ON THE ISOLATION, CHARACTERISATION, AND CRYSTALLISATION OF A HUMAN BENCE-JONES PROTEIN OF KAPPA TYPE

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The isolation and purification of the human Bence-Jones protein Rei is described. Physico-chemical data are given characterising the protein. It is shown that the protein could be crystallised. It is intended to use the crystals for single crystal X-ray diffraction studies.

1. Introduction

Edelman and Gally have shown urinary Bence-Jones proteins* to be identical with the light chains from the abnormal immunoglobulin present in the serum of the same patient suffering from myelomatosis. Human light chains can be divided into two classes, lambda and kappa, on the basis of the different amino acid sequences of the constant parts of the chains [2]. Although it is likely that the tertiary structure of a protein will be determined by its amino acid sequence the latter does not tell us how to work out the three-dimensional structure, which can, however, be determined by X-ray crystallographic methods. From the chemical point of view the main difficulty is to prepare single protein crystals of sufficient size for crystallography.

2. Materials and methods

For isolation of the BJ-protein, urine of a 65 years old male patient was collected in 25 l plastic bottles using toluene as a preservative. The urine was adjusted to a pH of 4.7 with 1.0 M acetic acid and the protein precipitated by adding ammonium sulfate (40% w/v). The precipitate was collected and dissolved in distilled water; the remaining residue discarded. The protein solution was reprecipitated with ammonium sulfate (40% w/v) and this procedure repeated four times until a white precipitate was obtained which was dissolved in a 0.05 M tris buffer, pH 8. The solution was kept frozen. Disc electrophoresis was carried out in glass tubes 6 mm in diameter using a 7% (w/v) aqueous polyacrylamide gel (Serva, Heidelberg). It was run in a 0.1 M tris-glycine, buffer, pH 8.5, at 3 mA per tube for 60 min.

The isoelectric point was determined by electrofocusing. The protein was run in a 7% (w/v) aqueous polyacrylamide gel (Serva, Heidelberg) in an Ampholine pH gradient (LKB) from 3 to 10 for 16 hr at 5 to 1 mA. The protein fraction was eluted from the gel into a few ml of redistilled water and the pH measured with a (pH) meter.

The sedimentation coefficients were determined at different concentrations in a Beckman-Spinco model E analytical ultracentrifuge equipped with Schlieren optics and electronic speed control. The diffusion constants were measured at various concentrations in a diffusion cell attached to an analytical ultracentrifuge, type Phywe U 50 L, using the Schlieren optical system.

The molecular weight of the protein was calculated from the sedimentation and diffusion constants using the Svedberg equation. A partial specific volume of 0.736 was used which was found for other BJ-proteins [3]. Alternatively the molecular weight was determined by the Archibald [4] and Yphantis [5] methods.

* The abbreviation BJ-protein is used for Bence-Jones protein.

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Immuno double gel diffusion against specific antisera (Behring-Werke) was carried out using 1% (w/v) agar (Behring-Werke) in a Michaelis buffer, pH 6.4, $I = 0.1$. Purification and separation of the protein was achieved on a 60 X 2.4 cm DEAE cellulose column (Whatman DE 32), loaded with approx. 200 mg protein, by means of a 0.05 M tris buffer, pH 8. The effluent was run through a Uvicord (LKB) and collected in 5 ml fractions. Reduction and alkylation [6] of the protein was carried out with dithiothreitol and iodoacetamide (Fluka). Crystallisation was effected by equilibrium dialysis. The concentration of the protein solution was varied between 5 and 10 mg per ml. The concentrations of the ammonium sulfate solutions were 0.1 to 2.0 M.

3. Results and discussion

The protein Rei was identified as a BJ-protein by its typical behaviour during heating and cooling; immunodiffusion against an anti kappa BJ serum showed a single and sharp precipitation line while the reaction with an anti lambda BJ serum was negative. The isoelectric point of the protein was found to be 4.7. The disc electrophoresis of the protein showed five main bands (fig. 1); based on this finding further purification was carried out on a DEAE cellulose column from which two main fractions could be eluted, they were called fraction 1 and 2 (fig. 1). Both fractions were concentrated by pressure dialysis and examined in the analytical ultracentrifuge.

It could be shown that the unseparated material as well as fractions 1 and 2 have the same sedimentation and diffusion constants i.e. $S_{20} = 2.6$ and $D_{20} = 10.0$ respectively. From the Svedberg equation the molecular weight was found to be about 25,000. The molecular weights determined by the Archibald [4] and the Yphantis methods [5] were both in good agreement with the value calculated from the Svedberg equation. In all the ultracentrifuge and diffusion experiments 0.15 M NaCl was used as solvent.

The frictional ratio f/f_0 was calculated [7] to be 1.08. Assuming the molecule to have a spherical shape the radius would be 19.4 Å. BJ-proteins normally exist in the dimeric form with a molecular weight varying from about 40,000 to 45,000 and have the coefficients $S_{20} = 3.6$ and $D_{20} = 7.7$. The constants of $S_{20} = 2.6$ and $D_{20} = 10.0$ for the BJ-protein Rei and the M.W. of 25,000 indicate that the protein exists as a monomer in solution. If the solutions are allowed to stand for several weeks at room temperature (or in the cold at 4°) the appearance of aggregates can be noticed. The exact assembly of the aggregates was not worked out but it is likely that they contain a mixture of different possible states of aggregation, as was shown by Gally and Edelman [8].

By running a freshly prepared solution using 0.15 M NaCl as solvent on a Sephadex G-75 column the protein was eluted in nearly the same fraction as an α-chymotrypsin (M.W. 22,000) as standard. A well known dimeric BJ-protein [3] as well as a solution of ovalbumin (M.W. 44,000) were eluted from the same column, under the same conditions, in front of the

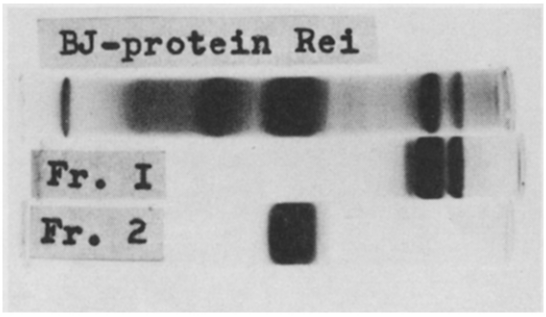

Fig. 1. Polyacrylamide disc electrophoresis of the BJ-protein Rei and of the fractions 1 and 2 separated on a DEAE cellulose column. See text for conditions.

Fig. 2. Photomicrograph of the crystallised fraction 2 of the BJ-protein Rei. The length of the crystal is approx. 1.5 mm.

BJ-protein Rei. When a solution containing a mixture of aggregates if Rei was exposed either to 0.5 M propionic acid or to 1.0 M acetic acid the aggregates were turned nearly completely into monomers, as could be shown by comparative elution from Sephadex G-75 columns.

A solution of BJ-protein Rei reduced and alkylated gave the same elution pattern on a Sephadex G-75 column as did the protein solution before reduction and alkylation. This indicates that the BJ-protein Rei normally exists as a monomer in freshly prepared solutions. Dissociation experiments show that aggregates are held together by non convalent interactions [8]. Experiments to crystallise the protein were carried out using the unseparated material as well as with each of the fraction 1 and 2. Crystallisation of the protein occurred best using the fraction 2; the concentration of the ammonium sulfate solution was 22% (w/v). Fraction 1 could not be crystallised. The presence of unseparated material or of fraction 1 did not interfere seriously with crystallisation of fraction 2 but the best crystals were obtained by using only a highly purified fraction 2 (fig. 2).

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