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CASE REPORT

A CASE OF TETRAMER BENCE JONES PROTEINAEMIA*

H. M. GREY AND P. F. KOHLER

Department of Experimental Pathology and Department of Allergy, Immunology and Rheumatology, Scripps Clinic and Research Foundation, La Jolla, California

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SUMMARY

A case of Bence Jones proteinaemia without concomitant Bence Jones proteinuria is described. The protein consisted of four λ type light chains with a molecular weight of 84,000. A pair of dimers were non-covalently bonded to form the tetramer. The dimers in turn were formed of disulphide linked monomers. The absence of proteinuria is discussed with regard to the renal clearance of a tetramer Bence Jones protein compared to that of a monomer or dimer protein.

INTRODUCTION

The present report describes the serological and physico-chemical characterization of a paraprotein which was present in the serum of a patient with a well-documented case of multiple myeloma. The unusual feature of the paraprotein was that, by antigenic analysis of the serum protein, it was classified as a λ type Bence Jones protein but despite this there was no evidence of Bence Jones protein in the patient's urine. This anomalous finding is probably explained by the fact that the Bence Jones protein circulated in the serum as a light chain tetramer rather than the usual monomer or dimer.

MATERIALS AND METHODS

Protein isolation

The paraprotein from the patient (C.G.) was first isolated by preparative starch block electrophoresis (Kunkel, 1954). Half-inch fractions were eluted and tested for the presence of λ light chains by means of immunoelectrophoresis. Those fractions that were rich in light chains were concentrated and further purified by gel filtration on a Sephadex G-200 column equilibrated with 0·1 M-phosphate buffer, pH 7·5.

Correspondence: Dr H. M. Grey, Scripps Clinic and Research Foundation, 476 Prospect Street, La Jolla, California 92037.

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Antisera

Antisera used to study the nature of the immunoglobulin present in the serum of the patient were hyperimmune rabbit antisera to γG, γA and γM as well as K and λ light chains made by immunizing rabbits with a suitable myeloma protein or Bence Jones protein in incomplete Freund's adjuvant and absorbing the antisera with other immunoglobulins in order to render it specific. Anti-whole human serum and anti-transferrin were obtained from Behringwerke Ag. Anti-γ D antiserum was kindly supplied by Dr J. Fahey. Antigenic analysis was performed by means of micro-immunoelectrophoresis (Scheidegger, 1955) and Ouchterlony immunodiffusion (Ouchterlony, 1953). Semi-quantitative estimation of different antigens was performed by Ouchterlony analysis of serial dilutions of the material under investigation and comparing the position and intensity of the precipitin bands with those of standards of known protein concentrations.

Urine collection

Twenty-four-hour urine samples were filtered through Whatman No. 12 filter paper and were then concentrated by either: (1) dialysis overnight against running tap water, followed by lyophilization; or (2) positive pressure ultrafiltration using an Amicon apparatus with a membrane which was impermeable to molecules of greater than 1000 molecular weight.

Starch gel electrophoresis was performed utilizing the vertical system of Smithies (1959). The gel was made up in 0·05 M-glycine buffer, pH 8·8, and electrophoresis was carried out for 5½ hr at 450 V in a discontinuous buffer system (Poulik, 1959).

Reduction and alkylation was carried out essentially as described by Fleischman, Pain & Porter (1962), using 0·2 M-2-mercaptoethanol as reducing agent and a 10% molar excess of iodoacetamide as alkylating reagent. De-gassing with nitrogen was omitted.

Analytic ultracentrifugation was performed in a Spinco Model E Ultracentrifuge. Calculations were made on a Gaertner comparator. Molecular weights were determined by the Archibald method (Schachman, 1957).

Iodination of all proteins was carried out with ^{131}I using a modification of the chloramine T method (McConahey & Dixon, 1966).

Case Report

C.G., a 62-year-old white female, was admitted to the Scripps Clinic and Research Foundation on 22 February 1966 for treatment of recently diagnosed multiple myeloma. Symptoms had begun in July 1965 with pain and tenderness involving the sternum and left anterior chest wall with radiation into the thoracic spine. By January 1966 she had become confined to bed because of generalized skeletal pain and was admitted to her community hospital for evaluation. Pertinent diagnostic studies included a normal urinalysis (no protein), a blood urea nitrogen (BUN) of 14 mg/100 ml and serum calcium (Ca) of 13·6 mg/100 ml. X-ray studies demonstrated several rib fractures, generalized oesteoporosis of the spine with vertebral compression fractures and multiple osteolytic lesions in the skull. The diagnosis of multiple myeloma was established by bone marrow aspiration and the patient was transferred to this institution. On physical examination she appeared dehydrated and both acutely and chronically ill. There was exquisite tenderness to palpation over the sternum, left anterior chest wall and the entire spine. The abdomen was distended and the spleen palpable 2 cm below the left costal margin. Urinalysis disclosed trace protein, the BUN was 28, Ca 16·0, phosphorus 4·8, uric acid 9·4 and creatinine 1·32 mg/100 ml. Serum protein electrophoresis demonstrated an 'M' component of 1·7 g/100 ml in the β region. Serum immunoglobulins, determined by single radial immunodiffusion, were γG 840, γA 104 and γM 28 mg/100 ml. Bone marrow aspiration

disclosed 48% total plasma cells with 20% abnormal forms. Treatment consisted of hydration with an oral intake of 4–5 litres, prednisone 60 mg and cyclophosphamide (Cytoxan) 150 mg/day. On this program there was a prompt improvement in renal function and 10 days after admission the Ca was 9·5, uric acid 4·7, BUN 10 mg/100 ml and the creatinine clearance 52 ml/min.

Her most recent admission was in February 1966 because of an acute bronchitis with paroxysmal coughing which aggravated the thoracic pain. The urinalysis was negative for protein, Ca 9·6 and BUN 20 mg/100 ml. The creatinine clearance was 45 ml/min and 24-hour urinary protein excretion 85 mg. The single occasion that proteinuria had been detected by the sulphosalicylate method was at the time of her first admission when hypercalcaemia and associated renal failure were present.

RESULTS

Paper electrophoresis of the serum from the patient, C.G., revealed an abnormally large, monoclonal β-globulin peak. On immunoelectrophoresis of the whole serum using antisera directed against γG, γA, γM, γD, K and λ light chains, as well as antisera against whole human serum, the only abnormality seen was the reaction with an anti-λ chain antiserum. Fig. 1 illustrates this reaction and shows a precipitin arc in the fast β-globulin region which

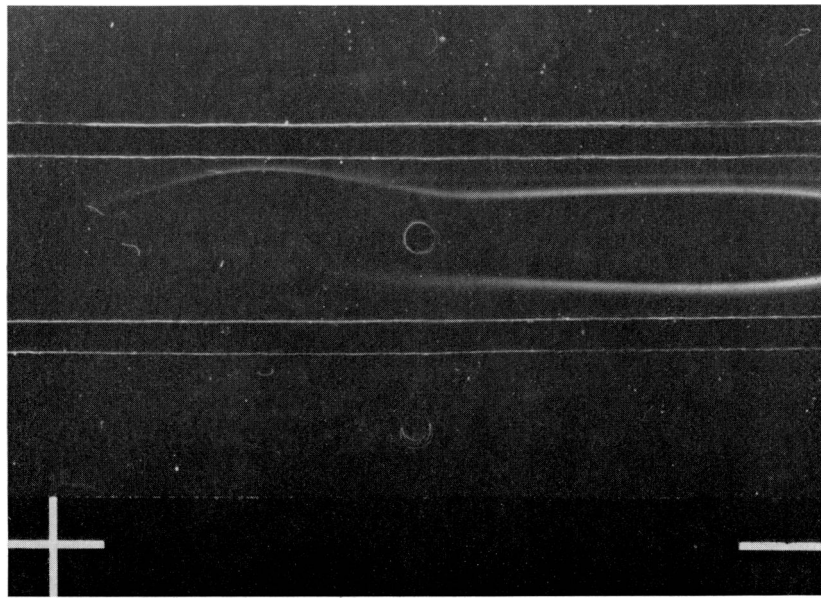

FIG. 1. Immunoelectrophoresis of C.G. serum. Upper trough: rabbit anti-λ chain antiserum; lower trough: rabbit anti-K chain antiserum.

is not seen when the serum is reacted against an anti-K antiserum. This set of observations was compatible with a diagnosis of Bence Jones proteinaemia. However, when the urine was examined no evidence of Bence Jones proteinuria was observed. Twenty-four-hour urine collections were made and the protein excretion measured. The urine contained a total of 85 mg/24 hr, which is within the upper limits of normal. When the urine was concentrated to 5·0 mg/ml and examined by immunoelectrophoresis the pattern observed

in Fig. 2 was obtained. The major protein detected by an anti-whole serum was albumin. When tested against anti-K and λ antisera, only small amounts of K chains were detected and no chains of the serological type of her serum paraprotein were observed.

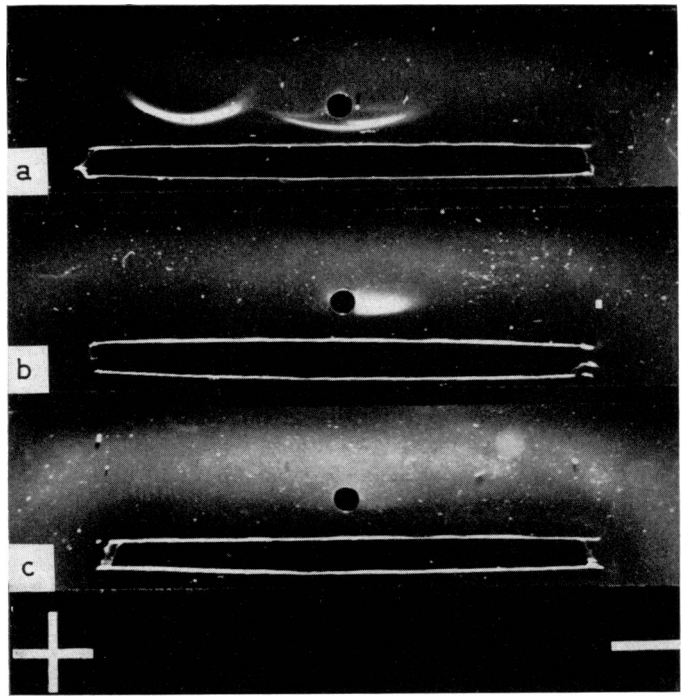

FIG. 2. Immunoelectrophoresis of concentrated C.G. urine (5 mg/ml) developed with: (a) anti-whole human serum, (b) anti-K chain antiserum, and (c) anti-λ chain antiserum.

Due to the unusual occurrence of Bence Jones proteinaemia without proteinuria, it was decided to further characterize the serum protein. The paraprotein was isolated by starch block electrophoresis and the β-globulin fractions containing λ light chain antigenic determinants were further purified on Sephadex G-200. A starch gel electrophoresis of the isolated protein is shown in Fig. 3. The paraprotein is indicated in the whole serum by the arrow. The isolated protein was contaminated with transferrin. As judged by semiquantitative Ouchterlony analysis and by relative intensity of staining on starch gel electrophoresis, the degree of contamination was 15–25%. Analytical ultracentrifugation of this material is shown in Fig. 4 and reveals a single homogeneous peak which had an $S^{\circ}_{20,w}$ of 4·9. There was no effect of dilution on the observed S rate at concentrations of 2–9 mg/ml.

In order to arrive at a value for the molecular weight of the serum Bence Jones protein, gel filtration as well as ultracentrifugal studies were carried out. Trace labelled bovine serum albumin (I*BSA) was added to a starch block isolated preparation of the Bence Jones protein and the mixture placed on a Sephadex G-200 column. The fractions were then assayed for radioactivity and ultraviolet absorbency, as well as examined for transferrin and λ light chains by immunodiffusion. The results are shown in Fig. 5. The distribution of transferrin and light chains was almost identical whereas the BSA peak was clearly eluted

after both transferrin and light chains. This indicated that the molecular weight of the λ light chains was greater than that of BSA (68,000) and very close to that of transferrin (90,000). A sample of the isolated light chains which contained approximately 20% transferrin was analysed by the Archibald method to determine its molecular weight. Calculations were done at the miniscus and bottom. Both calculations gave the same figure and indicated a molecular weight of 84,000 for the sample studied. No evidence of heterogeneity was seen in these molecular weight studies.

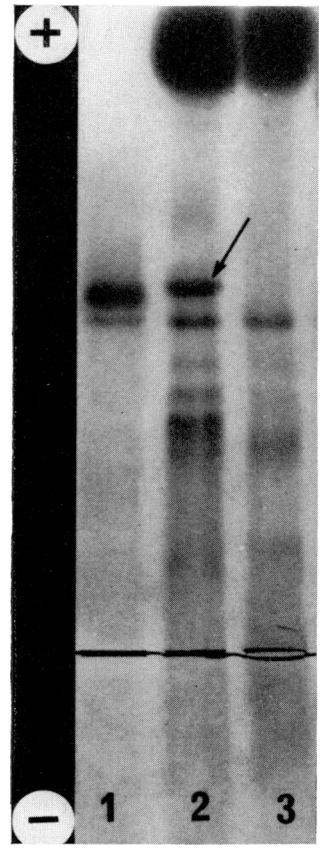

FIG. 3. Starch gel electrophoresis in glycine buffer, pH 8·8. Slot 1, C.G. protein isolated by starch block electrophoresis; slot 2, whole C.G. serum; slot 3, normal human serum. Arrow indicates the paraprotein band in whole C.G. serum. The isolated protein is contaminated with transferrin.

These two experiments indicated that the λ chains were most likely in the form of a tetramer consisting of four subunits of 20–25,000 molecular weight. In order to gain further information about the subunit structure, gel filtration experiments were done in the presence of a dissociating reagent, 1 M-acetic acid. The results are shown in Fig. 6. Bence Jones protein (and contaminating transferrin) isolated by starch block electrophoresis and G-200 gel filtration was concentrated, trace labelled I*BSA added, dialysed against

1 M-acetic acid and placed on a Sephadex G-100 column equilibrated in 1 M-acetic acid. Under these conditions transferrin and I*BSA were both eluted before the λ light chains, whereas when using the same column, partially reduced and alkylated light chains from normal

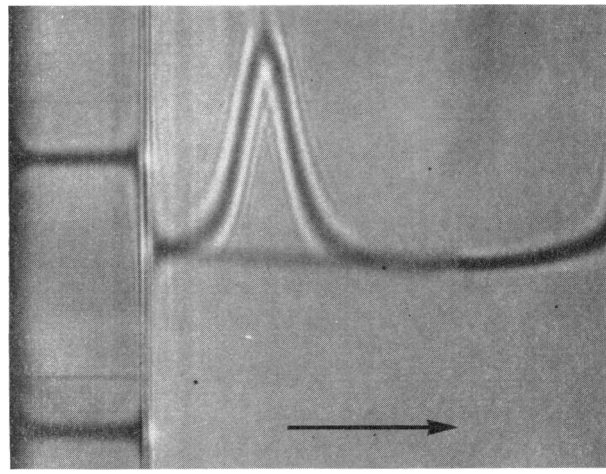

FIG. 4. Analytical ultracentrifuge analysis of C.G. protein. Direction of sedimentation is towards the right. Picture was taken 32 min after attaining a rotor speed of 59,780 rev/min.

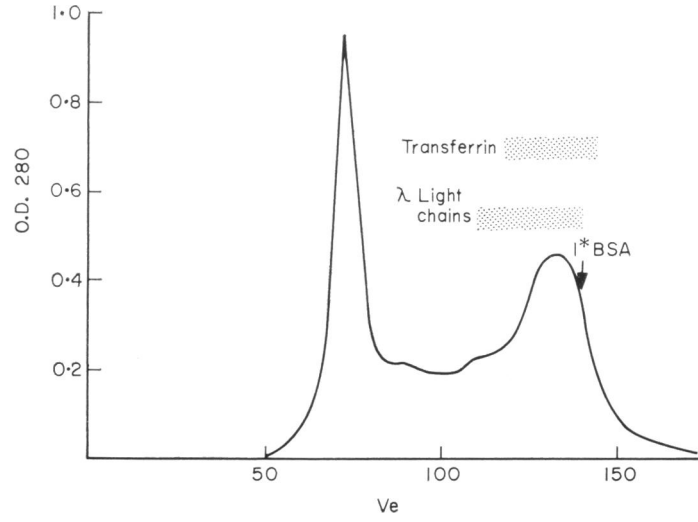

FIG. 5. G-200 gel filtration in 0·1 M-phosphate buffer, pH 7·2, of starch block isolated C.G. protein. Stippled horizontal bars indicate the fractions that contained antigenically identifiable transferrin and λ light chains. Arrow indicates the peak distribution of ^{131}I-labelled BSA which was added to the sample.

γ-globulin were eluted after the unreduced Bence Jones protein. This experiment indicated that the C.G. Bence Jones protein dissociated in the 1 M-acetic acid to a subunit with a size between monomer λ chains (23,000) and BSA (68,000). To determine whether di-

sulphide bonds were also involved in the subunit interaction, G-100 gel filtration in 1 M-acetic acid was performed on the partially reduced and alkylated Bence Jones protein. Fig. 7 shows these results and indicates that the reduced and alkylated Bence Jones protein is eluted at the same position as the trace labelled light chains obtained from normal γ-globulin, indicating that after reduction and acetic acid treatment the Bence Jones protein is broken down to a monomeric subunit the size of normal light chains.

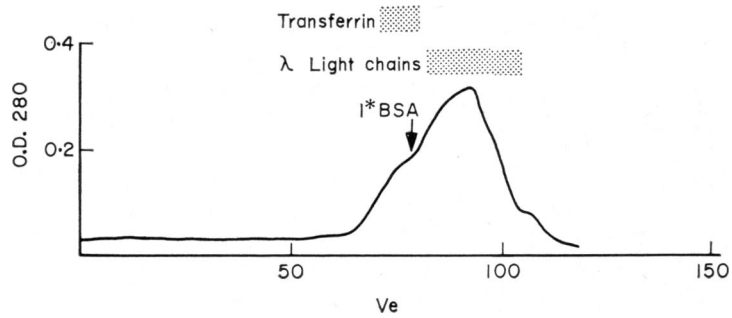

FIG. 6. G-100 gel filtration in 1 M-acetic acid of C.G. Bence Jones protein. Stippled horizontal bars indicate the fractions that contained antigenically identifiable transferrin and λ light chains. Arrow indicates the peak distribution of ^{131}I-labelled BSA which was added to the sample.

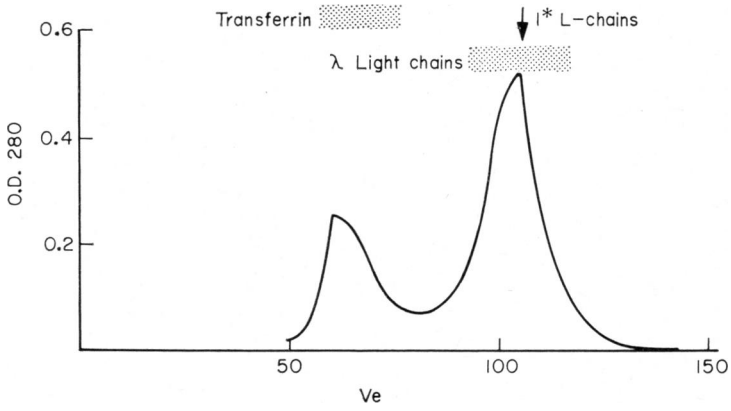

FIG. 7. G-100 gel filtration in 1 M-acetic acid of reduced and alkylated C.G. Bence Jones protein. Stippled horizontal bars indicate the fractions that contained transferrin and λ light chains. Arrow indicates peak distribution of ^{131}I-labelled λ chains from normal human γ globulin.

DISCUSSION

Previous studies on Bence Jones proteins and normal light chains (Bernier & Putnam, 1963; Milstein, 1964; Gally & Edelman, 1964; van Eijk, Monfoort & Westenbrink, 1963) have indicated that free light chains can exist in three different forms: as monomers of 20–25,000 molecular weight; as non-covalently bonded dimers of 45,000 molecular weight which are

converted into monomers when placed in dissociating solvents such as 1 M-acetic acid or 8 M-urea; and as disulphide bonded dimers. The present study indicates that another polymeric form is also possible; that is, a tetramer with a molecular weight of 84,000. The gel filtration experiments in 1 M-acetic acid on the reduced and unreduced C.G. protein suggest that the tetramer consisted of two dimers that were non-covalently bound to form the tetramer and that each dimer consisted of two disulphide linked monomers. Previous workers have also shown a correlation between the antigenic type of light chain and the polymeric form that the light chains possess in that most λ Bence Jones proteins appear to exist as disulphide bonded dimers whereas K Bence Jones proteins are usually present as monomers or non-disulphide bonded dimers (Bernier & Putnam, 1963; Milstein, 1964; Gally & Edelman, 1964). The present findings are in keeping with these generalizations in that the C.G. protein was of the λ type and it formed disulphide linked dimers.

The most likely explanation for the unique distribution of this Bence Jones protein to the serum and not to the urine of the patient is the high molecular weight of this protein compared to the more usual monomer and dimer forms of Bence Jones proteins. It has been shown by several investigators (Wallenius, 1953; Pappenheimer, 1953; Brewer, 1951; Hardwicke & Soothill, 1961) that the glomerulus acts as a molecular sieve and that the relationship between molecular weight and clearance shows a log-normal type distribution. Approximately a 100-fold difference or more in the renal clearance can be expected between a protein of 80,000 molecular weight and one of 20,000 or even 40,000 molecular weight. This means that a patient excreting 1 g of monomer or dimer Bence Jones protein in the urine per day, would under similar circumstances of protein synthesis and catabolism only excrete 10 mg of a tetramer.

Of course, other factors might also have contributed to the lack of Bence Jones proteinuria in this case, e.g. (a) the patient had relatively normal renal function, and (b) this particular Bence Jones protein might have had an abnormally fast catabolic rate.

Since most λ Bence Jones proteins exist as disulphide linked dimers and most K Bence Jones proteins exist as non-disulphide linked monomers and/or dimers, it might be expected that there would be a greater incidence of λ Bence Jones proteinaemia than K, since there should be approximately a three-fold difference in the clearance rates between monomer and dimer Bence Jones protein. It is of interest in this regard that seven of ten cases of Bence Jones proteinaemia reported by Solomon & Fahey (1964) were λ type proteins. This is approximately twice as many as would be anticipated from the relative frequency of the two types of Bence Jones protein in unselected cases of multiple myeloma with Bence Jones proteinuria (Korngold &Lipari, 1956; Mannik & Kunkel, 1962; Fahey & Solomon, 1963).

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