Comparison of Crystal Structures of Two Homologous Proteins: Structural Origin of Altered Domain Interactions in Immunoglobulin Light-Chain Dimers[†,‡]

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ABSTRACT: The sequence and structure of a second human κ_I immunoglobulin light-chain variable domain, Wat, has been determined. The *R*-factor is 15.7% for 1.9-Å data. One hundred and ninety-five water molecules were identified; 30 water molecules were located in identical positions in each of the monomers. Some of the water molecules are integral parts of the domains. This light chain is encoded by the same variable domain gene that encoded the previously characterized κ_I variable domain, Rei. Due to limited somatic mutation, the two highly homologous proteins differ in only 20 of the 108 residues. Wat crystallized in space group $P6_4$ while Rei crystallized in space group $P6_1$; in both crystals, the asymmetric unit was the noncovalent dimer. Although the basic domain structure is the same for both proteins, the relative positions of the domains within the two dimers differ. This difference is most likely accounted for by the replacement of Tyr36 in Rei by Phe in the Wat protein. Residue Tyr36 is part of the hydrogen-bonding network in the interface between the domains in Rei. Losing the hydrogen-bonding capability of residue 36 by replacement of Tyr by Phe alters the network of hydrogen bonds between the domains, resulting in a different domain−domain contact. The details of lattice contacts in the two crystals were compared. One type of contact that extends the β-sheet of the individual domains was conserved, but because it involved different symmetry elements within the crystal, different crystal packing resulted. In the Wat crystal, one of the contacts shows an example of how a symmetrical binding site can "bind" an asymmetrical object. Further, the examination of the Wat crystal also illustrates how the different crystalline environments of the domains of the dimer results in different distributions of temperature factors for the residues within the domains.

Human and murine immunoglobulin light-chain dimers have been used to further the understanding of mechanisms and diversity of Fab assembly. The variable (V_L)[1] and constant (C_L) domains that comprise light chains represent prototypical domains of the immunoglobulin superfamily; therefore, studying their structures and interactions increases the database for this important and diverse family of molecules. Based on amino acid sequences, several of the V-domain-related members of the immunoglobulin superfamily are most homologous to κ-type light-chain variable domains (Mostov et al., 1984; Ryu et al., 1990; Wang et al., 1990; Leahy et al., 1992). As a result, the structure of the κ variable domain Rei (Epp et al., 1975), the only human κ V-domain available to date, has been used to model the superfamily members.

A relatively large latitude has been found in the quaternary interaction (Schiffer et al., 1973; Furey et al., 1983; Chang et al., 1985; Schiffer et al., 1989) and affinities (Maeda et al., 1978; Stevens et al., 1980; Kolmar et al., 1994) of antibody light-chain variable domains. Diversity of domain interaction arises from amino acid substitutions that occur in both the heterogeneous complementarity determining regions (CDRs) as well as the more highly conserved framework regions (FRs). Several thousand naturally-occurring light-chain variable domain sequences have been archived (see Kabat et al., 1991), representing a large database of structurally compatible residue combinations that can occur in the interface. The contribution of individual residues can be tested by protein engineering. Some of these substitutions alter the affinity and kinetics of self-association and others also lead to changes in observed domain positions. These features, diversity of available primary structures, and correlated variations in thermodynamic and conformational

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[‡] The coordinates of the crystal structure of Wat have been deposited into the Brookhaven Data Bank (ident code 1WTL).

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[1] Abbreviations: V, variable; C, constant; V_L, variable domain of light chain; CDR, complementarity-determining region; Fab, antigen binding fragment.

properties suggest that naturally-occurring and recombinant light chains may serve as a good system for studying basic rules of domain−domain interactions.

Quaternary interactions of proteins are affected by many factors: complementarity of the surfaces in shape, hydrophobicity, hydrogen bonding, and charge−charge interaction potential. It is difficult to predict the results when one of the factors is changed and protein domains realign themselves to maximize the interactions between their surfaces. The rearrangement of the domains depends on what residue was changed and also on the other residues in the interface and on the solvent. Because the contributions of a single amino acid substitution are effectively doubled in the formation of a dimer, the modification has an enhanced effect since it occurs on both sides of the interface.

In this report, we describe the structure of a second human κ_I protein. We compare the structures of these homologous dimers and show the consequences of changing a tyrosine to phenylalanine—usually considered a conservative substitution—on the hydrogen-bonding network and the quaternary interactions. Because of the degree of sequence homology and the similarity of crystallization conditions, this is a well-defined system with which to analyze contributions of various amino acid substitutions to the conformation of the domains, to modes of dimer formation, to crystal lattice interactions, and to the relationship between crystal lattice packing and the observed interactions of domains.

MATERIALS AND METHODS

Protein Preparation. Bence Jones protein Wat was obtained from the urine of a patient who had multiple myeloma. Preliminary purification by Pevikon block electrophoresis and immunodiffusion identification of protein Wat as a κ_I light chain have been described previously (Stevens et al., 1981). The urinary Wat protein consisted of a mixture of intact light chains and V_L fragments; the Wat V_L domain was purified by gel filtration on a Sephadex G75SF column in a buffer consisting of 0.3 M NaCl, 0.01 M Tris, pH 6.8. The sample contained equal amounts of complete light chain and V_L fragments.

Amino acid sequence analysis was carried out as described by Eulitz et al. (1991).

Crystallographic Analysis. Crystals of the protein were obtained in 2−4 weeks by slowly concentrating the protein in a Zeppenzauer tube by dialysis against a solution of 20% dextran (73 000 molecular weight) in distilled water. For preservation, the crystals were transferred into a solution of 1.5 M $MgSO_4$ and 10% dextran (73 000). The crystals were hexagonal bipyramids that have been previously described (Stevens et al., 1981). Data were collected on a Siemens multiwire area detector at 4 °C. At 4 °C, the unit cell dimensions were $a = b = 82.25$ Å and $c = 61.20$ Å. Similar crystals, grown previously from 1.4 M ammonium sulfate at pH 6.5 and from 8% polyethylene glycol, had unit cell dimensions of $a = b = 82.6$ Å and $c = 60.3$ Å at room temperature and space group $P6_2$ or $P6_4$ (Stevens et al., 1981). Diffraction data were reduced using the XENGEN software package (Howard et al., 1987). The $R_{merge} = (\Sigma\langle F\rangle - F)/\Sigma F$ was 3.4%.

The structure was determined by molecular replacement using PC-refinement (Brünger, 1990) as implemented in X-PLOR, version 3.1 (Brünger, 1992). The Rei variable domain monomer and the dimer were used as search models with B-factors set to a uniform value (15 Å^2). A self-rotation function using the real-space method of Steigemann (Steigemann, 1974) as implemented in X-PLOR indicated a noncrystallographic 2-fold relationship between two monomers. A cross-rotation function using the monomer and the dimer did not produce significant solutions. The top two peaks of the monomer rotation function were not related by the noncrystallographic symmetry relationship seen in the self-rotation function.

Table 1: Amino Acid Sequence Differences between Proteins Wat and Rei

	res no.	res location	Rei	Wat
CDR1	24		Gln	Arg[a]
	30		Ile	Thr
	31		Lys	Asn
	33	internal	Leu	Val
	36	interdomain	Tyr	Phe
	39		Thr	Arg
	42		Lys	Gln
	46	interdomain	Leu	Val
CDR2	50		Glu	Gly
	53		Asn[a]	Ile
	55		Gln[a]	Glu
	56		Ala	Thr
	71	internal	Tyr	Phe
CDR3	92		Gln	Asp[a]
	93		Ser	Thr
	96	interdomain	Tyr	Leu
	100		Gln	Gly
	104	internal	Leu	Val
	105		Gln	Asp
	107		Thr[a]	Lys[a]

[a] Crystal contact.

PC refinement of the highest 6000 grid points of the dimer rotation function was carried out using the Rei dimer as the search model. The orientations and positions of the Rei dimer were refined with 15−4-Å resolution data. Two solutions emerged (7th and 41st highest grid points, respectively) that were significantly above background and that were related by the noncrystallographic symmetry. PC refinement modified the interdomain angles between the two monomers up to 9°.

A translation search of the PC-refined Rei dimer using the method of Fujinaga and Read (1987) as implemented in X-PLOR identified the $P6_4$ enantiomer as the correct one. The solution was 10σ above the mean and 1σ above error peaks. The orientation and position of both monomers were subsequently refined by rigid-body refinement, resulting in an R value of 37% at 8−3-Å resolution.

On the basis of a $(2F_o - F_c)$ Fourier series, the residues that differ between Wat and Rei (Table 1) were replaced using the program FRODO (Jones, 1978) on an Evans and Sutherland PS300 system. The structure was first refined using simulated annealing (Brünger, 1988) with 2.5-Å data; subsequently, the data were extended to 1.9 Å. Manual adjustments were made based on $(2F_o - F_c)$ difference maps. After several cycles of manual adjustments and refinements with PROLSQ (Hendrickson, 1985), the R-factor dropped from 28 to 23% with an overall temperature factor. After introducing individual temperature factors, the R-factor was 21% for 10−1.9-Å data. Water molecules were located in the $(2F_o - F_c)$ and $(F_o - F_c)$ maps. Electron density was accepted as a water molecule if it was within 3.5 Å of a likely hydrogen-bonding partner on the protein or another independently identified water molecule. First the occupancy

Table 2: Summary of Restrained Least-Squares Refinement Parameters in the Crystal Structure of Wat

	σ values	
	model	target
diffraction data		$0.52\langle\|\|F_o\| - \|F_c\|\|\rangle$
bonding distances (Å)		
bond length	0.017	0.020
angle-related distance	0.042	0.030
intraplanar (1−4) distance	0.050	0.050
deviation from plane (Å)	0.014	0.020
chiral volume (Å^3)	0.177	0.150
nonbonded contacts (Å)		
single torsion contact	0.198	0.300
multiple torsion contact	0.236	0.300
possible hydrogen bond	0.209	0.300
conformation torsion angle restraint (deg)		
planar (ω)	2.7	3.0
staggered	23.5	13.0
orthonormal	18.1	20.0
thermal factors (Å^2)		
main-chain bond	0.74	1.00
main-chain angle	1.29	1.50
side-chain bond	1.18	1.50
side-chain angle	1.80	2.00

Table 3: Results of Restrained Least-Squares Refinement

resolution (Å)	no. of observed reflections	percent of total[a]	*R* (shell)
10.0−4.3	1452	96	0.129
4.3−3.3	1842	95	0.122
3.3−2.9	1466	88	0.162
2.9−2.6	1529	78	0.189
2.6−2.4	1230	66	0.200
2.4−2.2	1428	60	0.209
2.2−1.9	1373	46	0.219
10.0−1.9	10320	72	0.157

[a] With F_o greater than $3\sigma(F_o)$.

and then the temperature factor of the waters were refined. The final occupancies of the waters ranged from 0.35 to 1.0, and their individual temperature factors varied from 16 to 47. A total of 195 water molecules were located; 179 are within 3.5 Å from a protein atom or another water molecule. Fifty-two water molecules are in contact with atoms of monomer 1; 72 are in contact with monomer 2. Thirty of these water molecules are located at identical sites in both monomers. Of the 52 waters near monomer 1, 12 also bind or form a bridge to monomer 2; out of the waters near monomer 2, three also bind to monomer 1. Sixteen waters are within 3.5−4 Å of a protein or water atom.

When the waters were included in the phase calculations, the electron density map revealed positions for all the side chains, including the terminal residues of the chains. For the refinement, reflections with $F > 3\sigma$ were used. The *R*-factor for 10 320 reflections between 10- and 1.9-Å resolution was 15.7%. The mean positional error based on Luzzatti plots (Luzzatti, 1952) was 0.2 Å. All but five of the residues fell in the allowed region of the Ramachandran plot; these residues were 51 and 30 of both monomers and residue 84 of monomer 2. The results of the refinement are shown in Tables 2 and 3. The coordinates have been deposited into the Brookhaven Data Bank (ident code 1WTL).

RESULTS

Amino Acid Sequence. The amino acid sequence of κ_1 protein Wat variable domain is shown in Figure 1. Protein

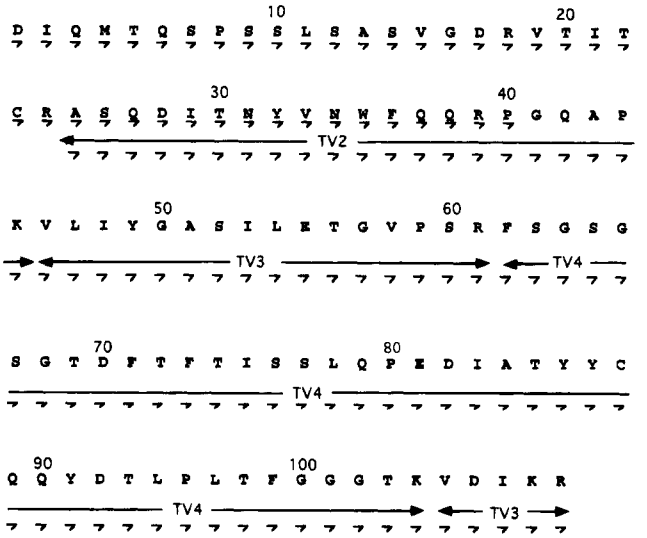

FIGURE 1: Amino acid sequence of the V-region of the protein Wat: the amino acid sequence is given in one-letter code. Tryptic peptides were indicated by bars and designated as TV2, TV3, etc. Half arrows indicate that the amino acids in these positions had been identified by either stepwise automatic degradation of the whole protein or the corresponding tryptic peptide.

Wat is derived from the same germ line V-exon (018−08) as κ_1 type protein Rei (Klein et al., 1993) but is rearranged with a different J-exon. As a result of somatic mutation and the alternative J segments, Wat and Rei (Palm & Hilschmann, 1973) differ in 20 out of 108 residues (see Table 1); 11 of these differences are in the complementarity-determining regions (CDRs). Three of the changes from Rei to Wat involve hydrophobic residues whose side chains are positioned within the core of the domains: 33 Leu → Val, 71 Tyr → Phe, and 104 Leu → Val. An additional three residues that differ between the two proteins are at the variable domain−domain interface: 36 Tyr → Phe, 46 Leu → Val, and 96 Tyr → Leu.

Description of Domain Structure. As previously described for other immunoglobulins, the Wat variable domain has a β-barrel or β-sandwich structure. One sheet consists of four antiparallel stands that form regular hydrogen bonds (see Table 4); the other sheet consists of three regular antiparallel strands and two additional shorter stands that are irregular. These latter two strands are connected by the CDR2 segment. There are also hydrogen bonds that involve the main chain between loops that include residues 29, 30, (from CDR1), and 68. The hydrogen bond between the carbonyl of residue 30 and the nitrogen of residue 68 might account for the unusual φ and ψ angles of residues 30 of both monomers in Wat (56°, −116°; 60°, −108°) and in Rei (79°, −78°; 72°, −67°). The first and last strands of each domain run parallel to each other; they form a parallel β-pleated sheet structure at one end (residues 9−13 and 102−107) and are connected or bridged by a string of four or five water molecules that are part of the domain structure at the other end (residues 2−6 and 95−101) (see Figure 2). These waters form hydrogen bonds with the main-chain atoms of the chain segments. In addition, the carbonyl oxygens of residues 93 and 95, and the side chain of residue Gln90 also coordinate to the most N-terminal of these water molecules.

Polar residues that point into the hydrophobic interior of the domain have their hydrogen bonding capabilities satisfied either by hydrogen bonding to each other, to the main chain, or to water molecules (see Table 5) Conserved residues Gln6

Table 4: Intramolecular Hydrogen Bonds in Wat and Rei (d < 3.5 Å)[a]

main chain to main chain		Rei		Wat		main chain to main chain		Rei		Wat	
N−H	O=C	chain 1	chain 2	chain 1	chain 2	N−H	O=C	chain 1	chain 2	chain 1	chain 2
26	3	3.27	3.23	2.74	2.97	89	34	2.89	2.91	3.11	2.80
5	24	2.86	3.06	2.74	2.64	35	48	2.98	2.94	2.50	2.73
24	5	2.94	3.04	2.97	2.96	47	35	3.01	2.81	2.93	2.82
7	22	3.10	3.00	3.10	3.22	48	35			3.35	3.24
22	7	2.75	2.77	2.94	3.00	36	87	2.61	2.61	2.57	2.85
103	9	3.36	3.22	2.93	3.06	87	36	2.91	2.77	2.95	2.77
11	103	3.14	3.00	3.25	2.91	37	45	2.89	2.92	2.69	2.97
105	11	3.02	3.01	3.02	2.86	45	37	2.84	2.80	2.60	2.86
13	105	3.33	3.26	2.38	3.00	38	85	2.87	2.74	2.91	2.85
107	13	2.53	3.48	3.07	2.83	85	38	2.95	3.07	2.89	3.26
17	14	2.97	3.08	2.99	3.28	42	39	3.24	3.19	3.03	2.92
16	78	2.90	2.90	2.96	2.81	55	47	3.33	3.32	2.99	2.86
78	17	3.25	3.20	2.52	2.93	49	53	2.95	3.04	2.94	3.01
19	75	3.24	3.07	2.71	2.90	52	49			3.35	
75	19	3.07	2.98	2.84	2.66	53	49	3.04	2.89	2.90	2.92
21	73	3.02	3.01	3.19	2.80	58	55	3.41		2.99	2.96
73	21	2.90	2.84	2.82	2.81	76	61	2.80	2.93	2.59	2.86
23	71	2.90	2.93	2.82	2.66	63	74	3.38	2.92	2.68	3.11
71	23	2.90	2.84	2.81	2.76	74	63	3.13	2.87	2.69	2.86
25	69	2.80	2.87	2.63	2.71	65	72	3.08	3.17	2.84	2.93
29	68	3.29	3.14	2.82	2.82	72	65	2.84	2.99	2.56	3.06
32	29	2.98	2.89	3.05	3.09	82	79	3.26	3.23	3.12	2.78
68	30	3.19	3.46	2.56	2.58	104	84	3.05	3.10	3.20	2.86
51	31			3.15	3.25	86	102	2.91	3.04	2.97	2.97
91	32	3.29	3.22	2.89	2.85	102	86	2.91	3.07	2.82	2.96
50	33			3.47	3.33	99	88	2.75	2.83	2.72	2.64
51	33	2.77	2.70	3.13	2.82	90	97	3.31	3.37	2.95	2.80
34	89	2.76	2.87	2.80	2.79						

		Rei		Wat				Rei		Wat	
main chain	side chain	chain 1	chain 2	chain 1	chain 2	main chain	side chain	chain 1	chain 2	chain 1	chain 2
2 O	Thr97 OG1	2.85	2.71	2.68	2.80	61 O	Ser76 OG			3.44	
3 N	Ser26 OG	3.24	3.41	2.81	3.24	68 O	Tyr71 OH(R)	3.26	3.22		
3 O	Ser26 OG		3.35	3.33	3.28	74 O	Ser63 OG			3.16	
5 O	Arg24 NH1(W)			3.26		76 O	Arg18 NH1				3.31
7 O	Thr22 OG1	3.14	3.39		3.34	79 N	Asp82 OD1	3.06	3.20	2.65	2.89
8 O	Thr102 OG1	2.77	2.67	2.59	2.85	81 O	Arg39 NH1(W)			3.44	2.47
9 O	Lys103 NZ	2.59				81 O	Arg39 NH2(W)			3.33	2.86
11 O	Gln105 NE2(R)		3.20			82 O	Tyr86 OH	2.71	2.84	2.43	2.61
13 O	Thr107 OG1(R)		3.20			83 O	Arg39 NH2(W)			3.11	3.28
14 N	Asp17 OD2	2.79	3.00	3.05	2.99	86 O	Gln6 NE2			2.93	3.05
16 O	Ser77 OG		3.33	3.38		88 N	Gln6 OE1	3.41	3.35	3.42	3.49
24 O	Thr5 OG1				2.82	88 O	Gln6 NE2	2.72	2.76		
25 O	Thr69 OG1		2.55			92 N	Gln90 OE1	2.88		2.83	2.71
30 N	Asp28 OD2	2.75	2.48	3.31		93 N	Gln90 OE1			3.07	3.16
30 O	Ser67 OG				3.35	95 O	Gln90 NE2	3.02		3.17	2.79
42 O	Gln38 NE2			3.01	2.56	96 O	Gln89 NE2				3.07
50 N	Tyr91 OH			2.56	2.90	101 N	Gln6 OE1			3.11	2.98
50 O	Asn31 ND2(W)				2.81						

		Rei		Wat				Rei		Wat	
side chain	side chain	chain 1	chain 2	chain 1	chain 2	side chain	side chain	chain 1	chain 2	chain 1	chain 2
Asp1 OD1	Thr97 OG1	2.94				Asn34 ND2	Gln89 OE1			2.91	2.74
Asp1 OD2	Gln90 NE2	3.16				Tyr36 OH(R)	Gln89 NE2	3.02	3.02		
Gln3 NE2	Thr5 OG1	3.19				Gln37 NE2	Tyr86 OH	3.25	3.38	2.95	3.03
Gln6 NE2	Thr102 OG1	2.85	2.96	2.79	2.93	Arg61 NH1	Glu81 OE2				3.23
Ser7 OG	Arg24 NH1(W)			2.86		Arg61 NH1	Asp82 OD2	3.40	3.36		2.42
Ser10 OG	Gln105 NE2(R)		3.32			Arg61 NH1	Asp82 OD1				3.20
Ser12 OG	Lys107 NZ(W)			3.04		Arg61 NH2	Asp82 OD1			2.60	2.40
Ser14 OG	Thr107 OG1(R)		3.39			Arg61 NH2	Asp82 OD2	2.67	2.87	2.62	3.19
Thr20 OG1	Thr74 OG1			2.97		Arg61 NH2	Gln79 OE1		2.73		
Arg24 NH2(W)	Asp70 OD1				2.78	Gln79 NE2	Glu81 OE2			3.21	
Gln24 NE2(R)	Asp70 OD1			2.51		Gln90 NE2	Thr97 OG1	3.21	3.28	3.11	3.04
Lys31 NZ(R)	Glu50 OE1(R)	2.89	2.38								

[a] (W) or (R) represents the residue for Wat or Rei only.

and Thr102 that point inside of the domain form hydrogen bonds with each other. In addition, Gln6 forms hydrogen bonds with the backbone of residues 88 and 86; Thr102 forms a hydrogen bond with the carbonyl oxygen of residue 8. Intradomain hydrogen bonds are also formed by Thr97 with the carbonyl oxygen of residue 2, by Ser26 with the nitrogen of residue 3, and by Asp17 with the nitrogen of residue 14. Side chains of Gln37 and Tyr86 form hydrogen bonds with each other. The Tyr86 hydroxyl group also forms a hydrogen bond with the carbonyl oxygen of 82. Asp82

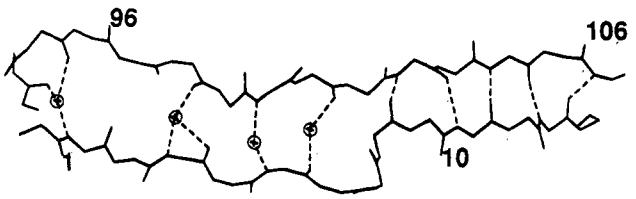

FIGURE 2: Hydrogen bonds and water molecules between the first and last strand, residues 2−13 and 95−107, of domain 1 are shown. The waters are slightly above the two chain segments.

Table 5: Hydrogen-Bonding Pattern of Internal and Conserved Polar Residues[a]

Gln6 (100)	Thr102 (97)	8 O
	88 N	
	86 O	
Trp35 (100)	Water	51 O
		65 O
Tyr86 (96)	Gln37 (96)	
	82 O	
Thr97 (92)[b]	2 O	
Asp17 (64)[c]	14 N	
Ser26 (95)	3 N	
Asp82 (99)	Arg61 (89)	
	79 N	

[a] The percentage of the special amino acids in human κ-type light chain is given in parentheses. [b] (Ser + Thr) 98% of residues at 97. [c] (Asp + Glu) 96% of residues at 17.

forms a salt bridge with Arg61; this conserved salt bridge is situated in what can be described as a depression in the outer surface of the domain. Asp82 also forms a hydrogen bond with the N of residue 79.

A buried water molecule is found between the buried side chain of Trp35 (NE1) and the carbonyl oxygens of residues 51 and 65 in both domains. This water has been observed in Rei (Epp et al., 1975), Rhe (Furey et al., 1983), and in M3 (Steipe et al., 1992) and can be considered to be an integral part of the domain.

A cluster of water molecules was observed at the framework end of the β-barrel in monomer 2 only. These waters form hydrogen bonds with several of the side-chain and main-chain atoms of residues 337, 339, 342, 343, 382, and 386 (a value of 300 was added to the residue numbers of the second monomer). Atoms of monomer 1 on the average have larger temperature factors; therefore, fewer of the water molecules bound to monomer 1 have sufficient occupancy to be identified as waters.

Comparison of the Domains in the Wat and Rei Proteins. In both Rei and Wat crystals, the two domains of the dimer

Table 6: Interdomain Hydrogen Bonds (Å)

	Wat	Rei
Gln38 OE1−Gln338 NE2	3.08	3.08
Gln38 NE2−Gln338 OE1	3.20	2.89
Gln38 NE2−Tyr387 OH	2.94	*a*
Tyr87 OH−Gln338 NE2	3.30	3.72
Gln89 OE1−Gln389 NE2	3.33	*a*
Gln89 NE2−Gln389 OE1	3.12	*a*
Tyr36 OH (Rei)−Gln389 OE1		3.19
Gln89 OE1−Tyr336 OH (Rei)		3.76
42 O−Tyr387 OH	3.48	3.42
Tyr87 OH−342 O	3.15	3.52

[a] $d > 4$ Å.

are related by local 2-fold axes. The root mean square deviation upon superimposing 107 α-carbon atoms of the Wat domains was 0.35 Å and for Rei domains was 0.36 Å. Superposition of the individual domains of Wat and Rei gives cross-comparison values of 0.51 and 0.56 Å for the α-carbon atoms, indicating that the domains from the same protein are more similar to each other than are the domains from the different proteins. The main differences between the two proteins are in the framework 2 and CDR2 sections; the number of main-chain hydrogen bonds also differs in those sections. Four out of seven residues in CDR2 differ between the two proteins, but it is not obvious how these differences correlate with the structural changes between the two molecules. Unusual ϕ and ψ angles in Wat (67°, −32°, and 70°, −32°) and Rei (61°, −50°, and 66°, −53°) were observed for residue 51 in the γ-turn of CDR2. That this unusual turn might be a conserved feature of CDR2 has been previously pointed out by Steipe et al. (1992), who found ϕ and ψ angles for residue 51 in M3 of 67° and −34°. The high-energy conformation for residue 51 was also found by Essen and Skerra (1994) in M29b (79°, −48°). The equivalent residue (52) in Rhe (Furey et al., 1983) has corresponding angles of 65° and −47°.

Comparison of the Dimer Structures in the Wat and Rei Proteins. In both the Wat and Rei structures, the asymmetric unit is a noncrystallographic dimer. The superposition of the two structures is shown in Figure 3. Though essentially the same face for each individual domain is involved in the dimerization, the modes of dimerization are different. Table 6 lists the hydrogen bonds between the domains. Because, in Wat, residue 36 of the dimer interface is Phe instead of the usually encountered Tyr, a rearranged hydrogen-bonding pattern is observed (see Figure 4). In the Rei dimer, Tyr36

FIGURE 3: Proteins Wat (thick line) and Rei (thin line) are superimposed by overlapping domains 1 of each molecule. Because of the different mode of dimerization, 11.8° and 1.3 Å would be required to superimpose the second domains.

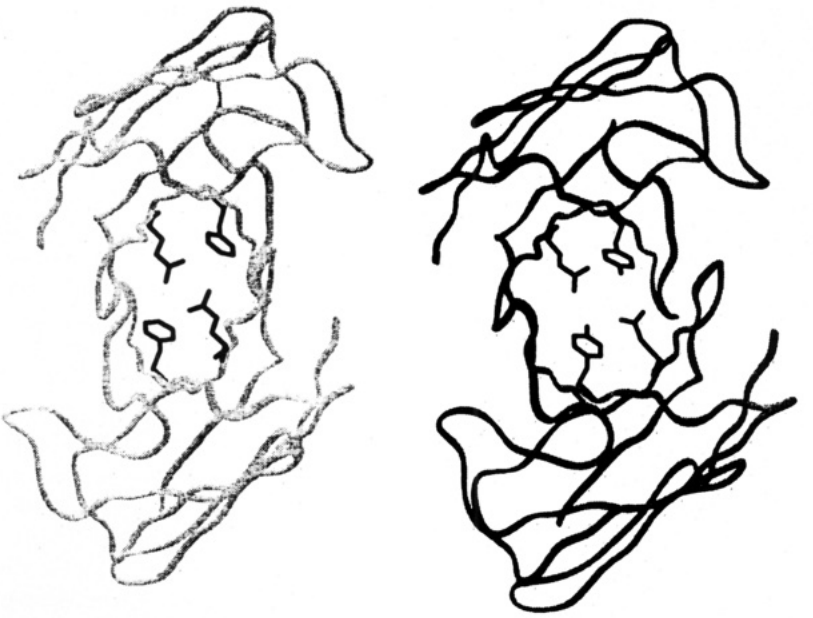

FIGURE 4: The backbones and residues 36 and 89 from both monomers of protein Wat and Rei are shown. In the Wat protein, Gln89 residues from both monomers hydrogen bond with each other. In Rei, hydrogen bonds are formed between Gln89 and Tyr36 residues from both monomers.

FIGURE 5: Conserved waters located between the two Wat domains are shown for one side. These are located between the side chain of Asn34 and N of 396, between the carbonyl oxygen of 44 and nitrogen of 398, and between the OH of Tyr87 and carbonyl of 342.

forms a hydrogen bond with Gln89 of the other monomer at the dimer interface. In the Wat protein, Phe36 cannot participate in a hydrogen bond; an apparent "sliding" of the domains brings the two Gln89 residues into position to form two interdomain hydrogen bonds. The hydrogen bonds between the generally conserved Gln residues at position 38, as well as the hydrogen bonds between the Tyr87 and the main-chain oxygen of residue 42, are maintained in both proteins.

The changes to smaller residues, Tyr → Leu at position 96 and probably the Leu → Val at position 46, may be required to achieve the observed domain pairing in the Wat dimer. Model building studies (data not shown) suggest that insufficient space is available to accommodate the bulkier Tyr side chains at positions 96 when the domains are arranged as observed in Wat.

In the Wat dimer, chain segments from neighboring domains are bridged by water molecules (see Figure 5). Six water molecules, three on each side, are integral parts of the interface. Water molecules on one side are located between the side chain of Asn34 and the nitrogen of Leu396, the hydroxyl of Tyr87, and the carbonyl oxygen of Gln 342, and the carbonyl oxygen of 44 and the nitrogen of 398. The water position near Asn34 has a high occupancy; therefore, the Asn at position 34 might be important for maintaining the dimer structure. Two of the six waters in similar positions were also identified in the Rei structure.

The sliding of the domains relative to each other alters the relative interdomain distances between the CDRs. The distances of the CDRs in the two molecules were compared by calculating the center of mass separation of the α-carbon atoms of the segments. We found that the CDR1s are ~2 Å closer in Wat than in Rei, the CDR2s are ~2 Å further in Wat than in Rei, and the CDR3s are ~4 Å closer in Wat than in the Rei dimer. The buried surface areas between the domains in both structures are very similar: 1498 and 1438 Å^2 in Rei and Wat, respectively. The differences in domain orientation can be assessed by using the method suggested by Colman et al. (1987). When one of the domains of Wat is superimposed on domain 1 of Rei, an 11.8° rotation and 1.3-Å translation is required to superimpose the other domain of Wat on domain 2 of Rei.

Comparison of Crystal Packing in the Wat and Rei Structures. Though the Wat and Rei dimers are closely

Table 7: Intermolecular Hydrogen Bonds with Symmetry-Related Molecules in the Crystal Structures of Wat and Rei

Wat			Rei		
atom 1	atom 2	d (Å)	atom 1	atom 2	d (Å)
$-x, 1-y, +z$[a]			$y, x-y, z-1/6$[a]		
Ser307 OG	Asp317 OD1	3.37	Ser7 OG	Asp317 OD1	2.92
Ser309 OG	Ser312 OG	2.85	Asp17 OD2	Ser307 OG	3.41
Ser309 OG	312 O	3.18	Thr107 OG1	Ser309 OG	2.98
309 N	312 O	2.79	9 N	312 O	2.86
310 N	312 O	3.46	10 N	312 O	3.33
310 O	312 N	3.08	10 O	312 N	2.93
			12 N	310 O	2.78
$y, y-x, z-2/3$			12 O	310 N	3.49
Asp17 OD1	Arg324 NH2	2.94	12 O	309 N	2.65
Asp17 OD2	Arg324 NH2	2.99	18 O	307 OG	3.18
Asp17 OD2	Thr369 OG1	2.72			
			$x-y+1, x+1, z+1/6$		
$1-y, 1-y+x, 1/3+z$			Asn53 ND2	Gln379 OE1	3.32
Asp92 OD1	Lys407 NZ	2.84	Gln55 OE1	Ser377 OG	2.77
Asp92 OD2	Lys407 NZ	3.30			
92 O	408 N	2.51	$y-x, 1-x, z-1/3$		
94 N	406 O	2.98	Thr69 N	Gln303 OE1	2.93
391 O	Arg408 NH2	3.24	Thr69 OG1	Gln303 OE1	3.08
391 O	Arg408 NE	3.00	Thr69 OG1	Gln303 NE2	3.46
392 O	Arg408 NH2	3.10			
Tyr349 OH	380 O	2.52			

[a] Symmetry operator of atom 2.

related, they crystallize in different space groups, $P6_4$ and $P6_1$, respectively. The Wat molecules are more tightly packed in the unit cell than are the Rei molecules; the crystal packing volume (Matthews, 1968) is 2.5 Å^3/Da for Wat and 3.3 Å^3/Da for Rei crystal. Table 7 lists the hydrogen-bonded contacts between asymmetric units in the Wat and Rei lattices. In Wat, an antiparallel β-sheet is formed by the crystallographic twofold axis that relates residues 309−312 of monomer 2 in two neighboring asymmetric units (see Figure 6). In the Rei unit cell, a similar interaction is also observed but different monomers are involved. In Rei, a local 2-fold axis relates residues 9−12 of monomer 1 with residues 309−312 of monomer 2 of the neighboring asymmetric unit located along the 6_1 axis of the unit cell. The interaction is strengthened by the hydrogen bonds formed by side chains of Ser7 and Asp17 from neighboring dimers. Wat and Rei have the same amino acids in this chain segment.

As noted above, chain segments of residues 9−13 form a parallel β-pleated sheet with residues 102−107 of the same domain. The crystallographic 2-fold axis in Wat (and the local 2-fold in Rei) extends this sheet and buries some residues between the neighboring molecules (Figure 6) in a manner similar to that observed in the packing of the constant domains in the λ-type light-chain dimer crystals (Schiffer et al., 1985). The buried surface area (Lee & Richards, 1971) calculated with a 1.4-Å probe radius of 820 Å^2 resulting from lattice contacts between the two domains in Wat compares with the 570 Å^2 buried surface area between the λ constant domains. This packing motif was also observed for other light chains, M3 (Steipe et al., 1992) and M29b (Essen & Skerra, 1994), and between the heavy chain variable domains in the NQ10/12.5 Fab (Alzari et al., 1990).

In the Wat crystal, the two dimers related by the 2-fold axis form a tetramer; in the Rei structure, the local 2-fold axis between monomers 1 and 2 results in an infinite helix along the 6_1 crystallographic axis, with a channel or pore parallel to the axis. The channel in the Rei crystal results in the observed lower packing density. The crystal packing along the 6-fold axis of Wat and Rei is shown in Figure 7.

Along the 6_4 axis in the Wat crystal, Asp17 forms a salt bridge with Arg324 and a hydrogen bond with Thr369 of the neighboring molecule. The surface area buried by this contact area is 300 Å^2. This intracrystalline contact could not exist in Rei since residue 24 in Rei is glutamine.

The two carboxyl-terminal residues, Lys407 and Arg408, of one dimer in the Wat crystal form a salt bridge and hydrogen bonds with the main-chain and side-chain atoms of the binding site of another dimer, which is related by the 3_1 axis involving the CDR3 regions of both domains (see Table 7 and Figure 8). The salt bridge is formed between Lys407 and Asp92. Both of these residues are different in Rei; residue 92 is Gln in Rei while residue 107 is Thr in Rei. Although the antigen binding site of the Wat dimer is symmetric, residues from CDR3 "bind" or form an asymmetrical crystal contact with the C-terminal residues of another monomer. The monomers of the second dimer bind different surfaces of a single domain of the first dimer. This is a good example of how a binding site with a 2-fold symmetry can bind an asymmetrical object. In addition to

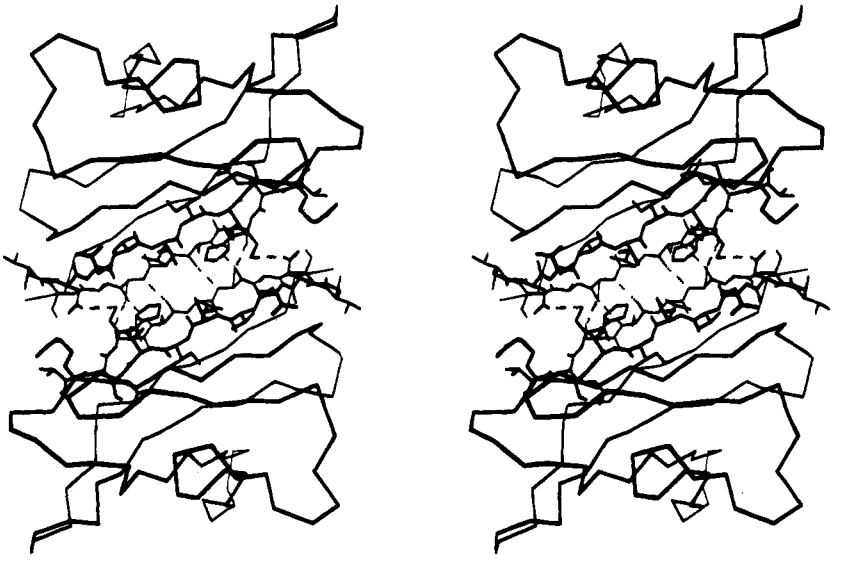

FIGURE 6: Antiparallel β-sheet formed between 2-fold axis related domains 2, showing the extension of the β-sheet and the surfaces buried between the domains.

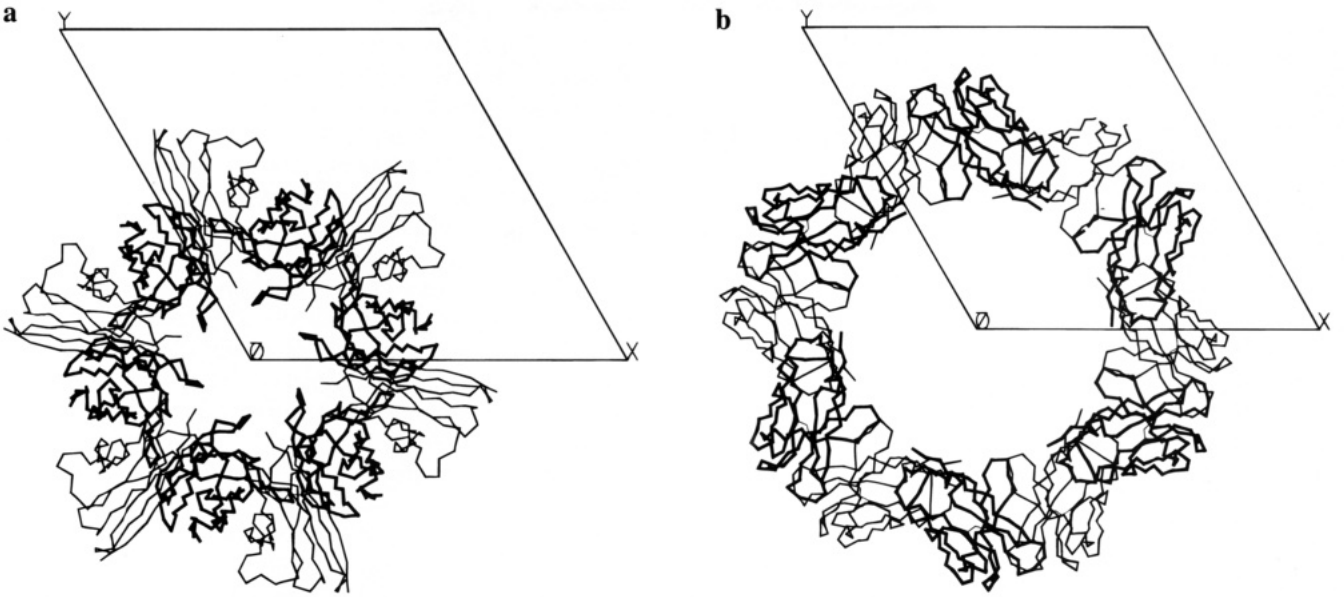

FIGURE 7: The packing of the molecules around the 6-fold axis in the Wat crystal (a) and in the Rei crystal (b) is shown projected onto the *xy* plane.

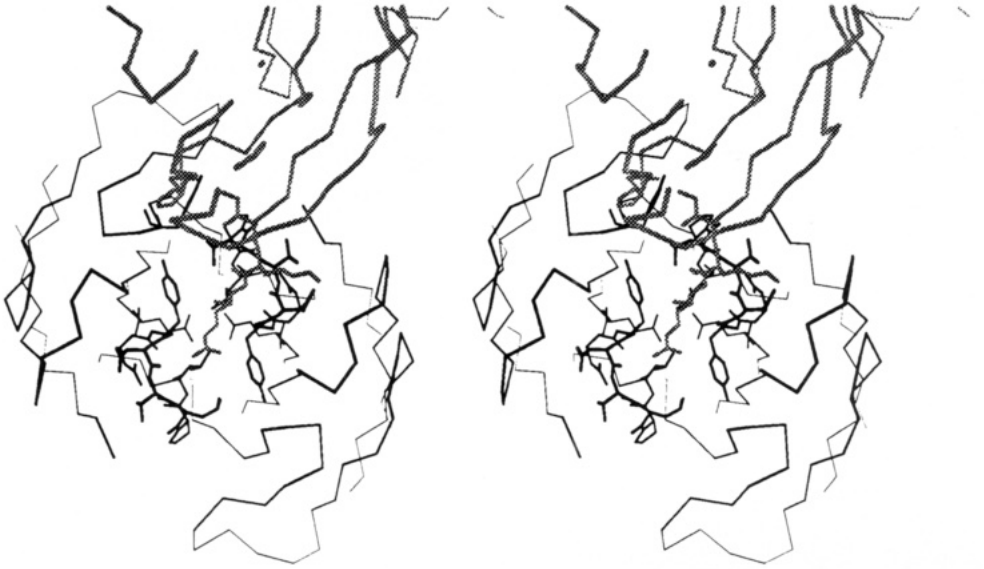

FIGURE 8: The crystal contact illustrating how one molecule binds the C-terminal residues (Lys407 and Arg408) of another molecule. Residues of CDR3 of both monomers Tyr349 and the "tail", residues 406−408, of the other asymmetric unit are shown, together with the backbone of both molecules.

the above contacts, a carbonyl oxygen from a loop on the C-terminal side of the domain (Pro380) of the first dimer also forms a hydrogen bond with Tyr349 of the 3_1 axis related dimer. The buried surface area in this crystal contact is 1156 Å^2, only 272 Å^2 less than that buried between domains 1 and 2.

Approximately 22% of the surface area of domain 1 is buried by lattice contacts, while 40% of the surface area of domain 2 is buried. The unequal distribution of close contact within the crystal of domains 1 and 2 probably results in the different distribution of individual temperature factors for the residues within the domains (as shown in Figure 9). The temperature factor distribution of the main chain for monomer 2 is as expected, with loops generally having the highest *B*-factors. In monomer 1, the CDR3 loop involved in a crystal contact has the lowest temperature factor, while the loop (residues 10−19) in the first framework region has the highest. Possibly the lack of crystal contacts permits several orientations of domain 1, which results in high temperature factors for the first framework loop that is furthest away from the crystal contact, CDR3.

In Rei (see Table 7), three residues that differ between the Rei and Wat proteins participate in the additional crystal contacts; these are Asn53 (Ile in Wat), Gln55 (Glu in Wat), and Thr107 (Lys in Wat).

DISCUSSION

The stability of the V_κ domain is maintained not only by the disulfide bond between chain segments from the two β-sheets but also by "internal" hydrogen bonds. These polar residues either in the interior of the domain or in a surface depression tend to be very well conserved in variable domains and in several members of the immunoglobulin superfamily. They form hydrogen bonds either with another polar side chain, a main-chain carbonyl or nitrogen, or an internal water molecule. Table 5 lists these residues, the percent conservation in human κ-type light chains, and their function in the domain. Similar observations were made for

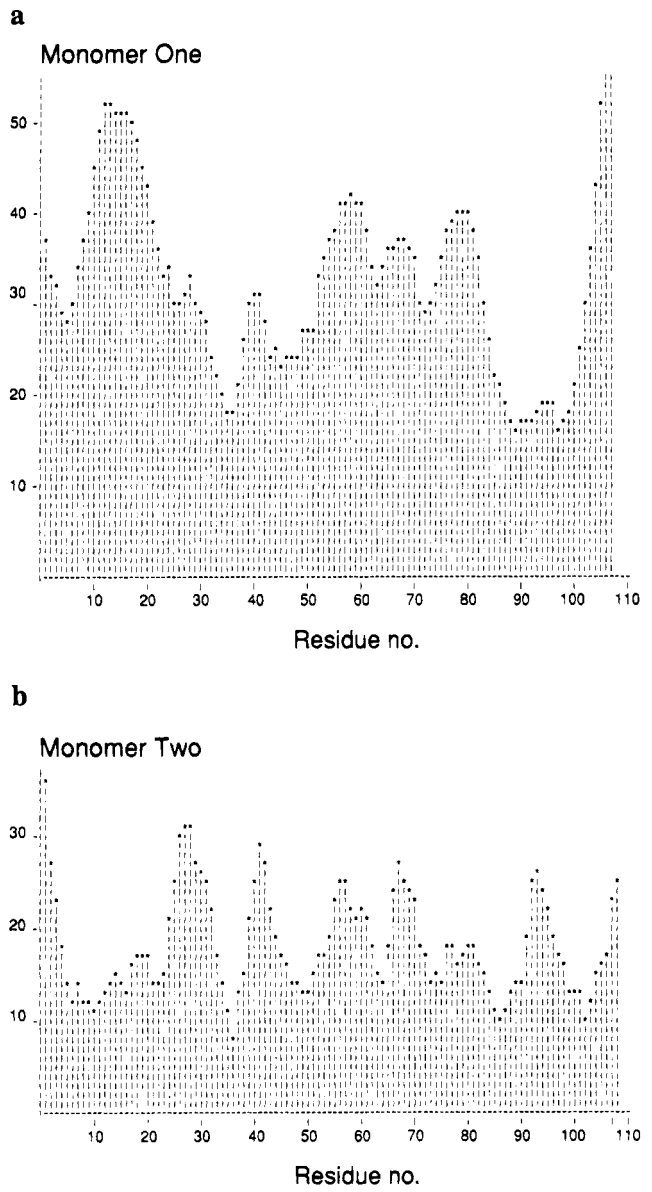

FIGURE 9: Average temperature factor for the main-chain atoms, of monomers 1 (a) and 2 (b). Twenty-two percent of the surface area of domain 1 and 40% of domain 2 are buried.

the hydrogen bonding and function of some of these residues by Steipe et al. (1992). The significant difference in the murine variable domain dimer, M3, is that the side chains of Gln6 and Thr102 do not hydrogen bond to each other, instead, Gln6 hydrogen bonds to a second internal water molecule. Since binding of Gln6 to Thr102 was observed in both the Rei (Epp et al., 1975) and Rhe (Furey et al., 1983) structures, Steipe et al. (1992) suggested that the Met in M3 instead of the well-conserved Ile at the internal position 21 could cause the different packing; indeed, the Wat protein also has Ile at position 21.

The change in the relative orientation of the Wat domains compared to that of the Rei domains and, therefore, the change from the usually observed mode of dimerization were surprising, since normally the substitution of one aromatic residue (Phe) for another aromatic residue (Tyr) is not considered a major change. With the change of residue 36 from a potentially hydrogen-bonding residue (Tyr) to a hydrophobic residue (Phe), there was a readjustment of the domain positions to maximize the numbers of hydrogen bonds between them. The observed domain positions in the Wat dimer are possible because there is a Leu at residue 96 and a Val at residue 46. In addition, there is an increased number of hydrogen bonds between the domains in Wat (see Table 6). The buried surface area between the Wat domains is 1438 Å^2, similar to that observed for the Rei dimer. Therefore, it is very probable that the domain association observed in the crystal is one that also occurs in solution. The question about the solution structure of the Wat dimer arises because, in the crystal, the binding site conformation appears to be a prerequisite for the crystal contact in which the C-terminal residues in one asymmetric unit interact with both monomers of the binding site of the other asymmetric unit. This contact involves a buried surface of 1156 Å^2 and includes six hydrogen bonds and one salt bridge, suggesting that it is a major contributor to the free energy of crystallization.

The Wat dimer structure suggests that the Tyr to Phe change, perhaps in collaboration with potential enabling substitutions at positions 46 and 96, drives the difference in the dimer structures between the Rei and Wat proteins. The Tyr to Phe alteration both changes the hydrogen-bonding possibilities (Connelly et al., 1994) and creates a void that is minimized by the observed domain shifts. About 6% of 175 sequenced human light-chain V_Ls (Stevens, unpublished observation) have Phe at position 36, while the majority (90%) have Tyr at this position. The germ-line gene also has Tyr at position 36 (Klein et al., 1993). The distribution of codon usage at this position (Kabat et al., 1991) also suggests that Phe was derived from Tyr. We have surveyed the function of Tyr in the crystallographic structures of Fab's deposited in the Brookhaven Data Bank. In 16 of the 24 structures, Tyr36 of the light chain formed a hydrogen bond with the peptide backbone nitrogen of residue 100K (Kabat numbering) of the heavy chain. This hydrogen bond appears to contribute to the interaction energy between heavy- and light-chain variable domains, which might explain the relative conservation of this residue.

Although Tyr is also the most frequent residue at position 36 in murine light chains, the somatic mutation to Phe has been found to be immunologically significant. An increased affinity to the hapten phenyloxazolone was observed in the late primary response, which correlated with the somatic mutation of the V_κ-Ox1 germ-line gene at position 36 from Tyr to Phe (Griffiths et al., 1984). Structural studies of the anti-oxazolone Fab NQ10/12.5 show that Phe36 forms part of the hydrophobic binding pocket of the phenyloxazolone hapten (Alzari et al., 1990), but there does not appear to be a significant domain rearrangement. It is of interest that the anti-oxazolone light chains have, and appear to require, Leu at position 96. Wat also has Leu at positionn 96. Leu at this position permits the observed mode of dimerization by eliminating steric hindrance to the domain shift that is imposed by Tyr in Rei. However, as shown by the conformation of Bence Jones protein Roy (Colman et al., 1977), a Leu residue at position 96 is not sufficient to generate a Wat-like domain arrangement.

The finding of an unusual domain interaction in protein Wat extends previous observations of domain rearrangements found in the λ_I protein Loc, in which a His residue at interface position 38, coupled with a Trp at position 91, created a dimer in which three quaternary conformations have been observed in different solvent conditions (Chang et al., 1985; Schiffer et al., 1989; Schiffer et al., unpublished observations). The Loc data provided a basis for the suggestion that the relationship between antibody variable domains was

more dynamic than had been previously suspected and that fluctuations in positions might contribute to antigen specificity and idiotypic properties of antibodies (Stevens et al., 1988). This concept was also indicated by small shifts observed in the complex of a Fab with neuraminidase (Colman et al., 1987) and has been recently demonstrated by a striking rearrangement of V_L and V_H in a Fab bound to HIV protease (Stanfield et al., 1993). Studies by Voss and co-workers (Voss et al., 1989; Werdner et al., 1992) have established the concept of metatype, an idiotype that is apparently created by conformational change associated with formation of an antibody:antigen complex.

Light-chain variable domain interactions have proven to be highly malleable, with relative positions responsive to amino acid alterations affecting small numbers of hydrogen bonds and steric restrictions. The properties of light-chain dimers have been found to be emulated in immunologically functional antibodies, and it can be anticipated, in other members of the immunoglobulin superfamily including T-cell receptors, cell adhesion molecules, and numerous other proteins involved in intercellular organization and communication. With limited three-dimensional structural data currently available for many important superfamily proteins, modeling studies are the only means for generation of hypothetical conformations of domains and their complexes. Crystallographic studies of additional light-chain dimers, both of natural origin or as modified by site-specific mutagenesis, should continue to expand our concepts of the modes of interaction available to all of the proteins of the immunoglobulin superfamily. Such studies should contribute to a more detailed understanding of the specific contributions of interface interactions and crystal lattice interactions to the ultimate quaternary interactions observed in many multisubunit proteins and protein complexes.

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