Luminescence Studies on Bence-Jones Proteins and Light Chains of Immunoglobulins and Their Subunits†

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ABSTRACT: To provide information on the tertiary structure of the antibody molecule we have investigated the luminescent properties of the light polypeptide chain of human immunoglobulins. The fluorescence and phosphorescence yields, spectra, lifetimes, and anisotropies of a large number of homogeneous light chains, i.e., Bence-Jones proteins and light chains derived from myeloma proteins, were measured. No two proteins gave identical tyrosyl or tryptophyl fluorescence spectra in comparative studies on over 75 proteins belonging to the four basic subgroups of κ chains and of λ chains. Spectral differences were apparent even among proteins exhibiting more than 85% amino acid sequence identity. The fluorescence yields of tyrosine and tryptophan varied 10- and 100-fold, respectively; the Stokes' shift of tryptophan ranged from 328 to 365 nm, but that for tyrosine was apparently invariant (305–307 nm). Emission as well as excitation spectra showed tyrosyl and tryptophyl residues interact minimally or not at all. Fluorescence lifetimes of the tyrosyl and tryptophyl contributions were measured separately, and the apparent natural lifetimes were calculated. Proteins could be grouped in accordance with similarities in fluorescence lifetimes and fluorescence yields; there was no evident relationship between these groupings and the light chain type (κ or λ), amino acid sequence, or tryptophan content. Also apparent were individual differences among κ light chains and among λ light chains in respect to their tyrosyl and tryptophyl phosphorescence spectra and phosphorescence lifetimes. Certain proteins showed an atypical, short-lived tryptophan phosphorescence decay time. Such variance in the luminescent behavior of the tryptophyl residue(s) indicates a conformational interaction between the V and C domains of light chains. Selective proteolytic cleavage of the light chain into V_L and C_L fragments permitted the comparison to be made of the luminescent properties of the V and C domains with those of the whole protein. The V domain and intact protein have luminescent features in common, whereas the C domain possesses features distinctive from that of the native protein. Data derived from fluorescence anisotropy spectral studies of intact light chains and their V_L-related fragments indicate that energy transfer between tryptophyl residues occurs in the C domain. The results of emission spectroscopic measurements performed at 220 and at 77 K indicate that the observed phosphorescence of light chains is mainly from a tryptophyl residue contiguous to a disulfide link. The potential for interdomain interaction in light chains is evidenced by the finding that the orientation of the tryptophyl residue(s) in the V domain can influence the tryptophyl–disulfide link interactions in the C domain; this interaction may account further for the extensive structural diversity of antibody molecules.

With the discovery that Bence-Jones proteins and light polypeptide chains of immunoglobulins were structurally comparable, Bence-Jones proteins were recognized as an important source of homogeneous material for studies on immunoglobulin structure. As summarized by Edelman (1970), the features that make Bence-Jones proteins excellent experimental models for studies on the tertiary structure of immunoglobulin light chains include: "(1) They represent an enormously diverse set of protein sequences. (2) The sequences are disposed around several basic patterns (i.e., κ and λ chains and their subgroups). Each pattern has presumably evolved to serve several functions, the V region for variation in shape, the C region for conservation of shape and interchain binding. (3) There is evidence (Edelman and Gally, 1962) to suggest that each sequence has a different tertiary structure. (4) They may be crystallized, and V regions can be cleaved from C regions and separately crystallized (Solomon and McLaughlin, 1969a)."

Although each Bence-Jones protein has a different primary sequence (implying a different tertiary structure), crystallographic studies have disclosed that all light chains examined possess a common conformational element, the immunoglobulin fold (Poljak et al., 1973). The molecular structures of a human Bence-Jones protein (Schiffer et al., 1973; Edmundson et al., 1974), a human Fab fragment (Poljak et al., 1973, 1974; Amzel et al., 1974), and a murine Fab fragment (Segal et al., 1974) are known in detail. Two compact domains constitute the light chain; the amino-terminal, variant half (V_L)[1] of the light chain constitutes the V domain, and the carboxyl-terminal, constant half (C_L) constitutes the C domain. The two modules or domains are linked by an extended section of the polypeptide chain identical to the previously termed "switch region" (Putnam et al., 1967).

Comparisons of the primary sequence of proteins that have homologous conformations have revealed no more than 15% sequence homology (Dayhoff, 1972). The tertiary structure

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[1] Abbreviations used are: V_L, variant (amino-terminal) half of the light chain; C_L, constant (carboxyl-terminal) half of the light chain; i.d., inside diameter.

of the light chain discloses remarkable conformational homology between the V and C domains for which sequence homology is less than 18% (Edmundson et al., 1975).

Proteins that contain tyrosine and tryptophan luminesce from these residues, and, indeed, the contribution by each component is readily separable from the complete fluorescence or phosphorescence spectra. A diversity of luminescence yields and lifetimes is observed for different proteins, as well as for different conformers of the *same* protein. The major diversity reflects variation in the mode and dispersion of the contribution by tryptophyl residues. The diversity of luminescence spectral character is not a simple consequence of the composition of the protein but rather it is a result of conformational influence.

Edelman and Gally (1962) noted that the fluorescence maximum of individual Bence-Jones proteins was unique. They speculated that the observed diversity was a consequence of differences in tertiary structure among light chains, but that the results of analyses of transition temperatures as a function of pH implied generic similarities in structure and indicated that more detailed studies might result in the discovery of factors that influence both protein structure and protein fluorescence.

We report the results of our investigation of the luminescent properties of a large number of immunochemically-defined (McLaughlin and Solomon, 1972) Bence-Jones proteins and light chains of known partial or complete amino acid sequence. These studies provide new information on the extent of similarity and diversity in the tertiary structure of the light polypeptide chain of human immunoglobulins and indicate relationships among these proteins which are not currently evident from their amino acid sequence.

Materials and Methods

Proteins. The methods for the isolation and purification of Bence-Jones proteins, the cleavage by pepsin of Bence-Jones proteins into V_L- and C_L-related fragments, the subsequent isolation of the V_L and C_L components, and the preparation and isolation of light chains have been described previously (Solomon and McLaughlin, 1969a).

The Bence-Jones proteins and light chains were classified structurally and/or immunochemically as belonging to either the κI, κII, κIII, or κIV basic subgroups of κ light chains or the λI, λII, λIII, or λIV basic subgroups of λ light chains (Milstein and Pink, 1970; Solomon and McLaughlin, 1969b; Schneider and Hilschmann, 1974).

Emission Spectroscopic Measurements. Fluorescence and phosphorescence spectra were obtained by using a laboratory-constructed spectrometer having as essentials a dual-beam digital ratio circuit and Becquerel tuning-fork dual choppers (Longworth and Rahn, 1967; Longworth and Battista, 1970). Phosphorescence intensity decay was monitored with a multichannel analyzer that utilized a mean algorithm to average several memory sweeps. Anisotropy of fluorescence was measured with a dual-prism detection system that incorporated digital ratio circuits for display. Fluorescence lifetimes were determined by a time-interval analysis of single-photoelectron events detected by a delayed-coincidence-timing spectrometer built from standard commercial instrumentation modules. A nanoflash was formed in hydrogen (0.5 atm) between zirconium electrodes in a quartz lamp mounted within a transmission line. The light source was a modification of one supplied by Unilux, Inc. Timing discrimination was achieved through a constant fraction of pulse-height with a differential discriminator functioning in its low-level timing mode. Processed signals were stored in a multichannel analyzer calibrated with a Tennelec, Inc. TAC time calibrator.

For all studies performed at room temperature, the Bence-Jones proteins and light chains were dissolved in water at a concentration of 1 mg/ml and were contained in a 10 × 10 × 45 mm quartz cell. Luminescence studies at liquid nitrogen temperature (77 K) were performed in either equivolume mixtures of water and glycerol or ethylene glycol at a protein concentration of 10 mg/ml with a volume of 0.1 ml in a 3-mm i.d. quartz tube. Fluorescence anisotropy spectra were determined at 220 K from solutions of glycerol–water (1:1) at a protein concentration of 10 mg/ml in a 5 × 5 × 45 mm quartz cell.

Protein fluorescence was excited at 280 nm with 7-nm fwhm and observed with 4-nm fwhm. The fluorescence light measured in anisotropy investigations was isolated with Schott, Inc. colored glass filters that pass light beyond either 305 or 335 nm (as appropriate). Fluorescence lifetimes were determined by exciting at 280 nm fwhm and then isolating the fluorescence through a Corning CS 054 (pass beyond 310 nm). Fluorescence quantum yields were estimated from peak intensities by comparisons with those obtained for proteins and compounds of established yield and are to be regarded as approximate.

Results

Fluorescence Spectra. The fluorescence spectra of κ and λ Bence-Jones proteins and light chains of immunoglobulins were determined at 298 K with excitation at both 280- and 293-nm wavelengths. Light at 293 nm selectively excites tryptophyl residues, whereas light at 280 nm excites both tyrosyl and tryptophyl residues. Tyrosine fluorescence with spectra maxima between 305 and 307 nm was common to all proteins investigated, whereas tryptophan fluorescence had maxima ranging between 328 and 365 nm. These differences in spectral maxima and widths allowed us to separate the differing contributions of tyrosyl and tryptophyl residues, and this separation made feasible the comparative studies of more than 75 light chain fluorescence spectra. Proteins representative of the four basic κ-chain subgroups and four λ-chain subgroups were examined and none of the spectra were found to be identical. The relative proportions of the tyrosine and tryptophan spectral components, as well as the absolute magnitude of each of the components, differed. The tryptophan fluorescence, which is closely related to Stokes' shift, varied in wavelength maximum and peak width. The relative fluorescence yields ranged between 0.02 and 0.24 for tyrosyl residues and between 0.005 and 0.65 for tryptophyl residues (i.e., a 10-fold variation for tyrosine and a 100-fold variation for tryptophan). The width of the tyrosine spectra was 420 ± 10 nm^{-1} and that of the tryptophan spectra ranged between 480 and 620 nm^{-1} with a mean of 565 ± 37 nm^{-1}. No correlation was apparent among peak wavelength, width, yield, or lifetime.

The spectra of four κIII Bence-Jones proteins are presented in Figure 1. The diversity of fluorescence observed within a single subgroup of κ chains is evident. Light chains that possess extensive sequence homologies can be distinguished on the basis of their fluorescent properties as shown in Figure 2. The κI proteins ROY, AG, and AU differ in only 11–13 amino acid residues (Dayhoff, 1972). All κ chains possess a tryptophyl residue at position 35 in the V domain and at position 150 in the C domain. The κI protein AU has an additional tryptophyl residue at position 96 in the third hypervariable region (Wu and Kabat, 1970) of the V domain; similarly, the κI proteins EU and HBJ4 have an additional tryptophyl residue at position 32 in the first hypervariable region of the V domain. The spectra of proteins EU and HBJ4 are also distinct and different

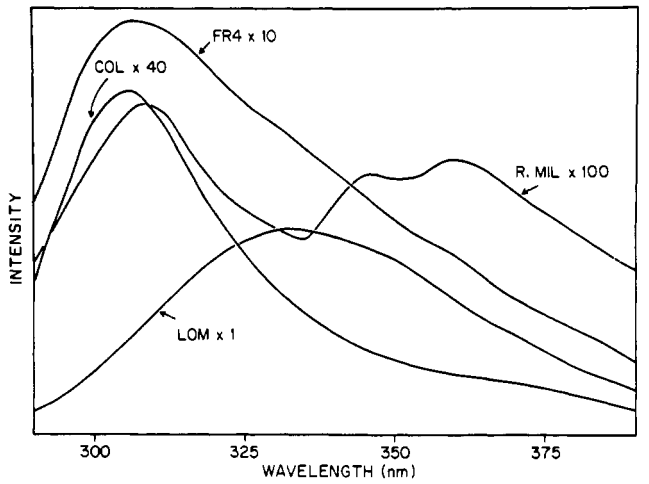

FIGURE 1: Fluorescence of κIII Bence-Jones proteins. Solvent, water (pH 7); temperature, 298 K; excitation wavelength, 280 nm. Protein LOM, gain × 1; protein FR4, gain × 10; protein COL, gain × 40; protein R. MIL, gain × 100.

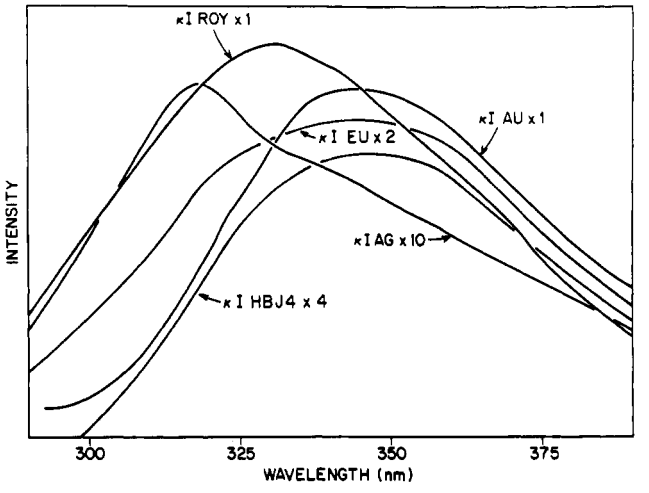

FIGURE 2: Fluorescence of κI Bence-Jones proteins. Solvent, water (pH 7); temperature, 298 K; excitation wavelength, 280 nm. κI AU, gain × 1; κI ROY, gain × 1; κI AG, gain × 10; κI EU, gain × 2; κI HBJ4, gain × 4.

from that of κI AU, as well as from κI ROY and κI AG (see Figure 2). The presence of an additional tryptophan apparently does not contribute to such diversity. A low tryptophan yield was found for certain κI, κII, κIII, and κIV proteins, as well as for certain λ chains belonging to different subgroups. The fluorescence from these proteins was attributable mainly to tyrosyl residues (e.g., see Figure 1, κIII FR4). However, a tryptophan contribution could be seen upon excitation at a wavelength of 293 nm or longer. Certain light chains had unusually large tyrosine yields with more atypical tryptophan yields (e.g., κIII COL); and it was the atypically large tyrosine yield that accounted for the predominant tyrosine fluorescence contribution. Other light chains were found to have a large tryptophan yield (e.g., κIII LOM); and still others had an extremely small yield for both tyrosine and tryptophan (κIII R. MIL). The fluorescence spectra of the two κ light chains examined by Pollet et al. (1972) were similar to the spectrum of κIII Bence-Jones protein COL, and the spectra of their two λ proteins resembled the spectrum of κIII Bence-Jones protein LOM.

Fluorescence Lifetimes. Fluorescence lifetimes were determined for many of our Bence-Jones proteins and light chains. The lifetimes were measured by single-photoelectron time interval analysis through a delayed-coincidence procedure. For proteins exhibiting prominent tyrosine and tryptophan fluorescence, both tyrosine and tryptophan decay components were observed. The majority of fluorescence decays were fitted with a single exponential value. A plot of the apparent tryptophan quantum yield (q_{fl}) at 280-nm excitation vs. the observed tryptophan fluorescence lifetime (τ_{fl}) for 280-nm excitation is shown in Figure 3. An analogous plot was obtained for tyrosine fluorescence. In both instances, the Bence-Jones proteins and light chains can be fitted into similar, but not identical, groups for which the apparent natural tryptophan or tyrosine fluorescence lifetimes ($\tau_0 = \tau_{fl}/q_{fl}$) vary 8-fold. There was no evident relationship between the light chain type (κ or λ) or V region subgroup and the apparent natural fluorescence lifetimes of either tryptophan or tyrosine.

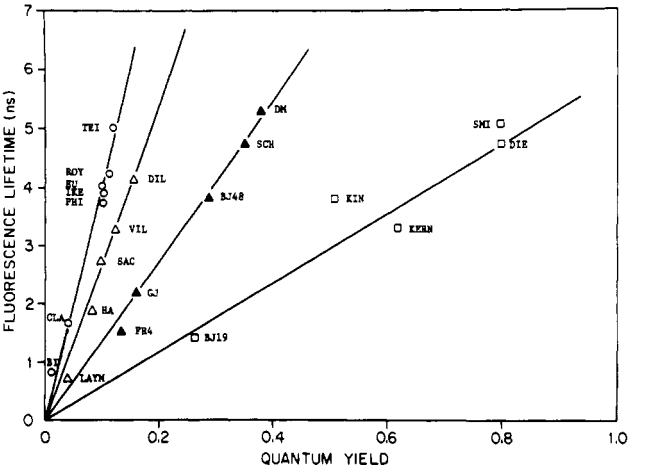

FIGURE 3: Fluorescence lifetimes of Bence-Jones proteins plotted vs. absolute quantum yields of fluorescence. Solvent, water (pH 7); temperature, 298 K; excitation wavelength, 280 nm. □, △, ▲, ○ represent individual κ and λ Bence-Jones proteins and light chains as follows: Bence-Jones proteins BI, CLA, ROY, TEI, SAC, BJ48, DM, BJ19, KIN, DIE, SMI, and light chain EU are κI proteins; Bence-Jones protein LAYM is a κII protein; Bence-Jones proteins FR4, DIL, IKE, PHI, SCH, and light chain GJ are κIII proteins; Bence-Jones proteins HA, VIL, and KERN are λI, λII, and λIV proteins, respectively.

Phosphorescence Spectra. Tyrosine and tryptophan phosphorescence spectra of more than 30 κ and λ Bence-Jones proteins were studied and as in the temperature fluorescence studies, the contributions of tyrosyl and tryptophyl residues were determined separately. The proportions of tyrosine and tryptophan spectral components, the Stokes' shift of tryptophan phosphorescence, and the width of the fine-structure peaks varied significantly among the proteins examined. A diffuse structure was noted in the tyrosine spectra of some proteins but not in others. The fine structure of tryptophan phosphorescence was absent in certain proteins such as κII LAYM (Figure 4).

Phosphorescence Lifetimes. The phosphorescence lifetimes of the tyrosine and tryptophan contributions were measured separately by the selection of appropriate excitation wavelength. Tyrosine lifetimes were a mixture of 0.82 ± 0.33 and 2.35 ± 0.65 s with either component the major species. Tryptophan lifetimes were either 1.00 ± 0.30 s, or a mixture of 0.95 ± 0.45 and 7.17 ± 2.10 s with the major species frequently being the long component. Of the κ and λ Bence-Jones proteins examined, each possessed a distinct tyrosine and tryptophan lifetime and a distinct short- and long-lived fractional com-

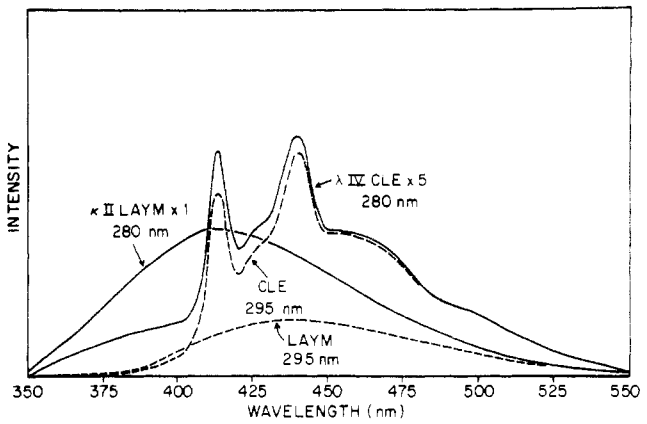

FIGURE 4: Phosphorescence of Bence-Jones proteins. Solvent, 1,2-ethanediol-water (pH 7); temperature, 77 K; excitation wavelengths, 280 and 295 nm. κII LAYM, gain × 1; λIV CLE, gain × 5; (—) 280 nm; (- - - -) 295 nm.

ponent. When the major species were the short-lived tyrosine and tryptophan components, the spectra were typified by the absence of fine structure.

Luminescence of Subunits of Bence-Jones Proteins. The ability to cleave selectively light chains into their respective V and C domains has permitted comparative studies of the V_L, C_L, and the intact light chain (Solomon and McLaughlin, 1969a; Karlsson et al., 1969). The κIV Bence-Jones protein LEN (Schneider and Hilschmann, 1974) was cleaved by pepsin and the V_L- and C_L-related fragments isolated for fluorescence studies. The fluorescence spectra of intact protein LEN and LEN V_L were similar; however, the fluorescence spectrum of LEN C_L differed from that of the intact protein LEN and LEN V_L by having greater tyrosine and tryptophan yields, as well as a larger Stokes' shift of tryptophan (Figure 5). The phosphorescence of intact protein LEN and LEN V_L had a peak at 411 nm, whereas the phosphorescence of LEN C_L had a peak at 409 nm. The long tryptophan decay time was 6.93 ± 0.01 s for intact protein LEN and 5.66 ± 0.003 s for LEN V_L, while LEN C_L had a long component lifetime of 7.11 ± 0.007 s. The phosphorescence of tyrosine for LEN V_L was comparable to that for LEN C_L but differed from that for intact protein LEN; the tyrosine decay times were 2.5, 2.6, and 2.2 s for V_L, C_L, and intact LEN, respectively. The V_L and/or C_L of κI, κIII, and certain λ Bence-Jones proteins were also examined. The luminescence of the V_L had many features in common with that of the intact light chain, whereas the C_L possessed markedly different features.

Discussion

In general, aromatic amino acids show a high degree of conservatism in sequence position or conformation. Conserved positions for tryptophyl residues have been found in all globins, cytochromes, flavodoxins, neurotoxins, dehydrogenases, lysozymes, and serine proteases. Likewise, the light chains of immunoglobulins possess conserved tryptophyl residues that are homologous to tryptophyl residues in the heavy chain domains (Dayhoff, 1972). Because tryptophyl residues occur infrequently in a protein sequence, tryptophan provides a unique spectroscopic probe.

We have examined the fluorescence spectra of over 75 light chains belonging to the four basic κ and λ chain subgroups and, in no instance, have we found two proteins with identical spectra; however, similarities in fluorescence lifetimes and fluorescence yields were observed. Furthermore, significant spectral differences were found among proteins of the same subgroup that had more than 85% sequence homology. Although an eightfold variation was found in the apparent natural lifetimes of Bence-Jones proteins, a separate study of the tryptophan fluorescence of a homologous family of globular proteins, the serine proteases, did not disclose such a wide variation. A similar investigation of the lysozyme–lactalbumin family revealed natural lifetime variations analogous to those noted for the light chains. The serine proteases possess a common natural lifetime comparable to that found for indole compounds. In order to explain the wide variation exhibited by Bence-Jones proteins and the lysozyme–lactalbumin family, it is necessary to consider the possibility of either an energy transfer between tyrosyl and tryptophyl residues or the presence of highly quenched tyrosyl and tryptophyl residues that make a negligible contribution to the total fluorescence. The former explanation does not apply to lysozyme because of its large tryptophan content. Chemical modification studies have shown that only two of the six tryptophyl residues of hen lysozyme fluoresce appreciably. By homologous conformational consideration, only one of the five tryptophyl residues in human lysozyme fluoresces. The fluorescence of tryptophyl residues in lysozyme is probably influenced by two quenching mechanisms, namely, the interaction of tryptophyl residues with lysyl residues or with intrachain disulfide links.[2]

The crystallographic analyses of the λI Bence-Jones protein MCG by Schiffer et al. (1973) and of the Fab′ fragment of λ NEW by Poljak et al. (1973) clearly reveal an interaction between a tryptophyl residue and a disulfide link in both V and C domains of the light chain and, parenthetically, an analogous interaction occurs in the two homologous heavy chain domains. From the observation that the phosphorescence spectra of certain Bence-Jones proteins (e.g., κII LAYM, Figure 4) are similar to that of human lysozyme, it may be inferred that a tryptophan–disulfide link interaction exists in such proteins analogous to that postulated in human lysozyme. Phosphorescence spectra of all the κ and certain of the λ Bence-Jones proteins studied, as well as that of human lysozyme, show an atypical tryptophan phosphorescence shortened decay time and no apparent fine structure. Since the C domains of all κ chains and the C domains of all λ chains possess an essentially identical amino acid sequence (implying identical C domain conformation), one might expect an atypical phosphorescence

[2] In model compound studies, Cowgill (1967) investigated the influence of disulfide links upon the fluorescence of tryptophan and, indeed, showed that the fluorescence of tryptophan was significantly quenched by disulfide links. We studied the luminescence properties of bisindole methylene disulfide and found a significant perturbation of indole phosphorescence as evident by an absence of fine structure, a shortened and nonexponential decay, and an unusually small yield. The fluorescence yield at 77 K was also small. A similar propinquity of an indole ring and disulfide exists in hen lysozyme, i.e., tryptophan at position 63 is contiguous both in sequence and conformation to a disulfide link. Human lysozyme has an atypical phosphorescence spectrum characterized by an absence of detectable fine structure and a significantly shortened luminescence decay. Denaturation with 5 M guanidinium hydrochloride restores the typical tryptophan behavior to human lysozyme. Chemical modification studies have shown that fluorescence of hen lysozyme originates from tryptophyl residues at positions 62 and 108. Since human lysozyme lacks a tryptophyl residue at position 62, by analogy one may suggest that only the tryptophyl residue at position 108 fluoresces in the human lysozyme. Because of the short phosphorescence decay time found in both hen and human lysozyme, it appears that the observed phosphorescence is largely from the tryptophyl residue contiguous to a disulfide link. Therefore, for hen lysozyme we suggest that the tryptophyl residue at position 108, which is conformationally contiguous to the tryptophyl residue at position 63, transfers its triplet energy to the tryptophyl residue at position 63 which then becomes the source of phosphorescence.

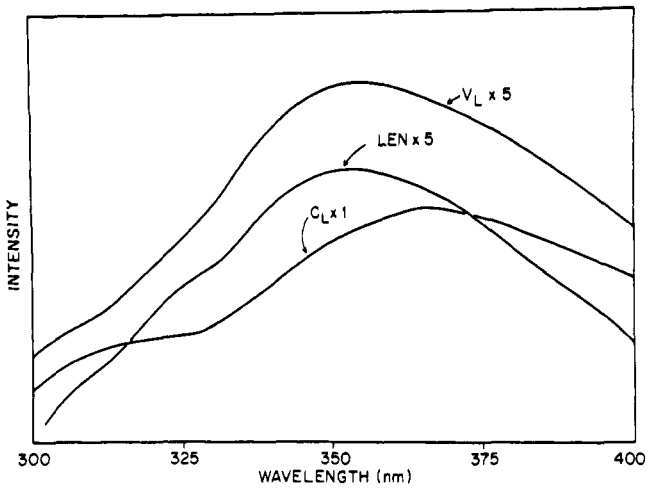

FIGURE 5: Fluorescence of the κIV Bence-Jones protein LEN and its V_L- and C_L-related fragments. Solvent, water (pH 7); temperature, 298 K; excitation wavelength, 280 nm. LEN, gain × 5; V_L, gain × 5; C_L, gain × 1.

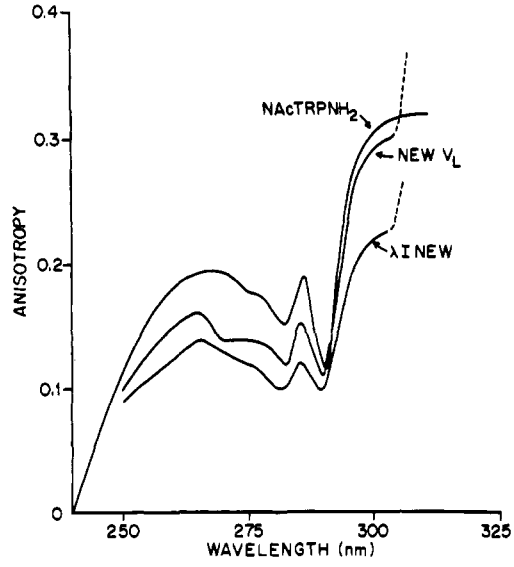

FIGURE 6: Fluorescence anisotropy of a Bence-Jones protein and its V_L-related fragment. Solvent, 1,2-propanediol–water (pH 7); temperature, 220 K, $NAcTRPNH_2$, *N*-acetyl-L-tryptophanamide.

component from all light chains. The observed diversity of luminescence characteristics must, therefore, reflect variance in the behavior of the tryptophyl residue(s) in the V domain. The absence of a short-lived phosphorescence in certain λ chains suggests that the different conformations of their C domains are a result of interdomain (V–C) interactions. The crystallographic data on the dimeric λ protein MCG (Schiffer et al., 1973) and on the Fab′ fragment of λ NEW (Poljak et al., 1973) reveal relatively few interdomain interactions, as compared with the intradomain interactions created by the V–V or C–C dimeric association. Consequently, it is possible that the lack of a pronounced short-lived phosphoresce in λ chains is a consequence of their dimeric form.

At least one additional tryptophyl residue is present in λ chains. We have studied the λIII protein SH (Dayhoff, 1972) that has three tryptophyl residues with the third tryptophan located in the C domain vicinal to the interchain disulfide link of the λ dimer (Schiffer et al., 1973). The tryptophan phosphorescence decay times of 1.85 and 7.68 s indicate that at least one tryptophyl residue probably interacts with a disulfide link.

Fluorescence anisotropy spectral studies were performed on the λI Bence-Jones protein NEW (Dayhoff, 1972) that has four tryptophyl residues (two in each domain) and evidence of energy transfer between tryptophyl residues was found. However, the study of the isolated V_L fragment of λI NEW did not reveal evidence of such electronic energy transfer. Thus, it would seem that if no significant conformational change affecting the transfer process occurs in the isolated V domain, the observed transfer must take place between tryptophans in the C domain because the distance is too great for efficient interdomain transfer (Schiffer et al., 1973). The fluorescence anisotropy of λI NEW and the V_L fragment of protein NEW is shown in Figure 6. We infer from the lower anisotropy of the intact protein NEW that energy is transferred between tryptophyl residues in this protein. Thus, the phosphorescence of C_λ, like that of C_κ, is from a tryptophyl residue located near the intradomain disulfide link.

The presence of variable fractions of radiationless tryptophyl residues in both domains is apparently responsible for the large natural lifetimes observed for certain light chains. One of our proteins (κI SMI) that had a high tryptophan fluorescence was studied at 77 K and two tryptophan phosphorescence lifetimes (0.8 and 6.5 s) were noted. This finding suggests that a quenched tryptophan is present in the C domain even though this protein was one of the most highly fluorescent Bence-Jones proteins that we examined. Analogous results have been observed for tyrosyl residues; we will discuss elsewhere the details of tyrosine luminescence of light polypeptide chains.

The fluorescence spectra of light chains are extremely diverse. The major source of this diversity results primarily from the different conformations of the V domain. Cowgill (1970) showed that orientation is a crucial element in the nature of the interaction between indole excited states and disulfide. We have found that at low temperature the vicinal disulfide link has no influence upon the fluorescence or phosphorescence. Thus, it appears that small variations in the orientation of the tryptophyl residue can significantly modify the nature of the interaction between that residue and the disulfide link. This finding assumes special importance because the crystallographic data indicate that certain V region aromatic amino acid residues (including the invariant tryptophyl residue located at positions 35 and 37 in the V domains of all κ and λ light chains, respectively) form an integral part of the antibody-combining site and also serve as contact residues in the binding of ligands (Edmundson et al., 1974; Amzel et al., 1974; Segal et al., 1974; Epp et al., 1974; Fehlhammer et al., 1975).

Interactions between the V and C domains of light chains may account further for the observed spectral diversity among light chains. The potential ability of the V domain to modify the C domain has been evident crystallographically (Colman et al., 1976) and immunochemically (reviewed in Solomon, 1976). Interdomain interaction was especially evident in our phosphorescence studies of dimeric forms of light chains, i.e., λ chains and certain κ chains. Light chains of human immunoglobulins of the IgA2, *Am2* subclass (a class of immunoglobulins found predominantly in secretions) exist as covalently-linked dimers and lack the characteristic disulfide bond to heavy chains (Grey et al., 1968; Jerry et al., 1970). The functional significance of the dimeric form of light chains has become evident through x-ray diffraction data on the structure and ligand-binding properties of crystals formed from the λ Bence-Jones protein dimer MCG (Schiffer et al., 1973; Edmundson et al., 1974; Edmundson et al., 1975). These studies revealed differences in the conformation of the monomeric

subunits (monomer 1 and monomer 2 resembled the Fab portion of the heavy chain and the light chain, respectively) and provided evidence that the dimeric light chains may serve as primitive forms of antibody. To relate structural and functional properties of light chains, we plan to extend our spectroscopic studies to include those light chains from which crystallographic data have been obtained and those light chains derived from monoclonal immunoproteins with defined ligand-binding (antibody) activity.

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