Rotational Allomerism and Divergent Evolution of Domains in Immunoglobulin Light Chains†

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ABSTRACT: The crystallographic structure of a human (Mcg) λ-type light chain (Bence-Jones) dimer has been correlated with the amino acid sequence determined by J. W. Fett and H. F. Deutsch ((1974), *Biochemistry 13,* 4102). Each light chain is composed of amino ("variable" or V) and carboxyl ("constant" or C) regions ("domains"), which are cylinders of β-pleated sheets enclosing hydrophobic interiors. The structures of the V and C domains support the hypothesis of a common ancestral gene. A comparison of sequences of the V and C domains, with the three-dimensional structures as a basis for alignment, confirms earlier views that sequence homologies have been obscured by divergent evolution. The types of changes that have occurred are indicated by the structures of the domains. For example, the polypeptide chains of the V and C domains appear to be rotational allomers. Among the α-carbon atoms of 38 pairs of selected residues, the angles of rotation between comparable V and C constituents are 163–167° in both monomers. The rotation axes pass approximately through homologous half-cystinyl residues of the intrachain disulfide bonds. The functions of the three- and four-chain layers of antiparallel β-pleated sheets constituting the basic "immunoglobulin fold" have changed during the evolution of the two domains. In the light chain dimer the three-chain layers of the V domains face each other across a solvent channel in which hapten-like molecules can be bound. The corresponding layers in the C domains supply most of the external surfaces of the dimeric module. Conversely, the four-chain layers furnish the external surfaces of the V_1–V_2 dimer, but interact across a solvent-free zone to help maintain the compact arrangement of C domains. Sequence patterns in parts of the pleated sheets can be correlated with these differences. In the V domains an alternating pattern of polar and apolar residues is broken in the three-chain layers by the substitution of aromatic for aliphatic polar residues in positions important for the maintenance of the general architecture of the hapten-binding sites. Similarly, the alternating sequence pattern in the four-chain layers of the C domains is interrupted by the substitution of hydrophobic for polar residues at sites of interactions in the C_1–C_2 interface. The polar constituents in the alternating sequences of the four-chain layers of the V domains are mainly seryl and threonyl residues, which form most of the surfaces of the dimeric module. The polar residues on the surfaces of the C dimer are more diversified. The differences in surface properties may partially explain why the V domains have been implicated in the formation of amyloid fibrils and in the characteristic thermal behavior of Bence-Jones proteins.

The serum IgG1 immunoglobulin and the urinary Bence-Jones protein from a patient (Mcg) with multiple myeloma and amyloidosis have been crystallized and characterized by chemical and crystallographic techniques (Deutsch, 1971; Deutsch and Suzuki, 1971; Fett et al., 1973; Fett and Deutsch, 1974; Schiffer et al., 1970, 1973; Ely et al., 1973; Edmundson et al., 1970, 1971, 1973, 1974a,b). The IgG1 protein consists of two light (mol wt 23,000) and two heavy chains (mol wt 50,000). The Bence-Jones protein is a dimer of λ-type light chains identical in amino acid sequence with their counterparts in the parent IgG1 molecule.

In contrast to most IgG proteins, the Mcg molecule does not have light–heavy or heavy–heavy interchain disulfide bonds because of a deletion of 15 residues in the "hinge" region usually supplying the appropriate half-cystinyl residues (Fett et al., 1973). The light chains are of normal length and are connected by an interchain disulfide bond both in the IgG1 protein and in the Bence-Jones dimer. The presence of a crystallographic twofold axis of rotation between halves of the IgG1 molecule indicates that the covalently linked light chains have identical conformations when associated with heavy chains (Edmundson et al., 1970). In the Bence-Jones dimer, however, the spatial relations between the V and C domains in the two light chains are strikingly different (Schiffer et al., 1973). The angle between the long axes of cylindrical envelopes drawn around the V and C domains is approximately 70° in monomer 1 and 110° in monomer 2. Differences in the "switch" regions (i.e., around Gly-111) are mainly responsible for these conformational variants (Edmundson et al., 1974b). Comparisons of the structures of the light chain dimer and Fab fragments (Poljak et al., 1973, 1974; Amzel et al., 1974; Padlan et al., 1973; Segal et al., 1974; Padlan and Davies, 1975) indicate that the conformation of monomer 1 is similar to that of the heavy chain, while monomer 2 closely resembles the light chain components.

The crystallographic analysis of the light chain dimer at 3.5-Å resolution showed that each domain consists of a basic layered structure, one layer with four antiparallel extended chain segments, and the second with three antiparallel segments (Schiffer et al., 1973). The two layers are covalently linked near the center of each domain by an intrachain disulfide bond. Similar layered structures have been found in the domains of both heavy and light chains in human and murine Fab fragments (Poljak et al., 1973, 1974; Amzel et al., 1974; Padlan et al., 1973; Segal et al., 1974) and in V domain dimers from κ-type Bence-Jones proteins (Epp et al., 1974; Fehlhammer et al., 1975).

Extension of the crystallographic analysis of the Mcg dimer to 2.3 Å indicated that the layers in each domain are composed of "twisted" β-pleated sheets (Edmundson et al., 1974a). These sheets enclose hydrophobic interiors.

When examined in isolation, the structures of the V and C domains strongly support the hypothesis of a common ancestral protein of about 110 amino acid residues (Singer

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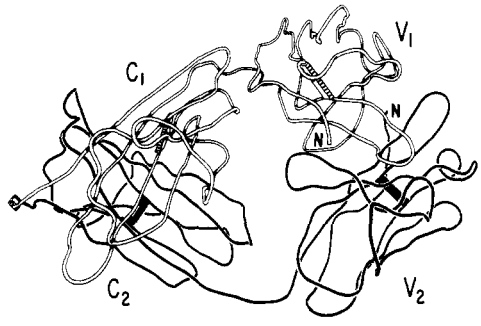

FIGURE 1: Tracing of a photograph of a model of the Bence-Jones dimer, with the polypeptide chains of monomers 1 and 2 shown in white and black, respectively. The solvent channel in which hapten-like molecules can be bound lies between the V domains on the right. The amino-terminal residue in each domain is labeled N, and the interchain disulfide bond between penultimate residues is located on the extreme left. Note the difference between the spatial relations of the V and C domains in the two monomers. Monomer 1 has a conformation similar to that in heavy chains of Fab (antigen-binding) fragments, while monomer 2 closely resembles the light chain components. This figure was adapted from Schiffer et al. (1973).

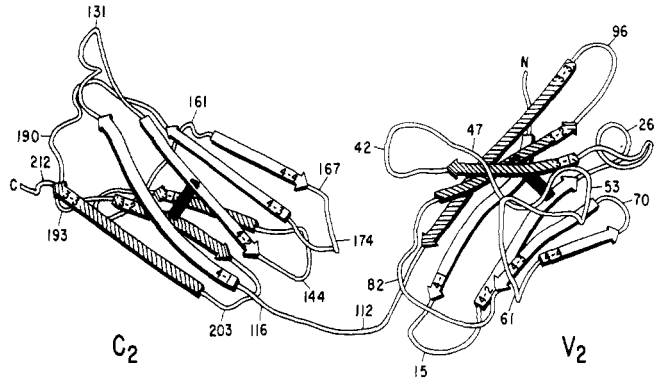

FIGURE 2: Schematic drawing of monomer 2, with directional arrows superimposed on segments participating in antiparallel β-pleated sheets. Three-chain layers are indicated by numbered striated arrows and four-chain layers by white arrows. Positions of representative residues are numbered to aid in the correlation of amino acid sequences with the three-dimensional structure.

and Doolittle, 1966; Hill et al., 1966). However, the three- and four-chain layers have markedly changed functions during the evolution of the V and C domains. In previous articles we did not attempt to define the structural basis for these functional changes. The availability of a 2.3-Å electron density map (Girling et al., in preparation) and the completion of the amino acid sequence determination (Fett and Deutsch, 1974) now permit this problem to be considered in the present report.

Materials and Methods

Details of the crystallographic study leading to the calculation of a nominal 2.3-Å electron density map will be published in another article. Using the amino acid sequence (Fett and Deutsch, 1974), Watson–Kendrew skeletal models were fitted to this map with the aid of an optical comparator (Richards, 1968). Atomic coordinates were measured from the model and subsequently used in Diamond's model building programs (1966).

Structural analyses, including comparisons of the V and C domains, were first performed by direct observation of the model. A least-squares procedure was then used to compare the α-carbon positions of selected residues in the V and C domains. The domains were superimposed by translation, and rotational parameters were adjusted until the sums of the squares of the distances between equivalent atoms were minimized (Huber et al., 1971; Drenth et al., 1972; Ohlsson et al., 1974).

Results and Discussion

For correlation with the sequences to be discussed, a tracing of a photograph of a scale model (2 cm = 1 Å) of the dimer is shown in Figure 1. Comparisons of amino acid sequences in light chain domains are difficult because of the apparent divergence of V and C genes (for reviews and sequence alignments, see Edelman and Gall, 1969; Milstein and Pink, 1970; Garver and Hilschmann, 1971; Dayhoff, 1972; Putnam, 1975; Poljak et al., 1974). With the aid of the following guidelines, however, the sequences can be compared within the framework of the three- and four-chain layers in the crystallographic structure. A schematic drawing of monomer 2, with directional arrows superimposed to indicate the locations of the three- and four-chain layers, is presented in Figure 2. The complete amino acid sequence of the Mcg light chain in reference to the three-dimensional structure is given in Figure 3.

The intrachain disulfide bonds are in similar locations in the two domains, and the corresponding disulfide loops are approximately the same size (~60 residues; see above reviews). Our sequence alignments were first confined to the most regular parts of the pleated sheets, since the three-dimensional structures of the V and C domains are most similar in these regions. About eight consecutive residues in each segment of the three- and four-chain layers were aligned in positions relative to that of an intrachain half-cystinyl residue. The chosen sequences, together with the patterns of emergence of side chains on alternating sides of the three- and four-chain pleated sheets, are illustrated in Figure 4. Residues selected for comparisons of α-carbon coordinates are underlined.

Less ordered regions and turns were compared next. Residues 23–36, 51–55, and 91–99, statistically defined as "hypervariable" in different light chains (Wu and Kabat, 1970; Kabat and Wu, 1972), were treated with caution. The hypervariable regions have few sequence identities with corresponding segments in the C domains, and include residues involved in insertions and deletions in other light chains.

Rotational Allomerism in the V and C Domains. The similarities in the cylindrical domains are illustrated in Figure 5. The schematic drawing of the C domain is rotated to approximate the orientation of the V domain.

Colman et al. (1974) have evidence for the presence of approximate twofold rotation axes (with translations) between the C_H2 and C_H3 domains of the Fc ("crystalline") fragment of an IgG immunoglobulin, and we looked for similar axes in the light chain monomers. The search was initially limited to observation of the atomic model in structurally important regions, in which sequence differences are minimal. Such segments are found near the intrachain disulfide bonds, which are shielded in each domain by a tryptophyl residue (37 and 152). The conformations of the tripeptide segments Val-Ser-Trp (35–37) and Val-Ala-Trp (150–152) bear an unmistakable resemblance to each other. As indicated schematically in Figure 6, these two sets of three residues are related by a noncrystallographic rotation axis, and a translation of 43–44 Å in monomer 2. This axis passes approximately through the side chains of Cys-22

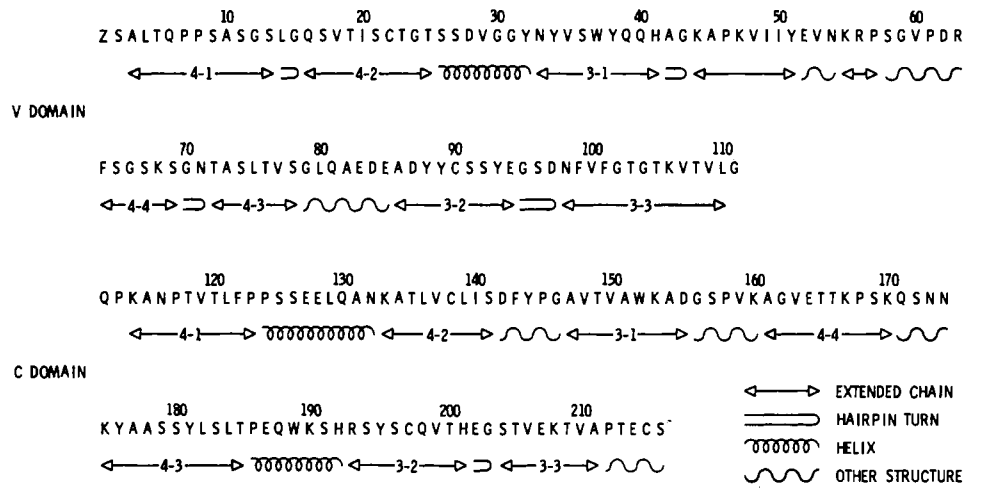

FIGURE 3: Amino acid sequence of the Mcg λ chain, as determined by Fett and Deutsch (1974). Residues are represented by one-letter abbreviations (Dayhoff, 1972). The three-dimensional structures assumed by the segments are indicated schematically under the appropriate sequences. Extended chains participating in the three- and four-chain layers are numbered as in Figure 2. The symbol for "other structures" indicates corners other than hairpin turns, as well as irregular structures not included in other categories.

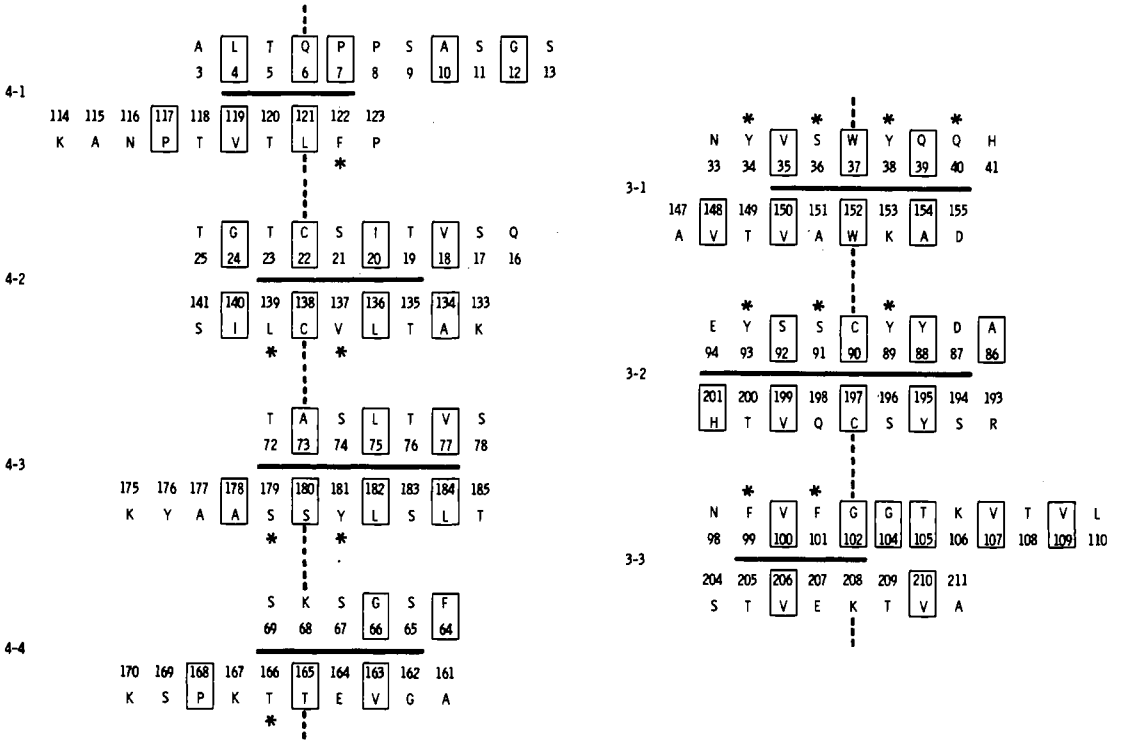

FIGURE 4: Amino acid sequences of segments in three- and four-chain layers of β-pleated sheets. Equivalent pairs of residues are listed, with the V components on top. Abbreviations of residues with side chains oriented toward the interior of each domain are enclosed in boxes. Residues marked with asterisks in the three-chain layer of the V domains are constituents of the sites in which hapten-like molecules are bound in the light chain dimer (see Figure 8). In the four-chain layer of the C domain the residues designated with asterisks occupy the interdomain space and help to stabilize the C_1–C_2 dimer (see Figure 9). The vertical dashed lines pass through positions approximately aligned with the intrachain half-cystinyl residues. There is a "kink" in chain 3-3 at Thr-103, which consequently is not in the same relative position as Thr-209. The latter is aligned with Gly-104 in the figure.

and -138.

Because of the differences in the spatial relations between the V and C domains (see Figures 1 and 7), the rotational allomerism is easier to visualize in monomer 2 than in monomer 1. Nevertheless, the above tripeptide segments of monomer 1 can also be related by rotation and translation.

When 38 pairs of residues (underlined in Figure 4) were compared by a least-squares procedure, the average deviation between the α-carbon positions of equivalent residues was 1.11 Å in the V_1 and C_1 domains and 1.16 Å in the V_2 and C_2 domains.[1] The average value for the angles of rotation between equivalent α-carbon atoms in the V and C domains were 163° for monomer 1 and 167° for monomer 2. These results indicate that the polypeptide backbones in the

[1] For the same 38 pairs of residues in like domains, the corresponding values were 0.94 Å for the V_1 and V_2 domains and 0.95 Å for the C_1 and C_2 domains. When all pairs of α-carbon atoms were compared, the angle of rotation between V_1 and V_2 components was 180°. The C_1 and C_2 domains were also related by a twofold axis of rotation.

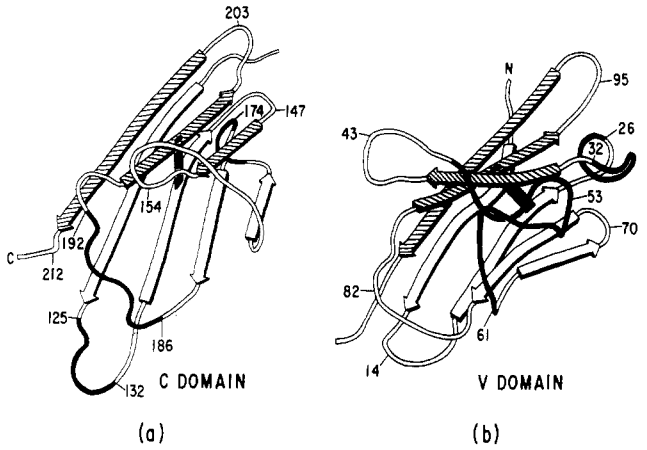

FIGURE 5: Comparison of the V and C domains of monomer 2. The C domain (a) is rotated to approximate the orientation of the V domain (b). The directional arrows in the three- and four-chain layers correspond to those in Figure 2. Chain segments present in only one domain or having different structures in the two domains are indicated by solid black lines. Positions of prominent residues, or their near-neighbors, are numbered for correlation with the text.

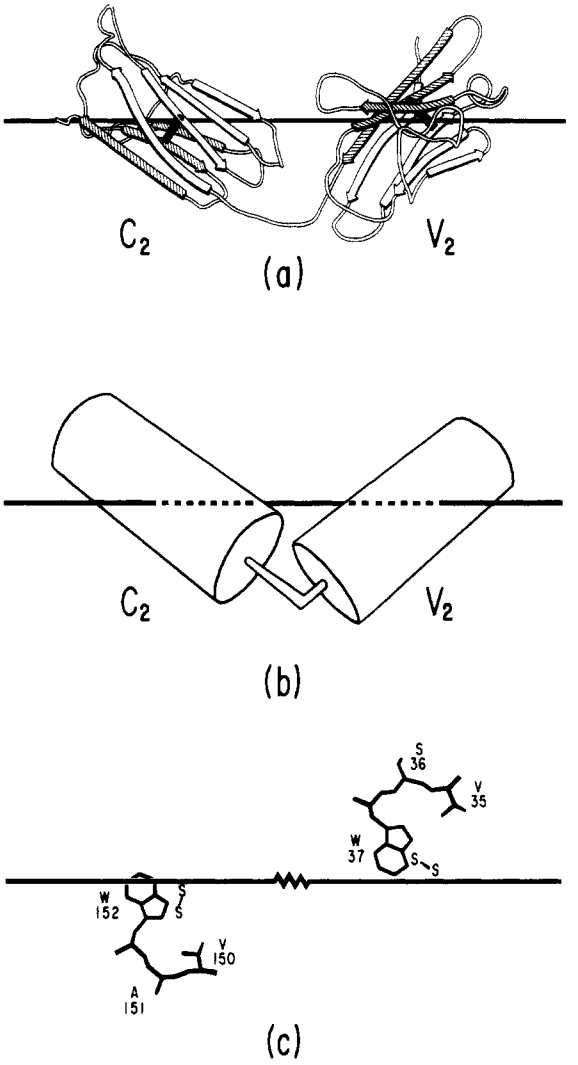

FIGURE 6: Schematic drawings showing the noncrystallographic axis of rotation between the V and C domains of monomer 2. (a) Monomer 2, as presented in Figure 2, with the rotation axis included. For α-carbon atoms of 38 pairs of residues selected from the three- and four-chain layers of the V and C domains, the angles of rotation are 163–167°. (b) Same view, with the domains represented by cylindrical envelopes. After rotation, corresponding structural elements in the two domains have to be translated 43–44 Å to bring them into coincidence. (c) Illustration of the rotational symmetry between two corresponding tripeptide segments near the intrachain disulfide bonds of the two domains.

β-pleated sheets of the V and C domains are closely similar in structure and in the characteristic twist of the chain segments. The similarities of monomers 1 and 2 to the heavy and light chain components of the Fab fragments strongly suggest that rotational allomerism is a fundamental feature of immunoglobulin structures.

Alternating Polar–Apolar Sequences in the β-Pleated Sheets. The recognition of rotational allomerism in the V and C domains permits the comparison of individual residues, particularly in the β-pleated sheet regions. In pleated sheets the side chains emerge at right angles to the direction of the polypeptide chain and on alternating sides of the backbone. Given a cylindrical domain with a hydrophobic center, we looked for sequence patterns with external polar residues alternating with internal apolar residues.

Such patterns are readily seen in the four-chain layers of the V domains and in the three-chain layers of the C domains (see Figure 4). In the V domains the polar residues in the alternating patterns are mainly serine and threonine, and the cylindrical surfaces are largely composed of side chains of these hydroxy amino acids. The surface residues of the C domains are more diversified.

The alternating sequence patterns are interrupted in important locations in the three-chain layers of the V domains and in the four-chain layers of the C domains. The significance of these interruptions will be considered after a discussion of the side chains directed toward the interior of each domain.

Internal Residues in the V and C Domains. Each cylindrical domain is filled with hydrophobic side chains, which are listed in Table I (also see Figure 4). Where possible, the residues are paired in equivalent positions in the two domains.

The intrachain disulfide bonds are well shielded from solvent by the side chains of Leu-4, Gln-6, Ile-20, Val-35, and Trp-37 in the V domains, and those of Val-119, Leu-121, Val-150, Trp-152, and Val-199 in the C domains. Gln-6, Gln-39, Ser-92, Thr-105, Thr-165, and Ser-180 are exceptions to the usual distribution of hydrophobic residues inside the cylinders.

The observations indicate that approximately one-fourth of the residues occupy internal sites in each domain. This fraction is comparable with that in the myoglobins and hemoglobins (Perutz et al., 1965), and is consistent with earlier conclusions based on calculations of average hydrophobicities and on the distributions of polar and apolar residues in the V and C domains of series of light chains (Welscher, 1969a,b; Edmundson et al., 1973). In the globins, which have similar three-dimensional structures, only a few (<6) amino acid residues have been conserved throughout phylogeny (Braunitzer, 1966; for tabulations and review, see Dayhoff, 1972). The common globin structure is preserved principally by the retention of similar, but not necessarily identical hydrophobic residues in ~30 key internal sites (Perutz et al., 1965; Watson, 1969). This conclusion also seems to be applicable to the maintenance of a common immunoglobulin fold.

Comparison of Three-Chain Layers in the V and C Domains. In the Mcg protein the main binding cavity in the solvent channel between the V domains is shaped like a

Table I: Internal Residues in the V and C Domains.

Four-Chain Layer			Three-Chain Layer			Connecting Chains		
Segment	V Domain	C Domain	Segment	V Domain	C Domain	Segment	V Domain	C Domain
4–1		Pro-117	3–1		Val-148	4–1 to 4–2		Pro-124
	Leu-4	Val-119		Val-35	Val-150			
	Gln-6	Leu-121		Trp-37	Trp-152	4–2 to 3–1	Val-29	
	Pro-7			Gln-39	Ala-154			Phe-143
	Ala-10							
	Gly-12		3–2	Ala-86		3–1 to 4–4	Ile-49	
				Tyr-88	Tyr-195		Ile-50	
4–2	Val-18	Ala-134		Cys-90	Cys-197		Pro-57	
	Ile-20	Leu-136		Ser-92	Val-199		Val-60	
	Cys-22	Cys-138			His-201			
	Gly-24	Ile-140				4–3 to 3–2	Leu-80	
			3–3	Val-100	Val-206			Trp-189
4–3		Ala-178		Gly-102				
	Ala-73	Ser-180		Gly-104				
	Leu-75	Leu-182		Thr-105	Val-210			
	Val-77	Leu-184		Val-107				
				Val-109				
4–4	Phe-64							
	Gly-66	Val-163						
		Thr-165						
		Pro-168						

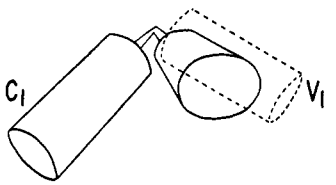

FIGURE 7: Drawing of cylindrical envelopes of the V and C domains of monomer 1 (—), with the V domain also drawn in the orientation (- - -) it would have assumed if the conformations of monomers 1 and 2 were identical. Because of these conformational differences, rotational symmetry of the type shown in Figure 6 is more difficult to visualize in monomer 1. For comparison with Figure 6, the distance between the intrachain disulfide bonds in the V and C domains is 25 Å in monomer 1 and 43 Å in monomer 2.

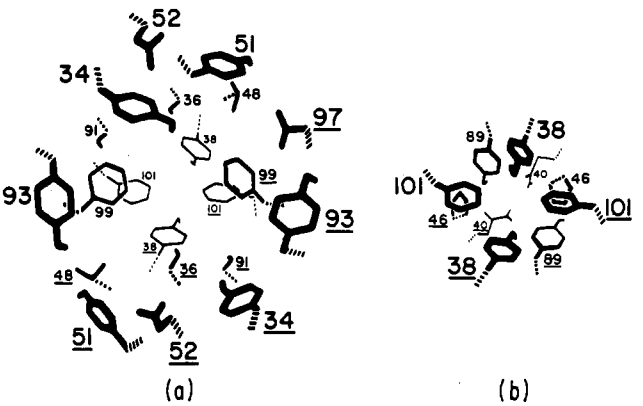

FIGURE 8: (a) Perspective drawing of the side chains lining the main cavity in the solvent channel between the V domains. The sequence numbers of the constituents of monomer 2 are underlined. Bonds between α- and β-carbon atoms are represented by dashed lines. All side chains except those of Val-48, Tyr-51, and Glu-52 emerge from segments of the three-chain layers (see Figure 4). The circular entrance to the cavity (see side chains in heavy, dark lines) is about 15 Å in diameter. The distance from the entrance to the four aromatic residues (38 and 101) at the base is about 17 Å; the Phe-99 side chains emerge at an intermediate level (11 Å from the entrance). (b) Perspective drawing of the side chains lining the deep pocket beyond the base of the main cavity.

truncated cone, 15 Å wide at the entrance and 17 Å deep. Schematic drawings of the side chains lining the solvent channel are presented in Figure 8a. The cavity contains the side chains of 21 residues (Edmundson et al., 1974b), including the constituents of regions statistically defined as "hypervariable" in different proteins (Kabat and Wu, 1972). Of the total, 12 side chains are aromatic and 15 are components of the three-chain layers (the exceptions being pairs of Val-48, Tyr-51, and Glu-52 from the two monomers).

At the base of the main cavity the solvent channel passes through a small opening bounded by Tyr-38 and Phe-101 of the two monomers (see Figure 8b). It then enlarges into a pocket lined by these four aromatic residues and pairs of side chains from Gln-40, Pro-46, and Tyr-89.

The presence of aromatic residues in such high proportions probably explains why the three-chain layers in the V domains form a cavity and pocket partly protected from solvent. Aromatic residues are not found in the corresponding positions in the C domains and a binding cavity and pocket are absent. For example, sites equivalent to those of Tyr-34, -38, -89, and -93 and Phe-99 and -101 are occupied by Thr-149, Lys-153, Ser-196, Thr-200, Thr-205, and Glu-207, all on the surface of the C_1–C_2 dimer. The aromatic residues alternate with internal residues which tend to be conserved in the C domains: Val-35 and Val-150; Trp-37 and Trp-152; Tyr-88 and Tyr-195; Cys-90 and Cys-197; and Val-100 and Val-206 (see Figures 3 and 4, and Table I).

The foregoing series of observations emphasizes the selection pressure to keep the internal regions of both domains hydrophobic, while allowing the formation of suitable binding sites along the solvent channel between the V domains. In terms of sequence differences, the polar–apolar stagger seen in the C domain is interrupted in the V domain, and tyrosyl or phenylalanyl residues are placed in key "polar" positions in the pleated sheet. The aromatic residues both maintain the architecture of the binding sites and act as contact residues in the binding of various hapten-like molecules (Edmundson et al., 1974b; see also Amzel et al., 1974; Segal et al., 1974; Epp et al., 1974). Other contact

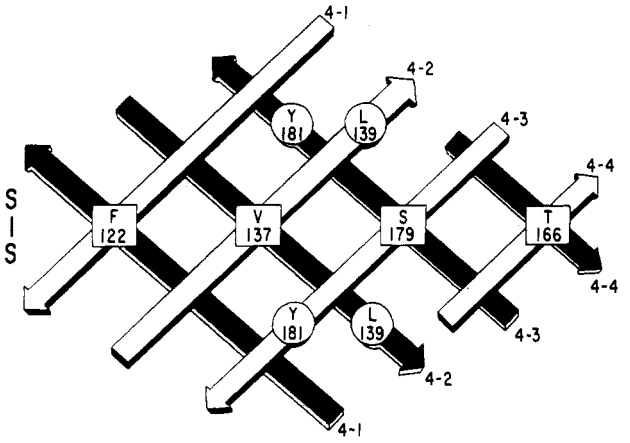

FIGURE 9: Schematic drawing of sites of interactions helping to maintain the compact arrangement of the C domains. The four-chain layers of the C domains of monomers 1 and 2 are represented by white and black arrows, respectively. The interchain disulfide bond is on the left. Abbreviations and numbers in squares indicate the approximate α-carbon positions of pairs of residues facing each other in the interdomain space. Other important residues contributing to the stabilization of the C_1–C_2 dimer are denoted by circles. The residues depicted here are marked with asterisks in the four-chain layers of Figure 4.

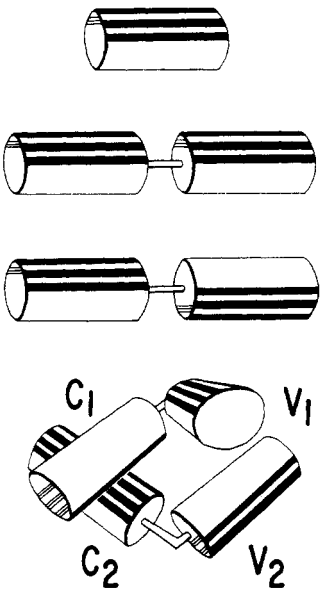

FIGURE 10: Possible evolutionary pathway from a primordial domain to the present Bence-Jones dimer. The four-chain layers are indicated by stripes. Three-chain layers occupy the parts left blank in the cylinders. Genetic changes in the sequences resulting in the stabilization of the present V and C rotational allomers are summarized in the text.

residues (e.g., Ser-36 and -91) are located on the same side of the pleated sheet and in the "polar" positions of the alternating sequence pattern. These observations suggest that binding specificity in immunoglobulins can be altered by only a few amino acid substitutions along one side of the three-chain pleated sheet.

Comparison of the Four-Chain Layers in the V and C Domains; Interactions Stabilizing the C_1–C_2 Dimer. Similarities in the four-chain layers can be seen by comparing Figures 3 and 4. The alternating hydroxy-apolar sequence pattern in the V domain is interrupted in the C domain in locations shown in Figure 4. Surface residues Ser-21 and Thr-23 in chain 4-2, and Ser-74 in chain 4-3 are replaced by Val-137, Leu-139, and Tyr-181. In addition Pro-7 is replaced by Phe-122. The C domain residues occupy key cross-over points at which side chains from the two monomers interdigitate in a solvent-free zone to help maintain the C dimer in its compact arrangement. These interactions are illustrated schematically in Figure 9. As in the three-chain layers, the hydrophobic character of residues oriented toward the inside of each domain is retained, and significant changes in the sequences are limited to one side of the pleated sheet (see Figure 4 and Table I). In two cross-over sites between C domains, the sequence differences with the V domains involve only interchanges between serine and threonine, but the functions are changed. The surface residues Ser-69 in chain 4-4 and Thr-72 in chain 4-3 are replaced by Thr-166 and Ser-179, which form pairs of opposing side chains in the interface of the C domains (see Figure 9). In addition to the conspicuous sites shown in Figure 9, there are other C_1–C_2 contacts which will be described after refinement of the present structure (Schiffer et al., unpublished results).

The interchain disulfide bond completes the principal zipper-like interactions stabilizing the C_1–C_2 dimer (see Figures 1 and 9). The penultimate half-cystinyl residues have no equivalents in the V domains.

In the last two sections we have presented evidence that the genetic changes leading to rotational allomerism of the V and C domains are critical in the evolution of immunoglobulins. Substitutions among important residues in both the three- and four-chain layers are probably required for a stable transition of one allomer to the other.

A proposal for the evolutionary progression from a single primordial domain to the present Bence-Jones dimer is shown schematically in Figure 10.

Comparison of Turns and Other Connecting Segments in the V and C Domains; Their Relation to Deletions and Insertions. The greatest differences in the two domains are found among the segments connecting the chains in the pleated sheet layers. For example, the helical part of the first hypervariable region and the entire second hypervariable region of the V domain are missing in the C domain (see Figure 5). Conversely, two helical segments at the distal end and one loop at the proximal end of the C domain have different structures or are not present in the V domain (see Figure 5). Localized differences, as well as strong similarities, are seen among the other connecting segments and turns. These will be discussed in order, starting with the connectors in the four-chain layer.

The corner between chains 4-1 and 4-2 in the V domain is a hairpin turn involving Leu-14 and Gly-15. Leu-14 corresponds to Leu-129, but the latter is a constituent of a helix containing residues 125–132 (see Figures 3 and 5).

The helical segment connecting chains 4-2 and 3-1 in the V domain seems to behave like an expandable spring. In monomer 1, chains 4-2 and 3-1 are farther apart (~2 Å) than in monomer 2 and the connecting segment is more extended. The equivalent, shortened corner in the C domain is a nonhelical loop centered around Tyr-144 and Pro-145. Tyr-144 is in van der Waals' contact with Gln-112 of the switch region (see Schiffer et al., 1973).

The corners at the carboxyl ends of the 3-1 chains in both V and C domains involve glycyl residues (43 and 156, see Figures 3 and 5). From this corner in the V domain the polypeptide chain loops forward to the second hypervariable region before returning to join chain 4-4 (see Figure 5). The loop containing the second hypervariable region contributes

Tyr-51 and Glu-52 to the principal binding site (A) in the main cavity (Edmundson et al., 1974b). Seven residues of this loop are deleted in the λ chain of the human New Fab′ fragment (Poljak et al., 1974), and the loop consequently does not participate in the binding site.

In the V domain of the Mcg protein the corner leading into chain 4-4 is similar in appearance to that in the C domain, but alignment of the two sequences involved is not straightforward because of the additional residues in the V domain (see Figures 3 and 5).

Between chains 4-4 and 4-3 in the V domain the polypeptide backbone makes a hairpin turn centered at Gly-70 (see Figure 5). Its equivalent in the C domain is a long segment which crosses almost the entire cylinder before switching back on itself in a tight loop at Asn-173 and Asn-174 (see Figure 5). There has probably been evolutionary pressure to preserve this additional segment since the position comparable to 174 in some rabbit light chains is occupied by a half-cystinyl residue forming a V–C interdomain disulfide bond with a second half-cystinyl residue in position 82 (see Figure 2 and Strosberg et al., 1972). However, if this disulfide bond were preserved in human light chains, the flexibility around the switch region would be markedly decreased. As a consequence of this loss of flexibility, it would not be possible for one of the molecules to assume the heavy chain type of conformation seen in monomer 1 of the Bence-Jones dimer.

Residue 82 is located on the wide bend between chains 4-3 and 3-2. This bend is tortuous between Glu-83 and Glu-85. There is a single turn of distorted helix in this region of the Rei κ-type V domain (Epp et al., 1974), and in the λ chain of the New Fab′ fragment (Poljak et al., 1974). In the C domain of the Mcg protein the segment connecting chains 4-3 and 3-2 contains about the same number of residues as in the V domain, but has a helical conformation (residues 186–192; see Figures 3 and 5).

The corner from chain 3-2 to 3-3 in each domain is a hairpin turn, but formed by different sequences (the hypervariable residues Gly-95, Ser-96, and Asp-97 in the V domain; Glu-202 and Gly-203 in the C domain).

In monomer 2, but not in monomer 1, the C-terminal segment makes a right angle turn at Pro-212 to meet monomer 1 at the interchain disulfide bond (Cys-215; see Figures 2 and 5). There is no equivalent turn in the V domain.

Genetic Variations within the C Domains. The C domains of λ chains are only relatively "constant" in their amino acid sequences. In addition to the Oz (Lys or Arg-193, + or −; Ein and Fahey, 1967) and Kern sites (Gly or Ser-156; Ponstingl and Hilschmann, 1969; Hess et al., 1971), substitutions at positions 116, 118, and 167 also occur in the Mcg protein and at least five other λ chains (Fett and Deutsch, 1974). The Mcg protein is Oz(−), Kern(+), and has Asn substituted for Ala-116, Thr for Ser-118, and Lys for Thr-167 (see Figures 2, 3, and 5). Changes at two other sites (147 and 175) were found in the Mz protein (Mcg, Ala-147 and Lys-175; Mz, Val-147 and Asn-175; Milstein et al., 1967).

All of the observed variants are on the surface of the C_1–C_2 dimer and with one possible exception do not appear to affect the structure significantly. The possible exception involves position 147, one of the few locations at which the V and C domains of monomer 1 are in close proximity. A side chain larger than that of valine would probably interfere with the interactions between the two domains.

The Kern and Oz sites are on adjacent chain segments, as are their equivalents in the V domain (Gly-43 = Kern Gly-156; Ala-86 = Oz Arg-193; see Figure 5). Gly-43 and Gly-156 occupy positions in turns, while Ala-86 and Arg-193 are situated at the carboxyl ends of coiled segments. However, the arginyl side chain is fully exposed to solvent, while the alanyl side chain is not. The differences in exposure, size, and charge explain why the arginyl, but not the alanyl side chain, can act as an antigenic determinant.

Among the remaining variants, Asn-116 corresponds to N-terminal glutamine (present as pyrrolidonecarboxylic acid) and Thr-118 to Ala-3. The residue corresponding to Ala-147 is still unidentified. Lys-167 and Lys-175 are part of a connecting loop which has no structural equivalent in the V domain (see Figure 5 and earlier section).

Possible Implications of V and C Differences in the Formation of Amyloid Fibrils and in Thermal Solubility Properties. In some patients with amyloidosis, the characteristic fibrils in the amyloid deposits are believed to be produced by proteolytic cleavage of peptide bonds in the V domains of light chains (Glenner et al., 1971a,b; Glenner, 1973). Although light chains in human immunoglobulins are usually distributed in a κ:λ ratio of 2:1, there is a preponderance of λ chain fragments among amyloid proteins (Pick and Osserman, 1968; Linke et al., 1973a,b). This preponderance is accentuated when fibril formation is simulated in vitro by the hydrolysis of Bence-Jones proteins with pepsin (Glenner et al., 1971a; Linke et al., 1973a,b). Among susceptible λ chains, subgroup I is represented to a significantly greater extent than other subgroups.

It should be noted that fibril formation in vitro can be induced in only about 10–20% of Bence-Jones proteins (Linke et al., 1973a). Some Bence-Jones proteins from patients with amyloidosis do not produce fibrils after peptic hydrolysis. This observation indicates that the specificity of pepsin is probably not identical with that of the enzyme(s) responsible for production of fibrils in vivo. However, the properties of the natural and induced products are quite similar. In both cases the ribbon-like filaments aggregate side by side to form fibrils which are 70–80 Å in diameter and 1000–2000 Å in length (Shirahama and Cohen, 1967; Glenner et al., 1968; Pras et al., 1969). The fibrils are sparingly soluble in water and are resistant to proteolysis. X-Ray diffraction of oriented fibrils indicates that the conformation consists of β-pleated sheets with antiparallel chains perpendicular to the long axis of the fibril (Eanes and Glenner, 1968; Bonar et al., 1969). This conformation is also consistent with the patterns obtained by infrared spectroscopy (Termine et al., 1972).

In the formation of fibrils by peptic hydrolysis, light chains are usually hydrolyzed at various sites within the intrachain disulfide loop of the V domain (Glenner et al., 1971b; Linke et al., 1973b). The locations of these sites have not been defined, but fragments of V domains with molecular weights of 4500 (Glenner et al., 1971b) and 8000 (Linke et al., 1973b) have been isolated from fibrils. Since the average molecular weight of amino acid constituents of light chains is about 100 (Edmundson et al., 1973), the cleavage points in these fragments are in the vicinities of residues 45 and 80 (see Figure 5). The presence of V fragments as the predominant components of both natural and induced fibrils may be related to the tendency of pepsin and other enzymes to hydrolyze C domains more completely at physiological temperature (Seon et al., 1972; Glenner, 1973).

The V domains have also been shown to have the proper-

ties responsible for the characteristic precipitation of Bence-Jones proteins when heated to 50–56° (Solomon and McLaughlin, 1969). Examination of such precipitates by X-ray diffraction and infrared spectroscopy indicates the presence of β-pleated sheets (Termine et al., 1972). Both the thermal reaction and fibril formation are therefore at least partly dependent on a pleated sheet conformation in the V domains, whether preexisting in the native state or induced by the thermal or enzymatic treatment. The optimum pH for the thermal transition of intact Bence-Jones proteins and isolated V domains is about 4 (Solomon and McLaughlin, 1969). The β-pleated sheet conformation in the amyloid-like fibrils is also favored by acidic conditions (Termine et al., 1972).

Amyloid deposits were found in the patient Mcg, but were not removed for comparison of the fibrils with the urinary λ chain dimer. Treatment of the latter with pepsin produces a precipitate, but not amyloid-like fibrils (J. Firca and R. LaFountain, unpublished observations). While a direct comparison cannot be made, it should be possible to correlate the general features of amyloid fibrils and heat-induced precipitates with the three-dimensional structure of the Mcg dimer. A basic point in the argument is the fact that the Mcg protein, like most Bence-Jones proteins, exists as a dimer under physiological conditions or in moderately acidic solutions at room temperature. Even after reduction and alkylation of the interchain disulfide bond, the Mcg dimer is not dissociated into monomers until the pH is lowered to about 2.5 (J. Firca and P. Kremser, unpublished observations). If the dimer is assumed to be the initial reactive species in the enzymatic or thermal reactions, the surfaces exposed to solvent (and to the enzymes) are different in the V and C domains; namely, the four-chain layers in the V dimer, and the three-chain layers in the C dimer. As emphasized earlier, these surfaces have different characteristics. In the V domains the surfaces are largely covered with side chains of hydroxy amino acids, and bulky residues suitable for peptic hydrolysis are generally confined to the interior (see Figure 4). The surface groups in the C domains are more varied, and enzymatic hydrolysis is expected to be less restrictive.

Among the sites that are susceptible to peptic hydrolysis in the V domains of amyloidogenic λ chains are the peptide bonds at the amino ends of residues 4 and 5 (Linke et al., 1973b). While the side chain of Leu-4 in the atomic model of the Mcg protein is oriented toward the interior, the peptide bonds involving both the imino and carbonyl groups of this residue are readily accessible. Hydrolysis in the vicinity of residue 45 would liberate a fragment containing chains 4-1, 4-2, and 3-1, after reduction of the intrachain disulfide bond. If cleavage occurs around residue 80, the released fragment would encompass the entire four-chain layer plus chain 3-1.

The surface characteristics of the Mcg dimer and the degradation studies of amyloidogenic proteins suggest that the four-chain layers of the V domains are the predominant contributors to the antiparallel β-pleated sheets of the amyloid fibrils. Even if denatured during or after enzymatic cleavage of the light chain, the segments of the four-chain layers have the most extensive alternating hydroxy-apolar sequences in the protein, and are well suited for the formation of micelles of highly ordered β-pleated sheets.

The latter argument may also be applied to the thermally induced precipitates of V domains, since the reactions probably include the disruption of existing hydrophobic interactions and subsequent aggregation (Solomon and McLaughlin, 1969). Some ordered structure is maintained or even increased, as evidenced by the enhancement of the characteristic pleated sheet bands in infrared patterns after heat treatment in acidic solutions (Termine et al., 1972). This enhancement has also been noted for enzymes having pleated sheets in their native three-dimensional structures (Termine et al., 1972).

In addition to the differences in the surface structures of the V and C domain dimers, there are other features which probably influence the thermal properties of Bence-Jones proteins. For example, different sets of residues will be involved in the disruption of interactions between like domains: i.e., constituents of the three-chain layers in the V dimer and the four-chain layers in the C dimer. As mentioned earlier, there are fewer hydrophobic interactions between the V domains in the Mcg protein than in the C_1–C_2 dimer.

Within the V and C domains of the Mcg dimer there are differences which may also affect the thermal properties. The layers in the V domains are less distinct and chain 4-1 is sufficiently close to chain 3-3 in one region to form hydrogen bonds between the polypeptide backbones (see Figure 5). These layers are also confluent in the Rei (Epp et al., 1974) and Au (Fehlhammer et al., 1975) V dimers, the murine κ chain of the McPC 603 Fab fragment (Padlan et al., 1973; Segal et al., 1974), and the human λ chain of the New Fab′ fragment (Poljak et al., 1973, 1974). After denaturation by heat treatment, therefore, both layers of the V domains may be in favorable positions to contribute to the observed pleated sheets in the precipitates.

Conclusions

The observations on the denatured, fragmented, and native proteins lead to a fairly comprehensive picture of the structure of an immunoglobulin light chain. The "immunoglobulin fold" of an individual domain is a cylindrical layered structure of β-pleated sheets with a hydrophobic interior. As in the globins, the hydrophobic character of key internal sites is maintained in immunoglobulin domains which differ substantially in amino acid sequences. The differences in sequences between the V and C domains provide a striking example of divergent evolution. The cylinders have rotated in such a way that homologous regions in the two domains perform different functions in their interactions with a second molecule of light or heavy chain. Regions present in only one domain are situated in positions appropriate for their functions, but not deleterious to the general structural integrity of a common fold.

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