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SIMPLIFIED METHOD OF ISOLATING β_2-MICROGLOBULIN FROM URINE

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β_2-Microglobulin (B_2-M), a protein first described by Edelman et al. [4], is a small molecule (mol. wt. 11,800) homologous in its primary structure with the Cγ3 domain of the immunoglobulin molecule.

Interest in this protein has increased in recent years, for it has been shown that it is a component of glycoproteins coded by the principal histocompatibility locus as a subunit. It has recently become evident that the role of cell surface glycoproteins in immunity is extremely important [3, 11]. However, the function of β_2-M is not yet known. It has recently been shown [2] that β_2-M activates T lymphocytes and increases the number of Fc receptors on their surface.

Methods of determination of β_2-M in human urine and serum are of great importance for the diagnosis of kidney diseases [1, 5] and of tumors [5, 7].

The technique of isolation of β_2-M was fundamentally researched by Beggard and Bearn [1]. As its successive stages it includes ultrafiltration, zonal electrophoresis, gel chromatography, and ion-exchange chromatography. The use of this technique calls for different kinds of apparatus and also, at the testing stage (Ouchterlony's method and immunoelectrophoresis), it requires anti-β_2-M antiserum. The use of SDS-electrophoresis [7], however, enables this test protein and test antiserum to be dispensed with. For the analysis of β_2-M in human blood and urine an expensive Phadebas β_2-microtest kit, containing nanogram quantities of human β_2-M, has been suggested by the firm of Pharmacia. For diagnostic and, in particular, for research work, larger quantities of human and animal β_2-M are necessary.

The development of a simplified technique for the isolation of β_2-M in amounts required for investigations of β_2-M in certain pathological processes, and also to study the role of this protein in the composition of the HLA-glycoproteins of the cell surface, is a matter of some urgency. This paper describes a study of the possibility of obtaining sufficiently purified β_2-M by methods of column chromatography. The sequence of stages of isolation of purified (92.3 ± 2.85%) β_2-M, with the characteristics of each stage, is described below.

All operations were performed at room temperature with the addition of sodium azide (final concentration 0.04%). The source of the β_2-M was urine from patients with Fanconi's syndrome (2.2-3 mg β_2-M/liter) and patients with transplanted kidneys (5-38 mg/liter).

The quantity of β_2-M in the urine and at all stages of its isolation was determined by the Phadebas β_2-microtest in accordance with instructions provided by the firm of Pharmacia. The protein concentration was determined by Lowry's method and from the optical density at 260 nm and 280 nm.

The samples of urine were desalted on columns (5 × 20 cm) with Molselect G-25 (from Reanal). Urine was applied to the column in a volume of 250 ml and the rate of elution was 60 ml/h. The eluant was a 0.001 M

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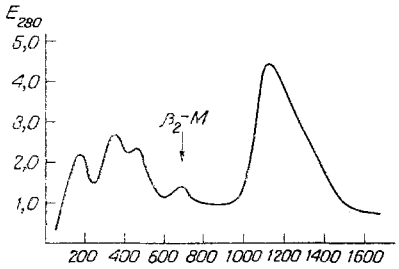

Fig. 1

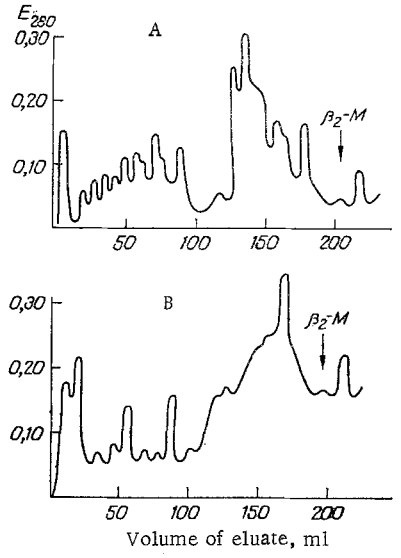

Fig. 2

Fig. 1. Chromatography of concentrated urine proteins (4.8 g) from a patient with transplanted kidneys on Sephadex G-100.

Fig. 2. Chromatograph of low-molecular-weight fraction of urine proteins (200 mg) on DEAE-Sephadex A-25. A) Patient with Fanconi's syndrome; B) Patients with transplanted kidneys.

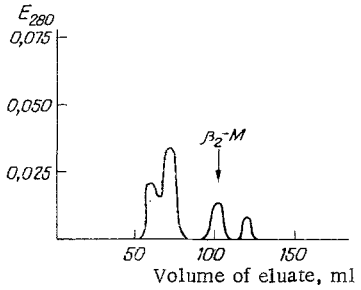

Fig. 3. Rechromatography of β_2M (215 μg) on DEAE-Sephadex A-25.

solution of Tris-HCl buffer, pH 6.8. A mixture of proteins, including β_2-M, came out with the outer bed volume of the column (690 ml), most of the protein material being present in the last 300 ml. This method of desalting is evidently the best, for the loss of β_2-M at this stage amounts to 50%.

Dialysis through ordinary dialysis membranes is accompanied by loss of 90% of β_2-M, whereas desalting on the IM-2 filter (Amicon) leads to loss of 70% of the β_2-M through adsorption of protein on the material of the filter.

The resulting product was lyophilized in a laboratory freeze-drier. The powder keeps well and can be used as required.

The powder produced was dissolved in 0.1M Tris-HCl buffer with 1M NaCl, pH 8.0 (concentration 20-30 mg/ml) and centrifuged to remove aggregates at 48,000g for 2 h (Beckman 2-65B centrifuge, 42.1 rotor).

Gel chromatography was carried out on a column (4.4 × 100 cm) with Sephadex G-100, and 80-100 ml of the solution of urinary proteins was applied. The same buffer solution was used as eluant. The rate of elution was 60 ml/h. Low-molecular-weight proteins (mol. wt. 7000-17,000), which were eluted in the inner volume of the column (35th-40th fraction each 20 ml in volume, Fig. 1), were adsorbed. The outer volume of the column used was 560-580 ml and the inner volume 880-900 ml. Losses of β_2-M at this stage were close to 65%. In the method of Beggard et al. [1], losses at this stage were a little less, possibly because the preceding stages of fractionation (repeated zonal electrophoresis) got rid of a considerable quantity of proteins.

Dialysis of the eluate against 0.01 M Tris-HCl buffer, pH 7.85, was necessary for subsequent fractionation on the ion-exchange resin. This was carried out in benzoylated fine-pore dialysis bags (Sigma, product No. D7884) for 48 h against 40 volumes of buffer. Losses of β_2-M at this stage amounted to 40%. If the special dialysis bags are not available, the proteins can be transferred into solution required for chromatography on a column with Molselect G-25, using 0.01M Tris-HCl buffer, pH 7.85, as the eluant.

Fractionation on a column (1.0 × 20 cm) with DEAE-Sephadex A-25 (Pharmacia) was carried out by applying 200 mg of total protein in a volume of 400 ml. Protein was eluted in a sodium chloride gradient (0-0.3M) in 0.01M Tris-HCl buffer, pH 7.85, at the rate of 8 ml/h. Typical patterns of fractionation of protein material from the urine of patients with Fanconi's syndrome and with transplanted kidneys are given in Fig. 2. β_2-M was eluted in fractions corresponding to an NaCl concentration close to 0.1 M. Losses of β_2-M at this stage amounted to 30%. The β_2-M was eluted in one peak together with several other proteins and, in particular, with transferrin [1]. For that reason rechromatography of the protein eluted in the peak corresponding to 0.09-0.11 M NaCl in the eluant is therefore necessary.

As a rule, the β_2-M leaves the column as a trace after a group of proteins present in the urine of these patients in much larger quantities than β_2-M; consequently, these proteins can be used as a guide for the determination of β_2-M.

Rechromatography was carried out on a column (0.5 × 6 cm) with DEAE-Sephadex A-25 (Pharmacia). Protein was applied to the column in a weight of 2 mg and in a volume of 8 ml. The sample was first dialyzed, using the dialysis bags mentioned above, for 12 h against 0.01M Tris-HCl buffer, pH 7.85. An example of fractionation is shown in Fig. 3. The concentration of β_2-M was maximal in the region of the gradient close to 0.1M NaCl. Losses of β_2-M at this stage amounted to 50%.

It will be clear from the above accounts that a purified preparation of β_2-M can be obtained in the course of two weeks by methods of column chromatography. During development of the method it is desirable to use a test protein and a technique of quantitative determination of this protein. We used small quantities of β_2-M labeled with ^{125}I and the Phadebas β_2-microtest. However, if these are not available, it is possible simply to use SDS-electrophoresis [10]. The yield of β_2-M from 4.8 g total urinary protein (corresponding to 1.3 liters of urine), when the 24-hourly sample of urine contained 3 mg, was 90 μg, or 3% of the initial quantity. The yield of β_2-M obtained by Beggard et al., [1] was 11%.

The quantity of β_2-M required is determined by the aims of the investigation. As a rule, however, it is necessary to obtain an antiserum against this protein. It will be recalled that a minimum of 300 μg protein is required to immunize a rabbit in accordance with the scheme suggested in [9].

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