5/19/95
86651

ANL/CMB/PP--86651

Three Quaternary Structures for a Single Protein

M. Schiffer, D.-B. Huang, C. F. Ainsworth, and F. J. Stevens

Center for Mechanistic Biology and Biotechnology
Argonne National Laboratory
Argonne, IL 60439-4833

The submitted manuscript has been authored
by a contractor of the U. S. Government
under contract No. W-31-109-ENG-38.
Accordingly, the U. S. Government retains a
nonexclusive, royalty-free license to publish
or reproduce the published form of this
contribution, or allow others to do so, for
U. S. Government purposes.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

For the first time, the structure of a multi-subunit protein (immunoglobulin light chain) was solved in three crystal forms, differing only in the pH and ionic strength of the solvent of crystallization. The three resulting "snapshots" of possible structures show that their variable-domain interactions differ, reflecting their stabilities in specific solvent conditions. The critical residues involved in the observed solvent response are tryptophans and histidines located between the two variable domains in the dimeric structure. Tryptophan residues are commonly found in interfaces between proteins and their subunits, and histidines have been implicated in pH-dependent conformation changes.

We have now determined the structure of the λ_1 type human light-chain dimer, Loc, in a third crystal form and have completed the refinement of the structures of the two crystal forms previously described.^{1,2} The immunoglobulin light chain consists of two domains, the N-terminal variable (V) domain and the C-terminal constant (C) domain. The two domains are connected by a flexible "switch" peptide. Light-chain dimers form by homologous association of the V and C domains of the two chains. The angle between the local twofold axes of the V domain dimer and the C domain dimer defines the "elbow" bend. The only unusual substitution in the Loc protein compared to previously studied light-chain or antigen-binding fragment (Fab) structures is the replacement of glutamine at position 38 by a histidine residue. Over 90% of light chains have Gln at framework position 38; the next most common residue at that position is His.³ In light-chain dimer and Fab structures studied previously, Gln38 (or its equivalent, Gln39, in the heavy chain) forms hydrogen bonds across the dimer interface with the equivalent Gln from the other chain.

The three crystal forms we studied are: LocAS,¹ grown from 1.8 M $(\text{NH}_4)_2\text{SO}_4$; LocW,

obtained by dialysis against distilled water² and LocNaKS grown from NaKSO₄ at ~pH 8. The unit cell and refinement parameters are listed in Table 1. Except for some variation in the complementarity-determining regions (CDRs) and other loops, the conformations of the domains, based on the comparison of α -carbon coordinates, are the same in the three structures (average RMS deviation of 0.6 Å). Therefore, the intra-domain arrangement of β -sheets and the curvature of the V-domain faces do not change. The molecular geometries are summarized in Table 2. The interactions between the C domains are essentially the same in all three crystal forms. The elbow bend is similar for the two high-salt forms, approximately 97°, and differs from the low-salt form which has an elbow bend of 120°. The main difference between the three forms occurs in the phi-psi angles of the switch peptides in the elbow bend, essentially localized to five residues. In one of the chains, monomer 2, the V and C domains are farther from each other than in monomer 1. In all three dimers, monomer 1 has the ball-and-socket joint as previously described for heavy chains in Fab fragments,⁴ except that Ser112 is replaced by Leu and Phe149 by Tyr in the Loc light chain.

The V-domain interactions are different in the three forms; the differences in V-domain arrangements are illustrated in Fig. 1. The two V-domains are related by a local twofold axis in LocW, but by local twofold screw axes with 1 Å and 3.5 Å screw components in LocNaKS and LocAs, respectively. In both high-salt forms, the V domain of monomer 2 is translated forward, away from the C domain, along the local twofold screw axis relative to the other V domain. Further, in the two high-salt forms, Trp91 residues from both monomers are close together at the interface, while in the low-salt form, the two V domains form a more "open" conformation in which the Trp91s are removed from each other. The arrangement of the V domains of LocW is most similar, though not identical, to that

which was observed for Mcg, another λ light-chain dimer crystallized from ammonium sulfate.⁵ In both structures, the V domains are related by local twofold axes and the side chains of residues 91 are not in contact with each other. The Mcg dimer structure is closely related to the Fab structures for which the interaction of the two V domains was described as an eight-stranded barrel that has a "three-layer packing."⁶ In the high-salt forms, especially in LocAS, the barrel formed by the two V domains is distorted. Its size is smaller, and it is asymmetric because of the screw axis relationship between the two domains, which permits two of the strands of monomer 1 to get closer to the concave surface of monomer 2. In the two high-salt crystal forms, the buried surface area is larger than in the low ionic strength form, both between the two V domains and also between the V and C domains as a result of the smaller elbow bend. The motion of the V domains relative to each other can be considered as a shear mechanism,⁷ while the relative positions of the V and C domains are reflected by the elbow bend, which is changed by a concerted hinge motion of the two chains.

On the basis of the first two crystal forms, LocAS and LocW, we speculated that the V-domain interactions were driven by the high-salt conditions, which could favor the hydrophobic interactions between Trp91 residues from both chains. Indeed, in the second high-salt form (LocNaKS), the two tryptophans have the same type of geometry. What we discovered by solving the structure of the third crystal form is that in addition to the difference in ionic strength, the pH at which the crystals were grown also contributed to these quaternary structures achieved by protein Loc. The pH of LocAS was less than 6, while the pH of LocW was adjusted to neutral pH with $\text{Ca}(\text{OH})_2$. The pH of crystallization presumably influences the ionization state of histidine 38 residue because the pK_a of histidine residues in proteins is between 6.5 and 7.0. Indeed, in crystals grown above pH 7, both at low ionic

strength (LocW) and at high ionic strength (LocNaKS), His38 is involved in hydrogen-bonding interactions with Tyr87 of the other monomer. His38 is not expected to be ionized in LocAS and is not involved in any hydrogen-bonding interactions. Hydrogen bonds involving residue 38 for the three Loc structures and the Mcg protein are shown schematically in Fig. 2. The ionization of the histidine and the resulting change of its hydrogen bonding potential appear to trigger the rearrangement of the interactions of the V-domains that leads to the LocW crystal form at low ionic strength and to the LocNaKS crystal form at high ionic strength. A pH "trigger" has been suggested to be involved in determining two crystal forms of BPTI.⁸ Major rearrangements caused by pH change have been observed in several systems.^{9,10} These rearrangements might have been affected by the change of ionization state of His residues, since there are His residues in or close to the segments where the rearrangement occurs. Apparent changes in pK_a of His residues are also implicated in several allosteric proteins reviewed by Perutz.¹¹

In the Loc V domain interface, there appears to be two groups, His38 and Trp91 at the two ends of the interface, that influence the packing of the domains in the different crystallization conditions. Despite differences in packing, in all three Loc dimers, a series of aromatic residues forms a herringbone pattern in the middle of the variable domain interface as has been described in Fab.¹² Trp91 and His38 are above and below these residues along the local twofold axis. In other multisubunit proteins, Trp and His residues, together with Phe and Tyr, occur as frequently in the subunit or domain interfaces as in their interiors.^{13,14} High ionic strength favors hydrophobic interactions and, therefore, the maximization of buried surface area that we observe in LocAS and LocNaKS relative to LocW. The resulting juxtaposition of the indole rings, sequestered from solvent, optimizes the electrostatic

interactions¹⁵ between Trp 91 residues of the two domains.

The possibility of the functional importance of relative movement¹⁶ of the two variable domains was observed crystallographically in comparisons of Fab structures with or without bound antigen.^{17,18} The contribution of isomerism to the dynamics of antibody interactions was confirmed recently by Foote and Milstein.¹⁹ Though it has not yet been observed for Fabs, multiple relative positions of V domains should be possible¹⁶ and may provide an allosteric contribution to antibody diversity. For the Loc molecule, at least three forms from the available repertoire can be crystallized. The conserved C-domain packing in the λ light chain crystal facilitates their crystallization regardless of the V-domain packing.²⁰ Therefore, these dual domain molecules, in both native and recombinant forms, offer a versatile system for study of fundamental structural and solvent contributions to the quaternary organization of proteins in general.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

References

1. Chang, C.-H., Short, M. T., Westholm, F. A., Stevens, F. J., Wang, B.-C., Furey, Jr., W., Solomon, A. & Schiffer, M. *Biochemistry* **24**, 4890–4897 (1985).
2. Schiffer, M., Ainsworth, C., Xu, Z.-B., Carperos, W., Olsen, K., Solomon, A., Stevens, F. J. & Chang, C.-H. *Biochemistry* **28**, 4066–4072 (1989).
3. Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S. & Foeller, C., Eds. In *Sequences of Proteins of Immunological Interest*, 5th ed., National Institutes of Health, Bethesda, MD (1991).
4. Lesk, A. M. & Chothia, C. *Nature* **335**, 188–190 (1988).
5. Schiffer, M., Girling, R. L., Ely K. R. & Edmundson, A. B. *Biochemistry* **12**, 4620–4631 (1973).
6. Chothia, C., Novotný, J., Bruccoleri, R. & Karplus, M. *J. Mol. Biol.* **186**, 651–663 (1985).
7. Gerstein, M., Lesk, A. M. & Chothia, C. *Biochemistry* **33**, 6739–6749 (1994).
8. Gallagher, W. H. & Croker, K. M. *Protein Science* **3**, 1602–1604 (1994).
9. Bullough P. A., Hughson, F. M., Skehel, J. J. & Wiley, D. C. *Nature* **371**, 37–43 (1994).
10. Bennett, M. J., Choe, S. & Eisenberg, D. *Proc. Natl. Acad. Sci. USA* **91**, 3127–3131 (1994).
11. Perutz, M. F. *Quarterly Reviews of Biophysics* **22**, 139–236 (1989).
12. Novotný, J. & Haber, E. *Proc. Natl. Acad. Sci.* **82**, 4592–4596 (1985).
13. Argos, P. *Protein Eng.* **2**, 101–113 (1988).

14. Janin, J., Miller, S. & Chothia, C. *J. Mol. Biol.* **204**, 155–164 (1988).
15. Burley, S. K. & Petsko, G. A. In *Advances in Protein Chemistry*, Vol. 39, Anfinsen, C. B., Edsall, J. T., Richards, F. M. & Eisenberg, D. S. (Eds.), pp. 125–189, Academic Press, Inc. (1988).
16. Stevens, F. J., Chang, C.-H. & Schiffer, M. *Proc. Natl. Acad. Sci. USA* **85**, 6895–6899 (1988).
17. Bhat, T. N., Bentley, G. A., Fischmann, T. O., Boulot, G. & Poljak, R. J. *Nature* **347**, 483–485 (1990).
18. Stanfield, R. L., Takimoto-Kamimura, M., Rini, J. M., Profy, A. T. & Wilson, I. A. *Structure* **1**, 83–93 (1993).
19. Foote, J. & Milstein, C. *Proc. Natl. Acad. Sci. USA* **91**, 10370–10374 (1994).
20. Schiffer, M., Chang, C.-H. & Stevens, F. J. *J. Mol. Biol.* **186**, 475–478 (1985).

Acknowledgments

This work is supported by the U.S. Department of Energy, Office of Health and Environmental Research, and by the U.S. Public Health Service. This research was supported in part by a grant from the Pittsburgh Supercomputing Center through the NIH National Center for Research Resources cooperative agreement. We thank G. Johnson for the structural figure and Dr. D. K. Hanson for critically reading the manuscript.

Table 1. Structural and Refinement Data for the Three Crystal Forms of Protein Loc

	LocAS ^a	LocW ^b	LocNaKS ^c
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
a (Å)	149.3	118.92	83.8
b	72.4	73.55	72.62
c	46.5	49.83	63.56
Volume fraction of protein	0.45	0.52	0.58
<hr/>			
Resolution (Å)	2.3	2.4	2.2
No. of reflections (>3σF)	14539	10327	14084
No. of waters	364 ^d	265	299 ^d
R-factor (%)	16.5	15.5	15.8
<hr/>			
<u>Deviations in:</u>			
Bond length (Å)	0.016	0.016	0.017
Angle-related distance (Å)	0.044	0.043	0.044
Deviation from plane (Å ²)	0.013	0.013	0.014
Chiral volume (Å ³)	0.190	0.179	0.193

^aCrystallized from 1.4M (NH₄)₂SO₄.

^bCrystallized from distilled water.

^cCrystallized from 0.95M Na₂SO₄, 0.4M K₂SO₄, and 0.04M Na₃PO₄.

^dNo sulfate ions found.

Table 2. Description of the Structures Relation of Variable Domains

	LocAS	LocW	LocNaKS
Elbow bend (°)	96.7	119.3	97.6
Rotational sym (°) V domains	179.9	180.0	179.8
Translation of V domains (Å)	3.5	0	1.0
Buried surface (Å ³) between V domains	1527	1212	1665
Number of H-bonds between V domains	1	2	4
Trp contacts	Yes	No	Yes

FIGURE LEGENDS

- Figure 1. Superposition of chain 1 variable domains of the three structures illustrates the differences in the relative positions of the domains. The three crystal forms are LocAS (white), LocNaKS (blue), and LocW (red). The figure was made using the program SETOR.
- Figure 2. Schematic illustration of the hydrogen bonds formed by residue 38 in LocW, LocAS, LocW, and for comparison, in the Mcg crystal. Additional hydrogen bonds found in the interface of Loc crystals are also shown. Three hundred was added to the residue numbers of chain 2.

Figure 1

Mcg

Loc W

Loc AS

Loc NaKS

Figure 2