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Note

Selective removal of β2-microglobulin from human plasma by high-performance immunoaffinity chromatography

MAKIO MOGI* and MINORU HARADA

Department of Oral Biochemistry, Matsumoto Dental College, Shiojiri 399-07 (Japan)

TOMOKO ADACHI and KOHICHI KOJIMA

Hatano Research Institute, Food and Drug Safety Center, Hadano 257 (Japan)

and

TOSHIHARU NAGATSU

Department of Biochemistry, Nagoya University School of Medicine, Nagoya 466 (Japan)

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Affinity chromatography using immobilized antibodies is a rapid and specific technique for the isolation of biologically active materials from a variety of different sources [1–4]. In addition, this method has been used to remove antibodies [5–7] and antigens [8] for medical application.

This technique can become even more useful when the controlled conditions of high-performance immunoaffinity chromatography (HPIAC) are applied. HPIAC is applicable to the rapid isolation and measurement of many different types of biological material [9–11]. Though the major disadvantage of the technique is the unavailability of a suitable packing material that can easily be used to immobilize antigens and antibodies, this problem can be overcome by using commercially available Affi-Prep 10 (Bio-Rad).

A significant correlation between the duration of hemodialysis and the incidence of carpal tunnel syndrome [12], or destructive cystic lesions of bone with shoulder and joint pain [13], has been previously reported Recently, amyloid deposition has been found to be associated with or directly to cause these lesions in most cases. This amyloid protein has been identified to be

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homologous with β2-microglobulin (B2-MG) [14]. B2-MG, a low-molecular mass, non-glycosylated protein (M_r = 11 800), which forms the light chain of the class I major histocompatibility complex, is present on the surface membrane of most nucleated cells [15] and in plasma. Since the proximal tubule cells of the kidney are the major site of B2-MG catabolism, this protein accumulates in the plasma of patients with advanced renal insufficiency and those on hemodialysis. It has been demonstrated that there is little or no clearance of this protein by cellulose or polyacrylonitrile dialysis membranes [16,17].

In an attempt to resolve this situation, we have used HPIAC to remove specifically B2-MG from human plasma. In this report, studies are described that were undertaken to determine the applicability and effectiveness of HPIAC on a column (Affi-Prep 10) bearing immobilized anti-B2-MG immunoglobulin G (IgG) as a procedure for plasma B2-MG elimination

EXPERIMENTAL

Materials

B2-MG, purified from human urine, was purchased from Calbiochem (La Jolla, CA, U.S.A) Anti-B2-MG antiserum was obtained from Dakopatts (Glostrup, Denmark) and Protein-A Sepharose from Pharmacia (Uppsala, Sweden). Affi-Prep 10 for affinity chromatography (30 mm × 4.6 mm I.D., 0.5 ml) and protein assay kit were from Bio-Rad (Rockville Centre, NY, U.S.A.). Human plasma samples were obtained from healthy individuals and stored at −20 °C. Anti-B2-MG IgG was isolated from the antiserum by use of Protein-A Sepharose (Bio-Rad). Human IgG was also purified from control plasma in the same way. Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO, U S.A.). All other chemicals used were analytical grade.

Preparation of immunoaffinity column

Anti B2-MG IgG was immobilized on the Affi-Prep 10 by following the standard protocol of Bio-Rad. The total coupling rate was 9.43 mg of IgG per 0.5 ml of Affi-Prep 10 gel.

Chromatographic conditions

The immunoaffinity column was installed into a Jasco L-800 high-performance liquid chromatographic (HPLC) system (Japan Spectroscopic, Tokyo, Japan) equipped with a Model 880-PU pump and a Model 875 UV detector. The system was also equipped with a Model 880-02 ternary gradient unit with a Model 801-SC system controller, which automatically controlled the elution profile of the system The column was jacketed and maintained at 4 °C in an ice-bath and was isocratically eluted with phosphate-buffered saline (PBS) (pH 7.2; 0.15 *M* NaCl) for 10 min at a flow-rate of 1.0 ml/min. Samples (human plasma or model samples) were loaded on to the column by means of the

ternary gradient unit/system controller Following the initial 10 min, during which the immobilized IgG reacted specifically with B2-MG and the unreacted material ran through the column, the B2-MG elution phase was started. For elution, a pH gradient was automatically generated from 7 2 to 2 5 by the addition of 0 5 *M* glycine–HCl buffer (pH 2 5) to the initial buffer over a 10-min period. The absorbance of the eluate was monitored at 280 nm with a detector sensitivity setting of 0 16 a.u f.s , and those fractions containing the eluted material were collected The B2-MG content in each fraction was determined by a sandwich enzyme immunoassay (EIA), which consisted of a solid phase (polystyrene beads) of immobilized anti-B2-MG antibodies and antibodies labelled with β-D-galactosidase from *Escherichia coli*, as described by Mogi et al. [18]. By this EIA, the protein A-purified anti-B2-MG IgG showed no cross-reactivity with purified IgG, IgM and albumin from human sera, indicating that this antibody is specific for B2-MG.

Two-dimensional electrophoresis was performed with O'Farrell's system [19] The protein concentration was estimated by the method of Bradford [20], with BSA as a standard.

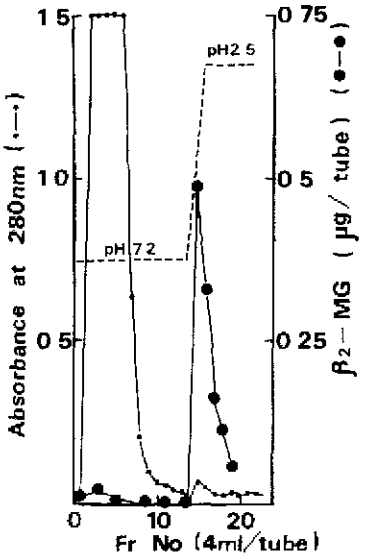

Fig 1 HPIAC chromatogram of B2-MG isolated from human control plasma (5 ml) The chromatogram was developed using an acid pH gradient elution from 7 2 to 2 5 The trace was obtained from an HPIAC column (30 mm×4 6 mm I D) eluted at 1 0 ml/min in PBS and monitored at 280 nm with the detector set at 0 16 a u f s The column was maintained at 4°C throughout the analysis The dotted line indicates the pH gradient profile The closed circles indicate the B2-MG content (μg/ml) determined by EIA

RESULTS

A typical elution profile, obtained by acid elution, is shown in Fig. 1. This chromatogram was produced by passing 5 ml of control plasma through the anti-B2-MG immunoaffinity column The primary, large peak contains the non-reactive plasma materials and the second peak indicates the B2-MG

EIA determination of the immunoaffinity-isolated B2-MG demonstrated that superior results were obtained, as shown in Tables I and II The reproducibility of total B2-MG trapped and B2-MG eluted was satisfactory. As shown in Table I, the percentage of B2-MG trapped (mean ± S D.) was 98 16 ± 0 13% [coefficient of variation (C V.) = 0.14%] and that of the material subsequently eluted

TABLE I

REPRODUCIBILITY OF AFFI-PREP 10-COUPLED ANTI-B2-MG IgG

Flow-rate, 1 0 ml/min, sample, 15 μg of B2-MG and 100–200 μg of BSA in 1 ml of PBS

Experiment No	Total B2-MG loaded (μg)	Total B2-MG in pass-through fraction (μg)	Total B2-MG trapped (%)	Total B2-MG eluted	
				μg	%
1	15	0 175	98 3	8 1	54 0
2	15	0 188	98 1	7 7	51 3
3	15	0 172	98 3	8 2	54 7
4	15	0 190	98 1	7 9	52 7
5	15	0 197	98 0	7 3	48 7
Mean ± S D		0 180 ± 0 01	98 16 ± 0 13	7 84 ± 0 36	52 28 ± 2 39
C V (%)		5 72	0 14	4 56	4 56

TABLE II

INFLUENCE OF FLOW-RATE AND B2-MG LOADED ON THE TRAPPING EFFICIENCY OF THE HPIAC COLUMN

Sample, 10–15 μg of B2-MG with 250 mg of BSA and 90 mg of IgG in 5 ml of PBS

Flow-rate		Total B2-MG loaded (μg)	Total B2-MG in pass through fraction (μg)	Total B2-MG trapped (%)
cm/h	ml/min			
90	0 25	15	0	100
360	1 0	10	0 392	96 1
540	1 5	10	0 164	98 4
720	2 0	10	0 644	93 6
900	2 5	10	1 196	88 0
1080	3 0	10	1 079	89 2
1260	3 5	10	3 194	68 1

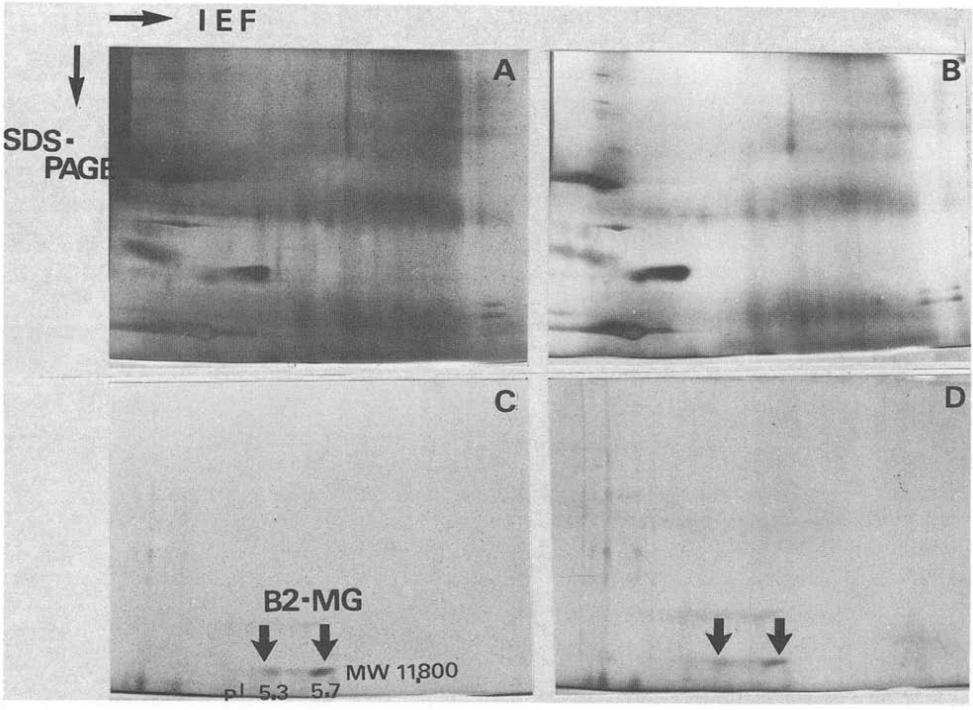

Fig 2 Two-dimensional gels obtained by silver staining of (A) a human plasma sample (150 μg), (B) the through fraction from the column (140 μg), (C) a B2-MG standard (50 ng) and (D) the fraction eluted with 0 5 *M* glycine–HCl (50 ng) Arrows indicate the positions of B2-MG p*I* coordinates were measured by comparing the migration of a standard p*I* marker (acetylated cytochrome c, Oriental, Japan) Molecular masses were determined by measuring the migration of low-molecular-mass standards (Bio-Rad, U S A)

TABLE III

HUMAN PLASMA (NORMAL OR NORMAL SUPPLEMENTED WITH B2-MG) LOADED ON HPIAC COLUMN

Flow-rate, 1 0 ml/min

Sample	Flow-rate (cm/h)	Total B2-MG loaded (μg)	Total B2-MG in pass-through fraction (μg)	Total B2-MG trapped (%)
Normal plasma (5 ml)	360	7 9 (1 58 μg/ml)	0 166	97 9
Normal plasma with 200 μg of B2-MG (5 ml)	360	207 9 (41 6 μg/ml)	4 81	97 7

was 52 28 ± 2.39% (C V. = 4.56%), using model samples of B2-MG (15 μg) and BSA (100–200 μg) in 1 ml of PBS

The flow-rate did not affect the trapping rate for model samples over the range 0.25–1.5 ml/min (Table II), but rates greater than 2.0 ml/min resulted in an inefficient binding of the B2-MG to the immobilized IgG during the initial phase of the immunoaffinity procedure. The pressure was below 6 kg/cm^2 in all cases. Although the B2-MG binding capacity of the column gradually decreased after ten column elutions and regenerations, we were unable to detect leakage of the immobilized IgG, checked by Western blot, even after fifty column recyclings. When samples were subjected to two-dimensional electrophoresis before or after HPIAC of 1 ml of plasma, no noteworthy differences were seen between the two gels, as shown in Fig. 2A and B. The maximum binding capacity of the column was 2.3 mg of B2-MG per 0.5 ml of Affi-Prep 10 under this condition. Table III summarizes our findings on 5 ml of normal plasma and that mixed with 200 μg of B2-MG

The concentration of B2-MG in the supplemented sample, 41.6 μg/ml, was the same as that in patients on long-term hemodialysis Over 97% of the B2-MG in plasma was adsorbed by the anti-B2-MG IgG immobilized on the Affi-Prep 10 column in each case. In addition, when we subjected samples eluted by acid buffer to two-dimensional electrophoresis, each sample showed two major spots in the pH range 5.3–5 7 in Fig 2D Both spots corresponded to the same relative molecular mass (M_r = 11 800), and their mobility was identical with that of standard B2-MG, as shown in Fig. 2C. These findings show that the HPIAC procedure is very effective and useful for specific removal of B2-MG from plasma.

DISCUSSION

The use of HPIAC provides a technique for removing B2-MG from plasma and releasing it from the column in short time. The technique can be used in any facility that has a simple HPLC system. Once made, the immobilized-antibody column remains viable for 50 analyses and usable, when stored refrigerated at 4 °C, for up to one year.

The anti-B2-MG IgG column showed only a 48–55% recovery of B2-MG (Table I). When we tested the time-dependent immunological stability of the B2-MG in acidic condition (0 5 *M* glycine–HCl pH 2.5 at 4 °C), no differences were seen in the samples between 5 min and 3 h. One possible explanation for this phenomenon may be that B2-MG remained bound to high-affinity subclasses of the polyclonal anti-B2-MG IgG and was not eluted by the pH 2.5 glycine buffer. The amount of immunosorbent, flow-rate, duration of perfusion and elution buffer were probably not optimal; so further experimentation may lead to a more efficient tool for therapeutic use.

Recently, plasma exchange has been advocated for the treatment of several

autoimmune diseases [21] and cancer [22] This approach is effective but uses large amounts of valuable blood products and exposes the recipient to additional risks of hepatitis and other transmissible diseases. Thus, alternative methods for removal of antibodies or antigens are desirable. This HPIAC procedure should prove useful for medical application and may have broad applicability for the elimination of any plasma components with antigenic or amyloidogenecity properties

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