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# Bence Jones Proteins and Light Chains of Immunoglobulins

## XIV. Conformational Dependency and Molecular Localization of the Kappa (κ) and Lambda (λ) Antigenic Determinants

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Solomon, A. Bence Jones Proteins and Light Chains of Immunoglobulins. XIV. Conformational Dependency and Molecular Localization of the Kappa (κ) and Lambda (λ) Antigenic Determinants. *Scand. J. Immunol.* 5, 685-695, 1976.

The region on the light chain molecule responsible for expression of the κ and λ antigenic determinants was determined by comparative immunochemical analyses of intact Bence Jones proteins and naturally occurring or enzymatically derived fragments of Bence Jones proteins that lacked extensive portions of the V region or part of the C region. The reactivity of these fragments with numerous antisera having specificity for light-chain antigenic determinants indicated the essentiality of the intact light polypeptide chain for expression of the κ and λ antigenic determinants. The conformational dependency of the κ and λ antigenic determinants was also evidenced by denaturation-renaturation studies on κ and λ chains. The V domain, C domain, and interdomain 'switch' region contribute to the expression of κ or λ antigenicity and to certain isotypic and allotypic specificities.

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Immunochemical and structural analyses (9, 13, 27, 34) of Bence Jones proteins, myeloma proteins, and Waldenström macroglobulins have provided evidence that the light polypeptide chains of all immunoglobulins consist of two chemically distinct types designated kappa (κ) and lambda (λ). As evident from sequence analyses of homogeneous κ and λ light chains — that is, Bence Jones proteins (12) — both types of light chains share certain characteristic structural features: each type has an amino-terminal portion of ∼107 residues of variant sequence ($V_L$) and a carboxyl-terminal portion of ∼107 residues of constant sequence ($C_L$). The $V_L$ and $C_L$ are under separate genetic control (17) and exist as two compact domains (designated V and C, respectively) linked by an extended ∼10-residue section of the light polypeptide chain termed the 'switch' region (31, 35). A given light chain may be distinguished as κ or λ on the basis of the amino acid sequence of either the V *or* C domain (9). However, the domain or region on the light chain responsible for the immunochemical recognition of the κ or λ antigenic determinants has not been established; it is not known whether these determinants reside on the $C_L$ or $V_L$ or whether their expression requires the intact light polypeptide chain.

The relation between immunoglobulin structure and antigenicity has been established through studies of the naturally occurring or enzymatically derived fragments and the chemically produced subunits of homogeneous immunoglobulins (Bence Jones proteins, myeloma proteins, and Waldenström macroglobulins) characteristically found in association with multiple myeloma and related plasma cell-lymphocyte dyscrasias (43). The identification and characterization of urinary low-molecular-

weight proteins antigenically related to the V or C regions of Bence Jones proteins (2, 4, 7, 10, 44, 48, 52, 53, 56), the availability of unusual Bence Jones proteins with extensive V region deletions (15, 42), and the ability to cleave specifically Bence Jones proteins into $V_L$- and $C_L$-related fragments (20, 45) have provided the means for determining immunochemically the region on the light-chain molecule responsible for the $\varkappa$ and $\lambda$ antigenic determinants.

## MATERIALS AND METHODS

*Proteins.* Urine samples containing Bence Jones proteins and fragments of Bence Jones proteins were obtained from our patients with multiple myeloma. The specimens were collected without preservative and were maintained at 0°C to 4°C throughout the 24-h collection period. Subsequently, a sample of each 24-h specimen was frozen and stored at −20°C or −70°C. The remaining urine specimen was lyophilized after extensive dialyses at 4°C in 23/32 Visking tubing (Union Carbide Corp., New York) against deionized double-distilled water.

*Preparative procedures.* Bence Jones proteins and fragments of Bence Jones proteins were isolated from urine specimens by zone electrophoresis on starch or Pevikon blocks and then purified by gel filtration through polyacrylamide columns as previously described (45). The $\lambda$ chain Mcg (14), $\lambda$ chain Sm (15), and $\varkappa$ chain Sac (42) were furnished by Dr. Allen B. Edmundson, Dr. Elliott F. Osserman, and Dr. Dorothy M. Parr, respectively. The methods used for the preparation and isolation of light chains and of the papain-derived Fab fragment of FII $\gamma$G-globulin were as described previously (45).

The Bence Jones proteins were cleaved by pepsin into constant half-related ($C_L^P$) and variant half-related ($V_L^P$) fragments (45). The digestions were performed with an enzyme to protein ratio of 1:100 in 0.05M glycine-HCl buffer, pH 3.4, at 37° C (or at 55° C) and terminated by raising the pH of each reaction mixture to neutrality by adding 1M Tris base. These light chain fragments were isolated by zone electrophoresis and purified by gel filtration (45).

*Immunochemical procedures.* Antisera were prepared in albino New Zealand rabbits to $\varkappa$- and $\lambda$-type Bence Jones proteins, to the light chains and the Fab fragment derived from FII $\gamma$G-globulin, and to intact FII $\gamma$G-globulin. The methods used for the preparation of antisera and for the immunoelectrophoretic and immunodiffusion analyses were as described previously (46). The Inv typing of $\varkappa$ chains was performed by Dr. Arthur G. Steinberg.

## RESULTS

Urine specimens from certain of our patients with multiple myeloma and Bence Jones proteinuria who have received high doses of corticosteroids as part of their treatment regimen were found to contain a low-molecular-weight protein related to the Bence Jones proteins but not identical to either the $V_L$ or the $C_L$ (48). The new components, designated $C_L^*$, were found to be structurally and antigenically most closely related to the carboxyl-terminal half of the light polypeptide chain, although each component was 6 to 25 amino acid residues longer than the $C_L$-related fragment derived in vitro by peptic cleavage ($C_L^P$) of the light chain (48). The amino-terminal sequences of $C_L^*$ isolated from urine specimens of Patients Wms, Oak, and Edw and the $C_L^P$ prepared by pepsin cleav-

Table I. In vivo ($C_L^*$) and in vitro ($C_L^P$) constant-half-related fragments of $\varkappa$ Bence Jones proteins

| Protein | Amino-terminus | |
|---|---|---|
| | Residue | Position† |
| $C_L^*$ Wms | Asp | 92 |
| $C_L^*$ Oak | Thr/Ile | 97/106†† |
| $C_L^*$ Edw | Val | 110 |
| $C_L^P$ Len | Ile | 117 |

† The numbers indicate the position of the residues along the amino acid sequence of $\varkappa$ Bence Jones protein Roy (9).

†† Protein Oak consisted of two components of approximately equal concentration; the amino-termini of components one and two corresponded to positions 97 and 106, respectively.

Table II. Amino-terminal sequences of κ Bence Jones protein fragments

| | 92 | | | | | | 100 | | | | | | V/C ↓ | 110 | | | | | | 120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_L^*$ Wms | Asp | Ile | Phe | Pro | Gly | Thr | Phe | Gly | Gln | Gly | Thr | Lys | Val | Glu | Ile | Lys | Arg | Thr | Val | Ala | Ala | Pro | Ser | Val | Phe | Ile | Phe | Pro | Pro |
| $C_L^*$ Oak 1 | | | | | | | | | — | — | — | — | Phe | Gly | — | — | — | — | — | — | — | — | — | — | — | — | — | — | † |
| $C_L^*$ Oak 2 | | | | | | | | | | | | | | | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| $C_L^*$ Edw | | | | | | | | | | | | | | | | | | | — | — | — | — | — | — | — | — | — | — | — |
| $C_L^P$ Len | | | | | | | | | | | | | | | | | | | | | | | | | | — | — | — | — |

† The solid lines indicate sequence identity to $C_L^*$ Wms.

age of Bence Jones protein Len were determined by Dr. J. Donald Capra. The amino-terminal residues and sequences of the $C_L^*$ components and the $C_L^P$ fragment are given in Tables I and II.

The reactivity of the $C_L^*$ components Wms, Oak, and Edw and the $C_L^P$ Len fragment was compared with that of intact κ Bence Jones proteins Wms, Oak, Edw, and Len by immunoelectrophoretic analyses of urine specimens Wms, Oak, and Edw (containing both $C_L^*$ and Bence Jones protein) and of a sample of Bence Jones protein Len cleaved partially by pepsin into $C_L^P$ and $V_L^P$ (45). Antisera prepared against the homologous Bence Jones proteins Wms, Oak, Edw, and Len, and antisera prepared against 35 heterologous κ Bence Jones proteins were utilized for these analyses. With anti-homologous protein antisera, the $C_L^*$ components and the $C_L^P$ fragment were antigenically deficient as compared with the intact Bence Jones protein (Fig. 1); absorption of these antisera with a heterologous κ chain left reactions only with the homologous intact Bence Jones proteins. Striking differences in the reactivity of the $C_L$-related components were found with the anti-heterologous κ chain antisera. Certain antisera reacted weakly or not at all with the $C_L^*$ components and the $C_L^P$ fragment. Differences were also evident in the intensity of the precipitin arcs formed by the different $C_L^*$ components. The results obtained with the homologous and two representative heterologous anti-κ chain antisera are also shown in Fig. 1. With the anti-κ Isr antiserum, the $C_L^*$ components and the $C_L^P$ fragment reacted with equal intensity, each forming a precipitin reaction of identity with the intact Bence Jones protein. However, the anti-κ Bre antiserum did not react at all with $C_L^P$ Len and reac-

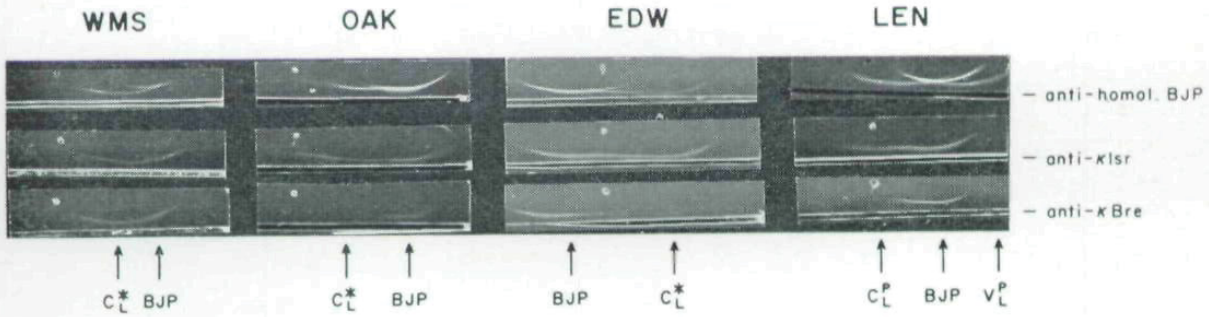



Fig. 1. Immunoelectrophoretic analyses of intact polypeptide chains and related fragments of four κ Bence Jones proteins. The antigen wells under the designations *WMS*, *OAK*, and *EDW* contained urine specimens from Patients Wms, Oak, and Edw, respectively; the antigen wells under the designation *LEN* contained a sample of Bence Jones protein Len subjected to limited pepsin proteolysis at 37°C. Each sample was tested against an antiserum prepared to the homologous Bence Jones protein (*anti-homol. BJP*) and against antisera prepared to heterologous κ Bence Jones proteins Isr and Bre (*anti-κ Isr* and *anti-κ Bre*). *BJP* = Bence Jones protein; $C_L^*$ = constant-half-related light-chain component found in vivo; $C_L^P$ and $V_L^P$ = constant-half- and variant-half-related light-chain fragments, respectively, produced in vitro by pepsin proteolytic cleavage of Bence Jones protein. The anode was located to the left.

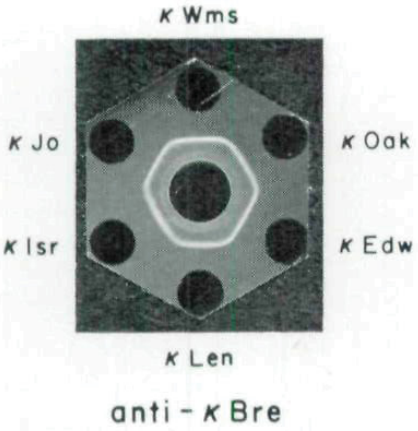



Fig. 2. Immunodiffusion analysis of six κ Bence Jones proteins. The peripheral antigen wells contained six different anti-κ Bence Jones proteins (κ *Wms*, κ *Oak*, κ *Edw*, κ *Len*, κ *Isr*, and κ *Jo*). The central antiserum well contained an antiserum prepared against κ Bence Jones protein Bre (*anti-κ Bre*).

ted only weakly with $C_L^*$ Edw and $C_L^*$ Oak, but a strong reaction occurred with $C_L^*$ Wms. The anti-κ Bre antiserum was a potent anti-κ antiserum; the precipitin reactions obtained with six κ Bence Jones proteins are shown in the immunodiffusion analysis depicted in Fig. 2. Differences in the reactivity among the $C_L^*$ components were evident with many other anti-κ-chain antisera; generally, the intensity of precipitin reactions among the $C_L$-related fragments followed the order: $C_L^*$ Wms > $C_L^*$ Oak > $C_L^*$ Edw > $C_L^P$ Len.

Other types of antisera with specificity for κ antigenic determinants also showed this same pattern of reactivity with the $C_L$-related components, as illustrated in Fig. 3 by the reactions obtained with antisera to intact FII γG-globulin and its subunits. The anti-FII light chains, anti-FII Fab, and anti-FII γG-globulin antisera did not form visible precipitin reactions with $C_L^P$ Len, and reacted only weakly with $C_L^*$ Edw and $C_L^*$ Oak; a more intense precipitin reaction occurred with $C_L^*$ Wms. All three antisera reacted strongly with intact κ (as well as intact λ) chains and, further, had the capacity to distinguish between κ and λ light chains. The results of immunodiffusion analysis of three κ and three λ Bence Jones proteins with the anti-FII Fab antiserum are shown in Fig. 4.

We have not as yet detected $C_L^*$-type components in urine specimens from our patients excreting λ Bence Jones proteins. For this reason we treated six different λ Bence Jones proteins with pepsin at 37°C or at 55°C (40) to generate $C_L^P$ components in an effort to test the reactivity of our anti-λ chain antisera with $C_L$-related fragments. The reactivity of intact λ

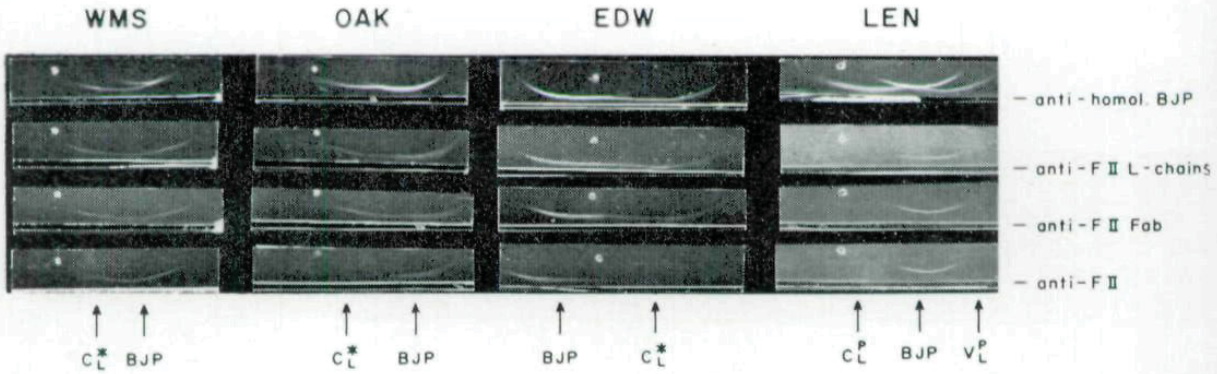



Fig. 3. Immunoelectrophoretic analyses of intact polypeptide chains and related fragments of four κ Bence Jones proteins. The antigen wells under the designations *WMS*, *OAK*, and *EDW* contained urine specimens from Patients Wms, Oak, and Edw, respectively; the antigen wells under the designation *LEN* contained a sample of Bence Jones protein Len subjected to limited pepsin proteolysis at 37°C. Each sample was tested against an antiserum prepared to the homologous Bence Jones protein (*anti-homol. BJP*) and against antisera prepared to the light chains of FII γG-globulin (*anti-FII L-chains*), the papain-derived Fab fragment of FII γG-globulin (*anti-FII Fab*), and to FII γG-globulin (*anti-FII*). *BJP* = Bence Jones protein; $C_L^*$ = constant-half-related light-chain component found in vivo; $C_L^P$ and $V_L^P$ = constant-half- and variant-half-related light-chain fragments, respectively, produced in vitro by pepsin proteolytic cleavage of Bence Jones protein. The anode was located to the left.

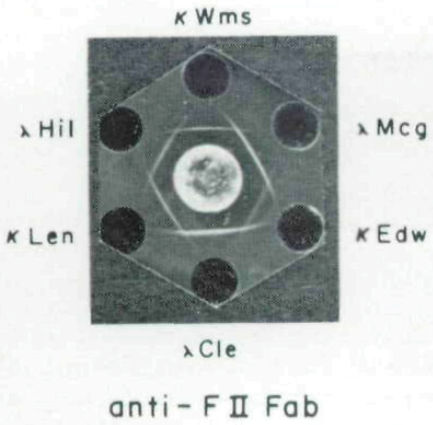



Fig. 4. Immunodiffusion analysis of three κ and three λ Bence Jones proteins. The peripheral antigen wells contained three κ Bence Jones proteins (κ *Wms*, κ *Edw*, and κ *Len*) and three λ Bence Jones proteins (λ *Mcg*, λ *Cle*, and λ *Hil*). The central well contained an antiserum prepared against the papain-derived Fab fragment of FII γG-globulin (*anti-FII Fab*).

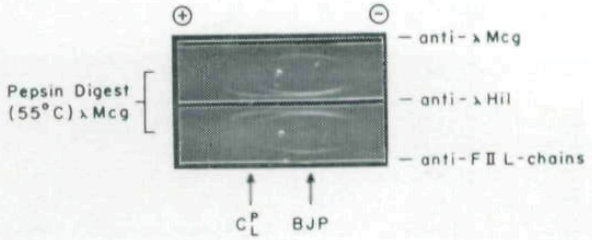
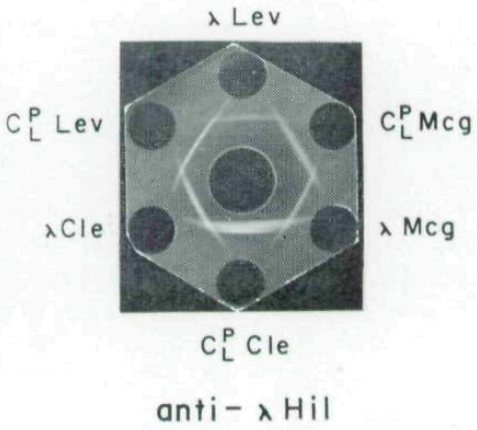
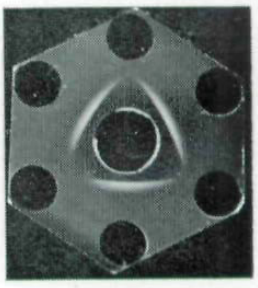



Fig. 5. Immunoelectrophoretic analysis of intact polypeptide chain and constant-half-related fragment of a λ Bence Jones protein. The antigen wells contained a sample of λ Bence Jones protein Mcg subjected to limited pepsin proteolysis at 55°C. The upper, middle, and lower antiserum troughs contained antisera prepared against λ Bence Jones protein Mcg (*anti-λ Mcg*), λ Bence Jones protein Hil (*anti-λ Hil*), and the light chains isolated from FII γG-globulin (*anti-FII L-chains*), respectively.

Bence Jones protein Mcg and $C_L^P$ Mcg (formed by partial peptic proteolytic cleavage of protein Mcg) is presented in Fig. 5. This immunoelectrophoretic analysis shows that the antiserum prepared to Bence Jones protein Mcg reacted only with the intact protein and not with the $C_L^P$ component. The antiserum prepared to λ Bence Jones protein Hil recognized both intact protein Mcg and its $C_L^P$, and, although not evident in the figure, the $C_L^P$ component formed a precipitin reaction of nonidentity with the intact Bence Jones protein. None of 20 antisera prepared against other λ Bence Jones proteins formed a visible precipitin reaction

Fig. 6. Immunodiffusion analysis of intact polypeptide chains and constant-half-related fragments of three λ Bence Jones proteins. The peripheral antigen wells in both patterns contained intact λ Bence Jones proteins (λ *Lev*, λ *Mcg*, and λ *Cle*) and their 55°C pepsin-derived constant-half-related fragments ($C_L^P$ *Lev*, $C_L^P$ *Mcg*, and $C_L^P$ *Cle*). The central well in the pattern on the left contained an antiserum prepared against λ Bence Jones protein Hil (*anti-λ Hil*). The central well in the pattern on the right contained the same antiserum absorbed with intact λ Bence Jones protein Lev (*anti-λ Hil* $\bar{A}$).



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