Immunosorbent for Removal of β_2-Microglobulin from Human Blood Plasma

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Immunosorbent removing β_2-microglobulin from human blood plasma was synthesized on the basis of sheep monospecific polyclonal antibodies to β_2-microglobulin and conditions ensuring effective regeneration of the immunosorbent for its repeated use were selected. Relationships between adsorption capacity and the volume of immobilized ligand, antigen concentration in the solution, and duration of incubation were studied. The adsorbent can be used for effective and specific removal of β_2-microglobulin from the plasma of patients on chronic hemodialysis.

Key Words: *β_2-microglobulin; immunosorbent; chronic renal insufficiency; hemodialysis*

The discovery of β_2-microglobulin (β_2-M) dates back to 1968, when Berggard and Bearn isolated it from the urine of patients with Wilson's disease and workers with chronic cadmium poisoning [3]. β_2-M was detected in other biological fluids and on the surface of almost all cells as the light chain of human and vertebrate class I HLA antigens [9]. β_2-M was used as a marker for the diagnosis of some diseases (renal failure, tumors, viral diseases, and autoimmune disorders). Recently a new type of amyloidosis was detected in patients treated by hemodialysis for 5-15 years; amyloid in these patients consists mainly of β_2-M and its fragments [5]. Membranes most often used in hemodialysis are virtually impermeable for β_2-M, and therefore its serum concentrations in patients with chronic uremia 20 and even 40 times surpasses the normal [6]. New highly permeable membranes can remove substances with molecular weight of up to 15 kDa, sometimes up to 25 kDa. But even many-week courses of daily hemodialysis cannot restore the balance of β_2-M and prevent deposition of β_2-M amyloid [4]. Removal of β_2-M from the blood of patients on hemodialysis is an important problem.

We investigated the possibility of reducing the level of β_2-M in human plasma by its selective removal with an adsorbent containing sheep monospecific polyclonal antibodies to human β_2-M as the ligand.

MATERIALS AND METHODS

Monospecific polyclonal antibodies to β_2-M were obtained by antiserum fractionation with ammonium sulfate followed by affinity chromatography on β_2-M Sepharose CL-4B [2].

Affinity antibodies were immobilized on Sepharose CL-4B (Pharmacia) by the standard method of bromocyan activation [8]. Adsorption capacity and specificity of the adsorbent were evaluated by column and batch chromatography.

Electrophoresis was carried out in a linear PAAG gradient (5-22%) under denaturing conditions at 20 mA current and 100 V voltage by a previously described method [10] with some modifications [7].

Protein content in the specimens was measured spectrophotometrically and by Mancini radial immunodiffusion.

The data in Tables (1-3) are presented as $M \pm SD$ for three independent experiments.

RESULTS

The immunosorbents used in extracorporeal blood purification meet special requirement [1]. Clinical effect

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can be attained only when the adsorbent possesses high adsorption capacity for the corresponding plasma component. High selectivity of the adsorbent rules out side effects caused by nonspecific binding of nonpathogenic plasma components. Moreover, immunosorbents are usually intended for repeated use and, therefore, it should regenerate and retain its adsorption capacity after repeated chromatography procedures.

The basic functional characteristic of the immunosorbent with immobilized antibodies is specific binding of the corresponding antigen in the presence of other proteins. We carried out affinity chromatography of human blood plasma on a column with anti-β_2-M sepharose CL-4B (Fig. 1). The protein bound to the adsorbent was eluted at low pH as a single peak. For evaluation of the adsorbent selectivity, the eluate was analyzed by vertical PAAG electrophoresis with sodium dodecylsulfate. Electrophoregram (Fig. 2) shows that the eluate contains a single band corresponding to a molecular weight of 12 kDa.

Then we selected optimal conditions for adsorption of β_2-M and regeneration of the adsorbent. In these experiments we used lyophilized concentrated urine of patients with chronic renal failure enriched with β_2-M (electrophoregram is presented in Fig. 2).

Buffer ensuring the most complete elution of β_2-M bound to the immunosorbent was selected as follows. Buffers of different pH and ionic strength were tested. Chromatography was carried out under conditions of *a priori* saturation of the adsorbent. Different buffer solutions were used as eluents. The maximum concentration of β_2-M in the eluate was attained with 0.05 M citrate buffer (pH 2.5) and 3 M $MgCl_2$ (Table 1).

We chose 0.05 M citrate buffer (pH 2.5), because its concentration was by 3 orders of magnitude lower than that of 3 M $MgCl_2$, and therefore its density, viscosity, and ionic strength were lower.

This, in turn, accelerated elution, *i. e.* regeneration of the adsorbent. In addition, high ionic strength of the solution can lead to irreversible changes in proteins and matrix of the adsorbent.

For final choice of the elution buffer (0.05 M citrate buffer, pH 2.5), we evaluated the adsorbent stability in a series of 10 chromatographies. The volume of β_2-M in eluate from the first and two last chromatographies was 0.405±0.018 and 0.393±0.032 mg/ml gel, respectively. Hence, the decrease in adsorption capacity by the 10th chromatography procedure was no more than 5%, which allows repeated use of the adsorbent without reducing its adsorption capacity. In further experiments we used 0.05 M citrate buffer (pH 2.5).

After choosing the elution buffer, we investigated the relationship between the adsorption capacity of the β_2-M adsorbent CL-4B Sepharose and the concentration of immobilized ligand. Monospecific polyclonal

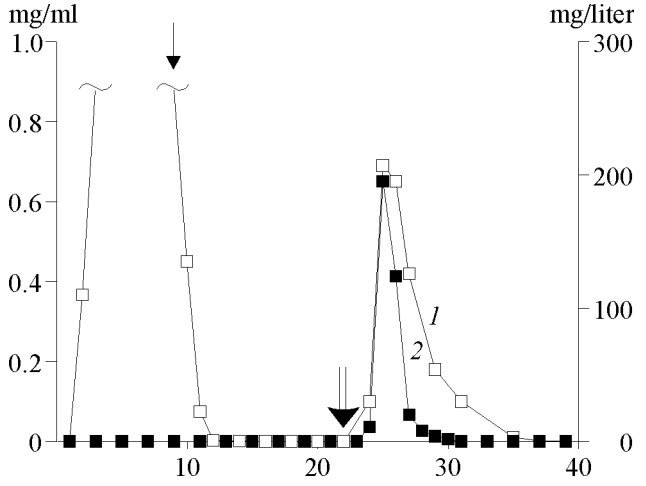

Fig. 1. Affinity chromatography of human plasma on adsorbent with sheep monospecific polyclonal antibodies to β_2-microglobulin (β_2-M). Washout with phosphate buffer, pH 7.4 (arrow), elution with 0.05 M citrate buffer, pH 2.5 (double arrow). Parameters of column chromatography: concentration of immobilized antibodies 12 mg/ml gel, adsorbent volume 1 ml, volume of processed plasma 20 ml, β_2-M concentration in the plasma 22 mg/liter, plasma flow 0.5 ml/min. *1*) concentration of total protein (left ordinate); *2*) concentration of β_2-M (right ordinate).

antibodies were immobilized on CL-4B Sepharose in concentrations 2-16 mg/ml. The relationship between the adsorption capacity of anti-β_2-M Sepharose CL-4B and the concentration of immobilized ligand was linear (Fig. 3). If the antigen is present in excess (zero order reaction by the antigen concentration), the linear relationship indicates that the rate of the antigen-antibody reaction is virtually not limited by diffusion. The relationship between the adsorption capacity of the adsorbent and duration of incubation with β_2-M-rich

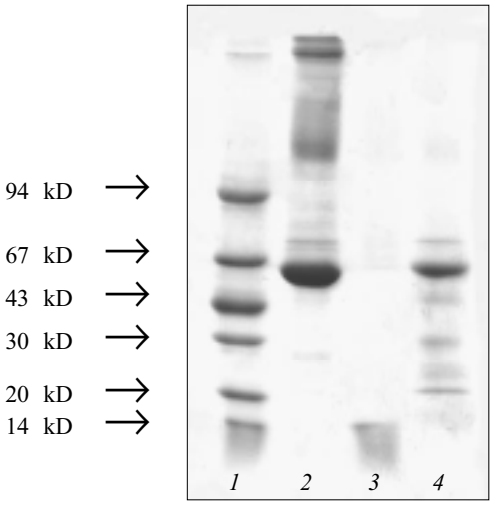

Fig. 2. Electrophoregram of eluate obtained by affinity chromatography on anti-β_2-microglobulin (β_2-M) Sepharose CL-4B. *1*) protein markers; *2*) plasma applied to the column; *3*) β_2-M preparation isolated from the plasma by affinity chromatography; *4*) β_2-M-rich fraction.

TABLE 1. Protein Concentration in Fractions Eluted from Anti-β_2-M Sepharose CL-4B with Different Eluents

Eluent	Protein concentration, mg/ml gel
3 M MgCl	0.89±0.05
2.5 M NaCl, pH 7.5	0.65±0.11
2 M KSCN	0.27±0.01
2.5 M KI	0.07±0.02
0.1 M $NaHCO_3$, pH 10.5	0.60±0.01
0.05 M citrate, pH 2.5	0.94±0.03

TABLE 2. Relationship between Adsorption Capacity of Anti-β_2-M Sepharose CL-4B and Duration of Incubation with β_2-M-Containing Solution

Duration of incubation, h	Concentration of β_2-M in eluate, mg/ml gel
0.5	0.53±0.05
1.0	0.50±0.03
3.0	0.55±0.06
5.0	0.57±0.04
24.0	0.56±0.04

TABLE 3. Relationship between Adsorption Capacity of Anti-β_2-M Sepharose CL-4B and β_2-M Concentration in Solution

Concentration of β_2-M, mg/liter	Concentration of β_2-M in eluate, mg/ml gel
13	0.59±0.06
36	0.70±0.07
53	0.73±0.07
66	0.72±0.06

fraction confirmed that antigen-binding capacity of the adsorbent virtually did not change (Table 2). Hence, β_2-M-binding capacity of anti-β_2-M sepharose CL-4B did not depend on the duration of the adsorbent incubation with β_2-M-containing solution.

The next step was evaluation of the relationship between the adsorption capacity of anti-β_2-M sepharose CL-4B and β_2-M concentration in the solution. The concentration of β_2-M in solutions passed through the column evaluated by Mancini radial immunodiffusion varied from 12 to 65 mg/liter (Table 3). The adsorption capacity negligibly increased at β_2-M concentrations of 12-34 mg/liter and virtually did not change at concentrations of 34-65 mg/liter. These results indicate that the reaction between immobilized

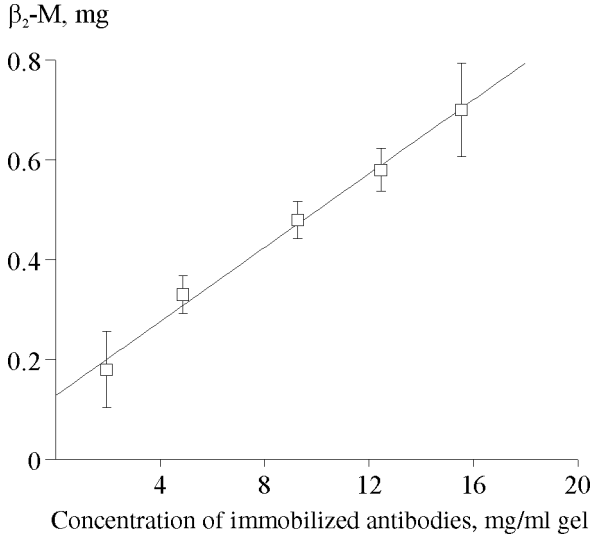

Fig. 3. Relationship between adsorption capacity of anti β_2-microglobulin (β_2-M) Sepharose CL-4B and concentration of immobilized ligand. Batch chromatography on 100 μm of adsorbent. Duration of adsorbent incubation with solution containing β_2-M was 1 h, β_2-M concentration in solution 54 mg/liter. Washing in phosphate buffer (pH 7.4), elution with 0.05 M citrate buffer (pH 2.5).

antibodies and β_2-M did not depend on the initial concentration of protein in the solution.

Hence, synthetic immunosorbent effectively and specifically removed β_2-M from human blood plasma. Application of this adsorbent under clinical conditions will reduce the level of β_2-M in the blood and prevent its further deposition in amyloid fibrils in patients on chronic hemodialysis.

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