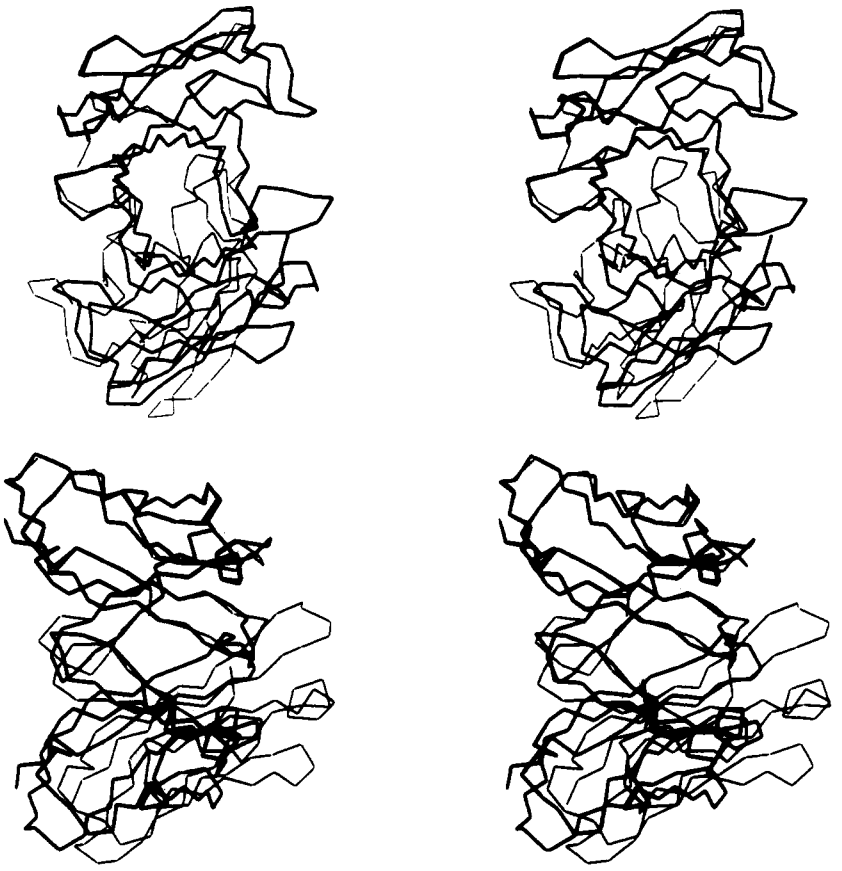

FIGURE 1: Comparison of the variable domain interaction geometries in Loc W (heavy line) and in Loc AS (thin line). The V_1 domain of Loc AS is superimposed on the V_1 domain of Loc W. In the upper figure, the view is along the local 2-fold axis between the variable domains of Loc W. In the lower figure, the 2-fold axis is horizontal.

Table III: Comparison of V–V Interaction Geometries in Light-Chain Dimers and Fabs

	M603	Mcg	Loc W	Loc AS	Nc41[a]
New	4.2 (0.7)[b]	8.8 (1.6)	22.7 (1.8)	31.7 (4.2)	12.2 (1.0)
M603		6.0 (1.3)	20.7 (2.5)	31.0 (4.9)	8.8 (1.3)
Mcg			16.4 (3.1)	34.5 (5.3)	
Loc W				36.4 (2.4)	

[a] Values from Colman et al. (1987). [b] The table lists the absolute value of the rotation (degrees) and translation (angstroms, in parentheses) calculated on the basis of the procedure of Colman et al. (1987) to compare interaction geometries of V–V domains. For the superposition of the domains, α-carbon coordinates of 26 residues in the V–V interface were used. These residues are 33–39, 43–47, 84–90, and 98–104 of the V_L domains and the structurally equivalent residues 34–40, 44–48, 88–94, and 103–109 from the V_H domains [using the numbering conventions of Kabat et al. (1987)]. In light-chain dimers, V_1 is equivalent to V_H, and V_2 is equivalent to the V_L of an Fab. First, a transformation matrix was calculated that superimposes the V_L (or V_2) domain of the molecule in the row on the equivalent domain of the molecule in the column. Then the transformation was applied to the V_H (or V_1) molecule of the row. Because of the different quaternary structures of the Fabs and light-chain dimers, an additional rotation and translation was required to superimpose the V_H (or V_1) of the molecule in the row on the molecule in the column. [For example, (1) calculate the transformation matrix that superimposes the New V_L on the M603 V_L, (2) apply the transformation to the coordinates of New V_H, which results in New V_H', and (3) calculate the rotation and the translation that are required to superimpose New V_H' onto M603 V_H.] Coordinates for proteins New (Saul et al., 1978) and McP603 (Satow et al., 1986) were obtained from the Brookhaven Data Bank.

Table IV: Comparison of Unit-Cell Characteristics of the Two Loc Crystals

	Loc W	Loc AS
cell dimensions (Å)		
a	120.2	149.3
b	73.8	72.4
c	50.5	46.5
space group	$P2_12_12_1$	$P2_12_12_1$
volume fraction protein	0.50	0.45

Gln-38 [numbering as in Kabat et al. (1987)] with a His. Whereas the two Gln residues of the two monomers form hydrogen bonds across the interface in Mcg (Edmundson et al., 1975) and in Rei (Epp et al., 1975), in Loc the equivalent His residue forms a hydrogen bond with Tyr-87 of the other chain. The relative positions of the two V domains in Loc W and Loc AS are compared in Figure 1. In Table III, the V–V interaction geometries of Loc W, Loc AS, Mcg, Fab fragment New, and McPC603 are compared with each other by using the procedure described in Colman et al. (1987). By this analysis, the light-chain dimer Mcg appears to be similar to the Fab fragment New and McPC603. The magnitude of the difference (16°) in domain orientations for Loc W compared to Mcg was surprising because, in both cases, the V domains are related by a 2-fold axis and the structures appear similar. As was discussed before (Chang et al., 1985), Loc AS differs from the other proteins in conformation; the angular difference is more than 30°.

The association of the C domains is very similar in the two crystal forms. The elbow bend of the water form is 120°, which is significantly larger than the 97° elbow bend observed in the ammonium sulfate form. By comparison, the elbow bend of the Mcg light-chain dimer is 113° when it is crystallized from ammonium sulfate (Schiffer et al., 1982) and 130° when crystallized from water (Abola et al., 1980), whereas the elbow bends of Fabs range between 132° and 172° (Davies et al., 1988).

Comparison of Crystal Packing of Loc W and Loc AS. The difference in the shape of the molecule in the two solvents is correlated with different unit-cell dimensions (Table IV) and

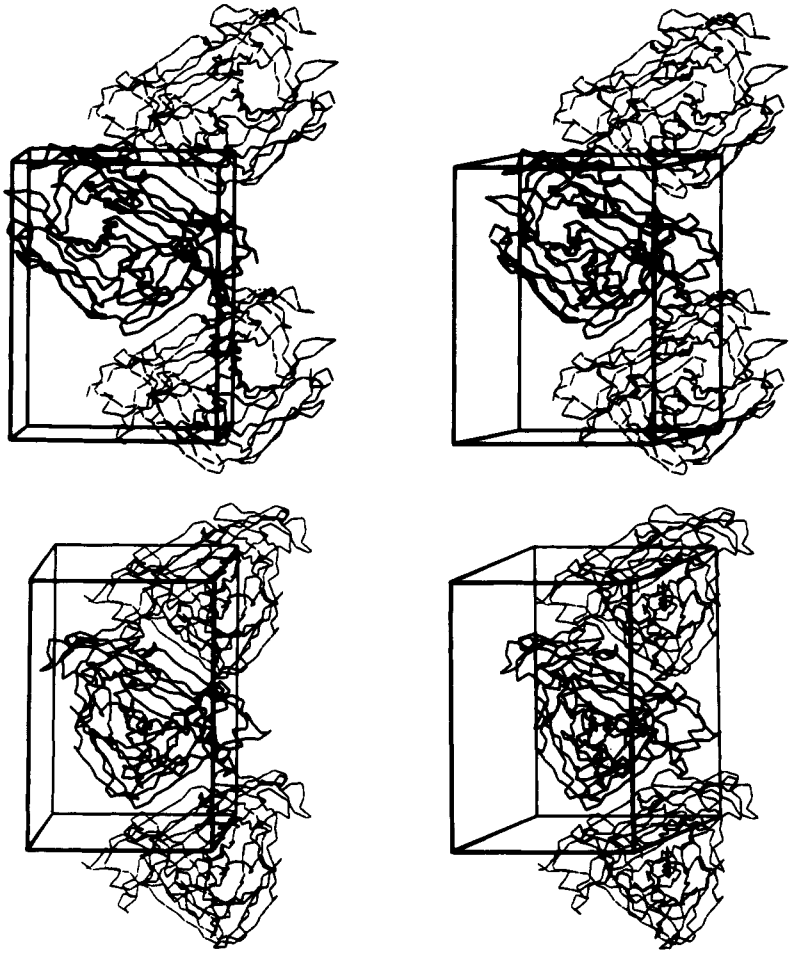

FIGURE 2: Illustration of molecular packing along the Y direction (vertical) of the unit cell. The Z direction is horizontal. Loc W is on the top; Loc AS is on the bottom. The 2-fold screw axis along the Y direction, at $Z = {}^3/_4$, relates the constant domains. This figure shows that the constant domains interact in a similar manner within the individual molecules and that their packing is essentially the same in both crystal forms. The size of the unit cell in the Z direction (horizontal) accommodates one molecule. The larger elbow bend at 120° compared to 97° and the open binding pocket in Loc W result in the increase of the cell dimension from 46.5 to 50.5 Å.

different packing interactions. Although the b cell dimension is very similar (1.4 Å longer), the a cell dimension is 29 Å shorter and the c cell dimension is 4 Å longer in the Loc W. These differences lead to an 11% smaller cell volume in which 50% is occupied by the protein, compared to 45% in Loc AS.

The similar b cell dimension results from similar packing of constant domains along the Y axis in both forms (see Figure 2). The neighboring constant domains are related by the 2-fold screw axis along Y, as was previously described (Schiffer et al., 1985), which leads to contact between C_1 and C_2 domains of the symmetry-related molecules. The local 2-fold axis between the constant domains makes an angle of ~20° with the Z axis in Loc W while the 2-fold axis makes an angle of ~15° with the Z axis in Loc AS (Chang et al., 1985).

The two variable domains, because there is a cavity between them, occupy a larger volume in Loc W than in Loc AS where the two variable domains are flatter. As is illustrated in Figure 3, Loc AS variable domains from two symmetry-related molecules can fit along the Z axis; the 2-fold screw along the Z axis relates the V domains from neighboring molecules. The neighbor–neighbor contact is between hypervariable residues (HVRs) of both the V_1 and V_2 domains of neighboring molecules. The third HVR of monomer 1 is close to the third and first HVR of monomer 2 of the neighboring molecule. The first HVR of monomer 1 is close to the first HVR of monomer 2. The second HVRs of adjacent molecules are in contact with each other. Together, these contacts in Loc AS shield the HVRs from solvent.

In Loc W (see Figure 4), the variable domains contact constant domains of symmetry-related molecules that are separated along the X axis and, to a lesser extent, variable domains that are separated along the Y axis of the unit cell. As can be seen by comparing Loc W with Loc AS in Figure 3, the close contact observed along the Z axis between the constant domains in Loc AS that are related by a unit-cell translation is relieved by the 4 Å longer cell dimension in the Loc W form. The size of the unit cell in the Z direction is such that it accommodates one molecule. The longer c dimension in Loc W accommodates the more open elbow bend and the increased volume occupied by the variable domains.

Variable Domain Interactions in Other Immunoglobulins. Variations in the V–V domain interactions of the Mcg light-chain dimer were also noted when the ammonium sulfate and distilled water forms were compared (Abola et al., 1980; Ely et al., 1983). Differences observed in both the elbow bend and the domain–domain interactions in Mcg were not as pronounced as in Loc. The observation of two different V–V domain interactions in the two crystal forms of Loc and Mcg is meaningful. The association constants of these light-chain dimers are high in solution, suggesting that domain–domain relationships are not readily altered by crystal lattice interactions. Although no known evolutionary selection underlies the mechanism of V_L domain self-association, the association constant that characterizes the V_L–V_L interactions in both Loc and Mcg is $\geq 10^6$ M^{-1} (Stevens et al., unpublished observations; Schiffer et al., 1982), which is comparable to the V_L–V_H

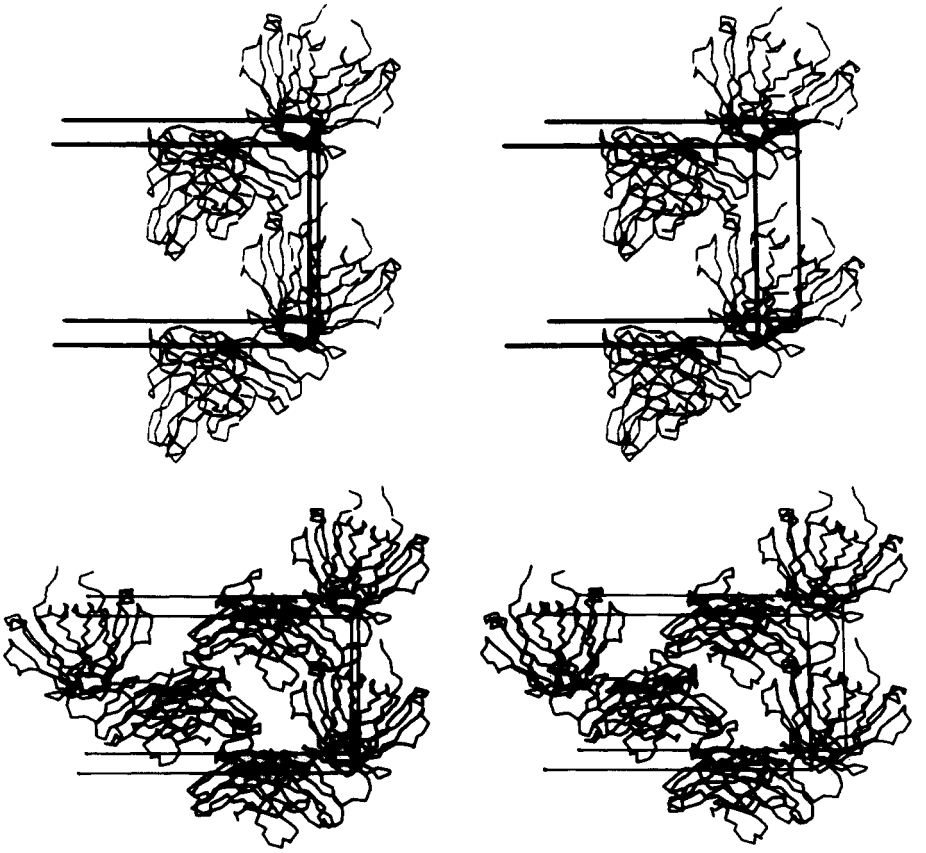

FIGURE 3: Illustration of molecular packing along the Z direction (vertical) of the unit cell. The X direction is horizontal. Loc W is on top, and Loc AS is on the bottom. In Loc AS, the variable domains are related by a 2-fold screw axis along the Z direction at $X = {}^3/_4$. In Loc W, the size of the variable region prevents this type of packing; the symmetry-related variable domains are displaced along the Y direction (see Figure 4). Unit-cell translation along the Z direction relates the constant domains at $X = 1$. In Loc W, the larger cell dimension along Z results in a larger separation of constant domains in comparison with Loc AS.

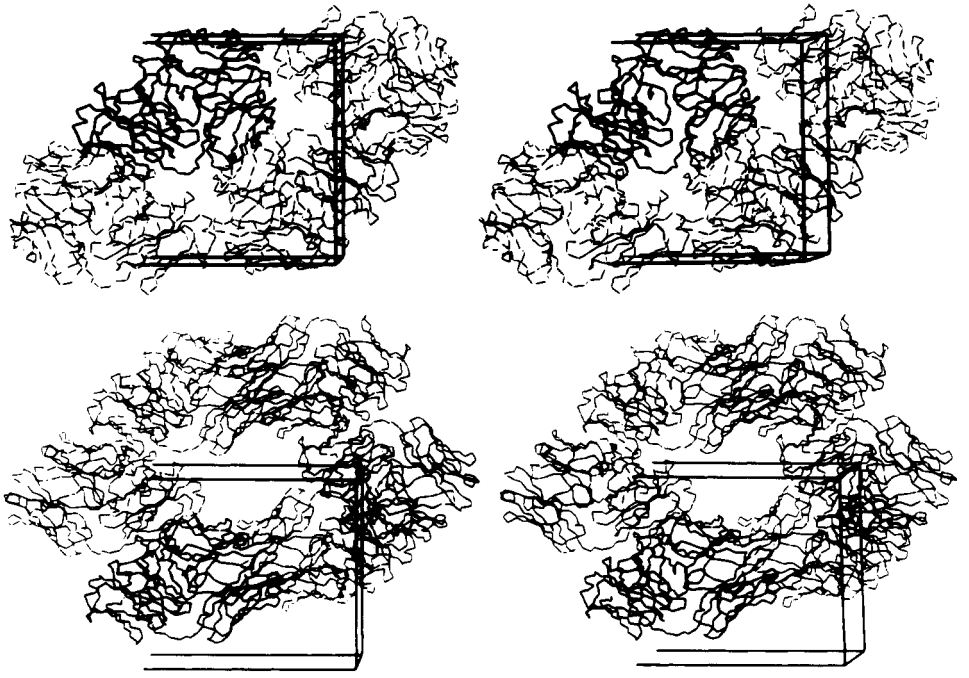

FIGURE 4: Illustration of molecular packing along the X direction (horizontal). The Y direction is vertical. Loc W is on top; Loc AS is on the bottom. In Loc W, there are contacts between the variable and constant domains along the X direction. In Loc AS, the contacts along the X direction are between neighboring variable domains, as was also illustrated in Figure 3. Though the crystals of Loc AS have a channel parallel to the Z direction, no small molecule can bind in this region because the crystal contacts are between hypervariable regions of the variable domains. In contrast, the hapten di-DNP-lysine was bound in the binding pocket of Loc W crystals.

interactions of 10^6–10^7 M^{-1} (Bigelow et al., 1974) observed for Fab fragments.

While variations in the elbow bend that might be a function of crystal packing have been observed (Matsushima et al., 1978; Sheriff et al., 1987; Prasad et al., 1988), the change that is required to convert the V–V domain interaction in Loc W

into the one found in Loc AS is so large that it appears unlikely to be the result of crystal packing forces. There are also indications that the structures differ in solution. The CD spectra in the aromatic region observed in solution vary in both Mcg and Loc, depending on the solvent (Firca et al., 1978; Ainsworth et al., unpublished observations), suggesting that the environment of the aromatic residues changes as a function of the solvent. Both of the observed conformations of the Loc light-chain dimers are quaternary structures that are physically attainable by this molecule. Rather than choosing which of the two structures presented by Loc is the physiologically relevant conformation, we have suggested that both are relevant (Stevens et al., 1988).

As noted above, it is possible that self-association properties of immunoglobulin light chains are, in some sense, fortuitous, and it is appropriate to be cautious in attributing observed characteristics of light-chain dimers to the corresponding antibody structure. On the basis of several crystallographic studies of isolated Fabs, the interactions between V domains have come to be considered essentially fixed (Novotny & Haber, 1985). However, several recent results (Roux et al., 1987; Colman et al., 1987; Gibson et al., 1988) indirectly suggest that multiple quaternary structures are also possible for Fabs. In this context, the dual crystallographic observations of the Loc protein indicate a mechanism for Fab conformational variability resulting from alterations of V domain interactions. Changes in relative V_L and V_H positions can lead to alterations in the conformation of the antigen binding site and, hence, specificity of the Fab, as well as a modification of idiotopes presented along the V_L–V_H interface (Stevens et al., 1988; Colman, 1988).

Relevance of the Two Loc Structures to Other Crystallographic Studies. The demonstration of two substantially different quaternary structures for the same protein raises several questions. Is each structure representative of the conformation attained in the individual solvents of crystallization? Do the solvent conditions differentially "select" conformational subpopulations on the basis of solubility? Do different packing "forces" that result from different near-neighbor interactions "trap" molecules of certain conformations? Of the single subunit proteins, structures of lysozyme (Phillips, 1967; Moult et al., 1976; Berthou et al., 1983) and bovine pancreatic trypsin inhibitor (Deisenhofer & Steigemann, 1975; Wlodawer et al., 1987) have been determined in crystals grown from different solvents. The basic tertiary structure in these cases was unaltered; however, change was observed in some of the surface loops and individual surface residues involved in the different lattice interactions. Hemoglobin is perhaps the multiple subunit protein most thoroughly studied crystallographically. Whereas the oxygenation state of the molecule leads to a crystallographically demonstrated change in the subunit interaction, solvent-dependent changes were not observed for methemoglobin and deoxyhemoglobin crystallized in high- and low-salt media (Perutz, 1946; Perutz et al., 1987). On the other hand, hemoglobin in an intermediate oxidation state has been "trapped" by lattice forces (Brzozowski et al., 1984). Trapping by crystal-packing interactions has been exploited in the study of conformational intermediates of enzymes such as hexokinase (Wilkinson & Rose, 1981) and aspartate aminotransferase (Arnone et al., 1982).

Our studies further underline the usefulness (Bennett & Huber, 1984) of studying proteins in different crystalline environments. Although apparent allosteric changes in the V–V interactions of antibodies have not been found, our studies of Loc crystallized from two standard crystallization solvents [described in Gilliland and Davies (1984)] showed the possibility of an equilibrium between at least two conformations. Interfacial interactions in a multisubunit protein provide a degree of freedom in which adjustments that minimize the free energy in a crystal system might be effected. Therefore, systematic analysis of multiple structures attained under various crystallization conditions might contribute significant new insight into the molecular mechanisms of protein–protein interactions.

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Molecular Cloning of the Human Casein Kinase II α Subunit[†,‡]

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ABSTRACT: A human cDNA encoding the α subunit of casein kinase II and a partial cDNA encoding the rat homologue were isolated by using a *Drosophila* casein kinase II cDNA probe. The 2.2-kb human cDNA contains a 1.2-kb open reading frame, 150 nucleotides of 5′ leader, and 850 nucleotides of 3′ noncoding region. Except for the first 7 deduced amino acids that are missing in the rat cDNA, the 328 amino acids beginning with the amino terminus are identical between human and rat. The *Drosophila* enzyme sequence is 90% identical with the human casein kinase II sequence, and there is only a single amino acid difference between the published partial bovine sequence and the human sequence. In addition, the C-terminus of the human cDNA has an extra 53 amino acids not present in *Drosophila*. Northern analysis of rat and human RNA showed predominant bands of 5.5, 3.1, and 1.8 kb. In rat tissues, brain and spleen had the highest levels of casein kinase II α subunit specific RNA, while skeletal muscle showed the lowest. Southern analysis of human cultured cell and tissue genomic DNA using the full-length cDNA probe revealed two bands with restriction enzymes that have no recognition sites within the cDNA and three to six bands with enzymes having single internal sites. These results are consistent with the possibility that two genes encode the α subunits.

Casein kinase II is a serine/threonine kinase that phosphorylates acidic proteins such as casein or phosvitin. The subunit structure is that of an a_2/b_2 or a-a′/β_2 tetramer, with the α subunit (M_r 40 000) possessing catalytic activity, and the β subunit (M_r 25 000) being autophosphorylated in vitro [for reviews, see Hathaway and Traugh (1982) and Edelman et al. (1987)]. In eucaryotic cells the enzyme is distributed predominantly in the cytosol but also has been found in mitochondria (Damuni & Reed, 1988), nuclei (Hathaway & Traugh, 1983), and coated pits (Bar-Zvi & Branton, 1986). Many enzymes have been identified as potential physiological substrates for casein kinase II, including glycogen synthetase, topoisomerase II, the R subunit of cAMP-dependent protein kinase, RNA polymerase II, acetyl-CoA carboxylase, and the B light chain of clathrin (Hathaway & Traugh, 1982; Edelman et al., 1987; Zandomeni et al., 1986; Haystead et al., 1988; Bar-Zvi & Branton, 1986). The possible role of this enzyme in RNA, DNA, and protein metabolic pathways has been

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