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Bence Jones Proteins: A Powerful Tool for the Fundamental Study of Protein Chemistry and Pathophysiology†

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On November 1, 1845, Dr. Henry Bence Jones received from Dr. Watson a specimen and an accompanying note which stated, "The tube contains urine of very high specific gravity; when boiled it becomes highly opake; on the addition of nitric acid it effervesces, assumes a reddish hue, becomes quite clear, but, as it cools, assumes the consistence and appearance which you see: heat reliquifies it. What is it?" (Jones, 1848). The answer was not elementary. Although the substance subsequently known as Bence Jones protein was chemically analyzed, its unusual temperature-dependent solubility described, and its potential diagnostic relevance for the hematopoietic neoplasm now known as multiple myeloma predicted, more than 100 years passed before Bence Jones protein was identified as the light-chain component of human immunoglobulin (Edelman & Gally, 1962).

Bence Jones proteins are typically found in patients with monoclonal plasma cell or related B-cell immunoproliferative disorders, i.e., multiple myeloma, light-chain-associated amyloidosis (amyloidosis AL), and, occasionally, benign gammopathy, lymphoma, and leukemia. Because of their monoclonal origin and resulting chemical homogeneity, as well as their ready availability, much of the fundamental information on immunoglobulin structure came initially from analysis of Bence Jones proteins and light chains isolated from monoclonal immunoglobulins. Amino acid analyses of these components revealed an N-terminal variable (V) and a C-terminal constant (C) domain (Hilschmann & Craig, 1965; Titani & Putnam, 1965), a major conceptual step in understanding the molecular basis of antibody function. Continued study of light chains helped identify within the V domain the hypervariable segments that account for the specificity and diversity of antibodies (Wu & Kabat, 1970). Over the past 3 decades, the primary structures of hundreds of light chains (complete and partial), from human and other sources, have been determined, aligned, and archived (Kabat et al., 1987).

Bence Jones proteins and V_L dimers were the crystallizable homogeneous proteins which provided much of the early three-dimensional conformational data that helped explain the structural basis of antibody function (Schiffer et al., 1973; Epp et al., 1974; Fehlhammer et al., 1975; Colman et al., 1977). Remarkably, a single Bence Jones protein in two solvent systems (low and high ionic strength) (Chang et al., 1985; Schiffer et al., 1989) exhibited significant differences in the interactions of the two monomeric subunits comprising the dimer, resulting in substantial variation in the structure of the antigen-combining site under the two solution conditions. This observation led to a prediction that heterogeneity of domain interactions may contribute to antibody–antigen interactions (Stevens et al., 1988). Experimental support for this hypothesis has come from comparisons of the detailed structures of antibody–antigen complexes (Colman, 1988; Bhat et al., 1990). The interactions at the interface of the V_L dimer are a function of primary structure and solution conditions. Study of these interactions has led to new information on the relationship between protein structure and function and, as discussed below, can account for specific pathophysiological properties associated with Bence Jones proteins.

The variability in V_L primary structure accounts for the diverse and idiosyncratic pathological characteristics of Bence Jones proteins. In many patients, the morbidity of the disease is aggravated by apparent structure-dependent polymerization and deposition tendencies of these proteins, which lead to organ impairment and failure (Solomon, 1982, 1986; Feiner, 1988; Gallo et al., 1989; Buxbaum et al., 1990). The molecular mechanisms leading to pathological phenomena that include cast formation in nephron tubules, and to polymerization and deposition on tubular and capillary basement membranes, or to amyloid formation accompanied by systemic or organ-specific deposition, are not understood. Although unusual temperature-dependent solubility (Jones, 1848; Putnam et al., 1959) and polymerization (Neet & Putnam, 1966) properties of Bence Jones proteins have been previously characterized, the relationship of these phenomena to the in vivo toxicity has not been evident. Through the use of an experimental animal system in which human Bence Jones proteins are injected into

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mice, we have shown that these proteins can be deposited in the mouse kidney as casts, basement precipitates, crystals, or as amyloid, comparable to that found in the kidneys of the patients from whom the Bence Jones proteins were obtained. Further evidence for the central role of Bence Jones proteins in the histopathological process has been evidenced by the fact that injection into mice of clinically nonnephrotoxic Bence Jones proteins fails to induce significant nephropathology (Solomon & Weiss, 1988; Solomon et al., 1991).

In an attempt to account for the capability of certain Bence Jones proteins to form in vivo proteinaceous casts within renal tubules, we have applied an HPLC technique to determine the tendency of these proteins to polymerize in vitro and have found a significant correlation between in vitro aggregation characteristics of these proteins and their in vivo deposition tendency. We posit that these oligomerization properties of Bence Jones proteins are involved in the mechanisms underlying light-chain deposition or amyloid formation (Stevens et al., unpublished results). To date, our studies have shown that Bence Jones proteins obtained from patients with light-chain-associated nephropathy are capable of higher order aggregation (presumably through interaction between dimers) under size-exclusion chromatography conditions that are representative of the microenvironmental sites of in vivo deposition. In contrast, Bence Jones proteins for which no tubular cast formation, basement membrane deposition, or amyloid formation was found clinically have shown no tendency to aggregate under the same conditions (Myatt and Stevens, unpublished results). Differences in characteristic dimerization and oligomerization properties of proteins produced by individual patients necessarily originate in the limited number of amino acid substitutions that differentiate these molecules of highly conserved three-dimensional structure. The magnitude of these interactions, i.e., the thermodynamic free energy change that accompanies aggregation, is a function of pH, salt concentration, and buffer composition.

The clinical significance of understanding the mechanism of Bence Jones protein pathology provides a new rationale for basic biophysical study of these polypeptides. The clinical challenge posed by such molecules addresses central issues of protein chemistry: the structural bases of biomolecular recognition and the assembly of protein subunits. In this respect, Bence Jones proteins represent a unique model for the study of fundamental protein chemistry.

Dimerization of Bence Jones proteins provides the basis of the ability of these molecules to mimic the Fab portion of an antibody, which comprises both the light chain and the corresponding V and C domain segments contributed by the immunoglobulin heavy chain. The affinity of the interaction between V_L domains (Maeda et al., 1978; Stevens et al., 1980) is dependent upon the primary sequence; significant differences in affinity were found as a consequence of amino acid substitutions at a single position in the third hypervariable segment (Stevens et al., 1980). In the absence of known biological function for light chains, other than their role as an antibody constituent, it has been assumed that self-association of light chains was a fortuitous occurrence, presumably reflecting the ability of the V_L domain to associate with the structurally homologous V_H domain. Recent evidence suggests that light chains are capable of expressing a B-cell regulatory function (Joshua et al., 1988; Ionannidis et al., 1989). If this is the case, then dimerization of light chains may itself have a natural biological significance. Higher order aggregation of Bence Jones proteins provides a pathological functional correlate of primary structure.

Advances in molecular biology make it possible to determine the structural basis of Bence Jones protein pathophysiology and, in so doing, to establish the value of these proteins for experimental analysis of fundamental protein structural and functional relationships. Differences in multiple (solvent-sensitive) interaction properties can be attributed to limited amino acid substitutions that differentiate individual Bence Jones proteins. Because functional immunoglobulin light chains can be expressed in *Escherichia coli* (Skerra & Pluckthun, 1988; Ward et al., 1989; Sharon, 1990; Anand et al., 1990; Glockshuber et al., 1990a,b), recombinant techniques can be used to construct single amino acid variants to test structure–function hypotheses to identify surfaces responsible for interactions between these proteins. Recombinant human light chains will facilitate quantitative modeling of the consequences of site-specific substitutions of single amino acids. The change in the free energy of interaction that results from a single-residue substitution provides the primary data needed for detailed theoretical understanding of conformational and functional alterations due to changes in amino acid composition. Design of variant recombinant light chains will be guided by the several hundred archived amino acid sequences (Kabat et al., 1987) that effectively catalog biologically permissible substitutions. Because of their role in immunoglobulin structure, Bence Jones proteins and other light chains represent the most broadly studied family of related proteins.

Combined biophysical, structural, and genetic engineering studies focused on Bence Jones proteins offer a unique experimental strategy to investigate systematically the detailed structural and functional consequences that arise from single amino acid substitutions. Development of this capability has direct significance for protein engineering and for understanding the biomedical consequences of genetic mutation. Knowledge of the detailed structural and functional effects of single amino acid substitutions will help make it possible to predict the three-dimensional structure of a protein based on its amino acid sequence.

The clinical objective associated with basic biophysical study of Bence Jones proteins is the development of new therapies to counter the formation of amyloid and other toxic aggregation products. A small molecule that binds specifically to one of the light-chain surfaces involved in the pathological aggregation process would limit the development of protein deposition. Thus, if light-chain pathologies are related to protein oligomerization, a conceptual basis exists for the identification or design of drugs that could retard or reverse oligomerization-related problems. For the same reasons that analysis of Bence Jones proteins should contribute significantly to understanding energetics and mechanisms of protein folding and macromolecular recognition, this family of proteins represents a significant tool for basic research into the design of synthetic protein-specific drugs. The experience that can be obtained during identification of pharmaceutical agents that modulate experimentally accessible interactions between immunoglobulin light chains will be relevant to other disease processes that involve pathological assembly of identical protein subunits such as found in nonimmunoglobulin forms of amyloidosis, e.g., systemic familial amyloidosis, senile amyloid, Alzheimer's disease, Down's syndrome, diabetes-associated amyloidosis, β_2-microglobulin (dialysis-associated) amyloidosis, and others. Interactions between identical protein subunits are also a feature of the assembly of viral capsids. Thus, systematic study of interactions between small molecules and the diverse family of immunoglobulin light chains may contribute significantly to the development of noncytotoxic drugs

capable of attenuating specific viral infections.

In summary, continued research on Bence Jones proteins provides new insights into the pathogenesis of human light-chain-associated renal and systemic diseases. These studies should also result in fundamental contributions to our understanding of the molecular basis of protein structure and interaction, to protein engineering, and to design of protein-specific synthetic drugs.

Perhaps, by November 1, 1995, the 150-year anniversary of Dr. Watson's seminal observations and query regarding an unusual urinary substance, it will be possible to identify candidate drugs that will reduce the clinical complications associated with the proteins that bear the name of his correspondent, Dr. Henry Bence Jones.

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