

Isolation and characterization of some Bence-Jones proteins

For several years we have been investigating the proteins in the urine of patients suffering from myelomatosis¹⁻³. This note concerns our patients Nos. 101, 109, 110 and 111. Tested by the method of PUTNAM⁴ the urine of each patient contained proteins, coagulating at 56° and redissolving at 100°.

The results of free-boundary electrophoresis and starch-gel electrophoresis⁵ of the crude protein mixtures showed that the samples were far from homogeneous. Several of the fractions discernible in the latter method appeared to be normal serum proteins (see ref. 3).

The major Bence-Jones fractions were isolated from the crude mixtures by means of ion-exchange chromatography on DEAE-Sephadex A-50 or CM-Sephadex C-50 and finally by gel filtration on Sephadex G-100 and on Sephadex G-200. The buffer systems used in ion-exchange chromatography were phosphate solutions of increasing molarity (0.005–0.1 *M*) and decreasing pH for DEAE-Sephadex and acetate solutions of increasing molarity (0.01–0.1 *M*) and pH for CM-Sephadex. The results obtained with these ion exchangers are assembled in Fig. 1*. The shadowed major fractions were submitted to gel filtration on Sephadex G-100 (Lot To 33, 140–400 mesh) and on Sephadex G-200 (Lot 120, 140–400 mesh) in 0.01 *M* Tris-HCl buffer (pH 7.6). The results are shown in Fig. 2*. The fractions shadowed are, according to their starch-gel electrophoresis diagrams, sufficiently purified for a chemical analysis. The analytical results are assembled in Table I*. They show that proteins Nos. 101, 109 and 110 have the same α -amino terminal amino acid (aspartic acid) and the same carboxyl terminal amino acid (leucine), while these amino acids are tyrosine and alanine, respectively, in protein No. 111. The 4 amino acids preceding the carboxyl terminal amino acid differ from protein to protein. We also determined the percentages by weight of some amino acids in the proteins, by specific methods, as well as their content of glucidic components and their sedimentation constants. As Table I shows, these quantities were also different.

A full account of this work will be published.

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TABLE I
SOME ANALYSES OF PURIFIED BENCE-JONES PROTEINS
References concern methods used. Contents of glucides and amino acid residues expressed as g/100 g protein.

Protein No.	α -Amino terminal amino acid ^{a,7}	Carboxyl terminal amino acid ^b and preceding amino acids ^c	Glucidic components (%)				Amino acid residues (%)				<i>S</i> ₂₀ (uncorrected)	
			Hexose ¹⁰	Hexosamine ¹¹			Cy + CysH ¹³	Lys ¹⁴	Arg ¹⁵	Tyr ¹⁶		Trp ¹⁷
				Sialic acid ¹²								
101	Asp	(Val, Ala, Glu, Asp)-Leu	1.5	0.07	0.2	1.66	3.58	0.57	5.35	1.06	2.4	
109	Asp	(Val, Ser, Ala, Glu)-Leu	0.4	0.03	0.1	1.05	2.47	1.11	3.30	0.88	3.4	
110	Asp	(Glu, Ala, Thr, Ser)-Leu	0.3	0.2	0.3	2.14	4.57	0.82	5.30	1.06	3.6	
111	Tyr	(Lys, Glu, Ser, Val)-Ala	1.4	0.4	0.3	2.03	2.43	0.48	5.30	2.00	3.7	

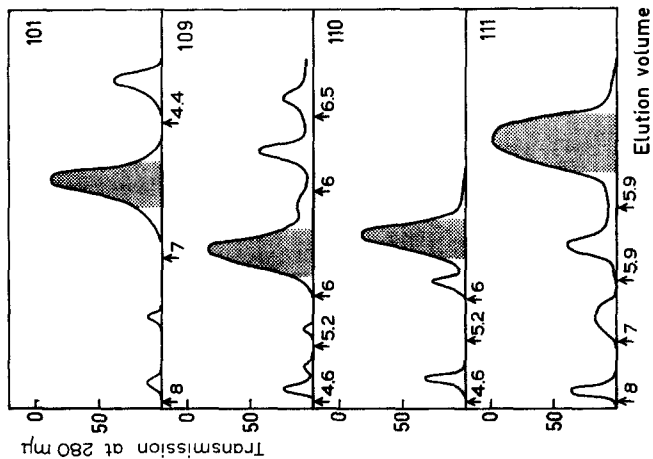


Fig. 1. Ion-exchange chromatography of crude protein mixtures on DEAE-Sephadex and CM-Sephadex. Discontinuous eluting systems, Nos. 101 and 111 on DEAE-Sephadex, Nos. 109 and 110 on CM-Sephadex. Arrows indicate introduction of buffer of pH marked.

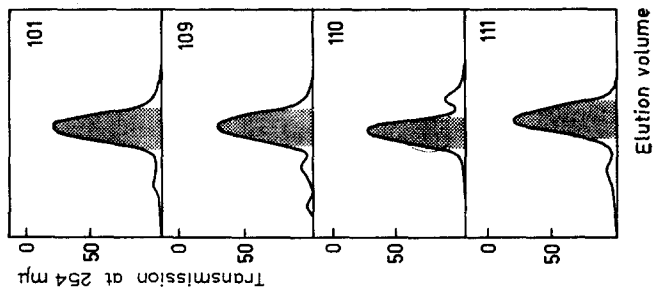


Fig. 2. Gel filtration of fractions shadowed in Fig. 1 on Sephadex G-100. Similar curves were obtained with Sephadex G-200.

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Structural relationships among normal human γ -globulin, myeloma globulins, and Bence-Jones proteins

In the study of structural relationships among normal and pathological globulins, Bence-Jones proteins should provide a valuable link since they exhibit many general relationships to normal human serum γ -globulin. These include common antigenic determinants, similar amino acid composition, and a common site of synthesis—the plasma cell¹. From isotopic studies we had earlier postulated that Bence-Jones proteins represent abortive products of myeloma-globulin synthesis². By comparison of the tryptic peptides we have now obtained the first chemical evidence for structural similarities among Bence-Jones proteins, the autologous abnormal globulin, and normal human γ -globulin. We propose that Bence-Jones proteins represent incomplete or aberrant polypeptide chains of normal human γ -globulin. This accords with the fact that 80 % of Bence-Jones proteins have an $s_{20} = 3.5 \text{ S} \pm 0.25$ with a molecular weight of 45 000⁴ and thus resemble in size the fragments obtained by oxidative³, reductive^{3,4} and enzymic cleavage of human γ -globulins⁵⁻⁷.

Peptide maps have been compared for pooled human γ -globulin, 7 purified pathological globulins, and 8 Bence-Jones proteins. The abnormal globulins represented 3 immunological types and several end-group types and included myeloma globulins and a macroglobulin. 5 of the Bence-Jones proteins were of immunological Type A and 3 of Type B (ref. 8). Like the myeloma globulins the Bence-Jones proteins differ individually in physical and chemical properties.

Fig. 1 contains a tracing of a composite peptide map* for a mixture of a 7-S γ -type myeloma globulin and the Bence-Jones protein from the same patient¹⁰. Fig. 2 has a similar composite peptide map for the same myeloma globulin and normal human γ -globulin. The peptides identified in the separate digests are so marked that a cross-hatched area results where the 2 proteins have a spot in common. Only peptides designated B₁-B₂₀ were found in the Bence-Jones protein which was of antigenic Type B. It is evident that almost all the peptides of this Bence-Jones

* The proteins were oxidized with performic acid and digested with trypsin for 18 h at 37°. The peptides were separated by chromatography in butanol-acetic acid-water followed by electrophoresis at pH 3.7 in pyridine-acetate buffer for 1 h at 2000 V as described by KATZ *et al.*⁹. The increase in leucine equivalents when measured by the ninhydrin method varied from 100-160/160 000 g for normal γ -globulin and the myeloma globulins and was about 25-30/45 000 g for the Bence-Jones proteins. The peptide spots were developed with ninhydrin and photographed immediately. Thus far, only a few peptides have been eluted, hydrolyzed, and analyzed with the automatic analyzer, but more than 100 peptide maps have been made.