PREPARATION OF RABBIT β_2 -MICROGLOBULIN

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(Received March 18, 1980)

β_2 -Microglobulin was prepared from the urine of Na-chromate injected rabbits by a simple method using gel filtration and ion exchange chromatography. The amino acid composition and isoelectric point of the rabbit β_2 -microglobulin were determined.

β_2 -Microglobulin is a small protein (M. W.=11,800) which occurs in biological fluids such as urine, serum and cerebrospinal fluid¹⁾. It is one component of histocompatibility antigen, but its biological function is still unknown.

For studying the biological function of β_2 -microglobulin, it is very useful to define similar proteins in species other than man. Some forms of mammalian β_2 -microglobulin have been isolated from the urine of dogs, guinea pigs and rabbits²⁻⁴⁾. This paper describes a simple method for isolating β_2 -microglobulin from the urine of rabbits with proteinuria.

MATERIALS AND METHODS

Rabbits (female) were given a single injection of sodium chromate (50 mg/rabbit; body weight, 2.5 kg) subcutaneously and kept in a metabolic cage. A 24-hour urine specimen was collected in a bottle at 4°C and tested for proteinuria with a Labstix strip (Miles O). Such specimens were stocked at -20°C until use. Specimens (2 to 5 days after the injection) which showed severe proteinuria were used for the preparation of β_2 -microglobulin. The urine specimen was centrifuged at 9,000 rpm for 15 min under refrigeration. The supernatant was applied directly to a column of Sephadex G-100 (7.0×145 cm or 10.0×145 cm), equilibrated with 0.02 M ammonium carbonate. The column was run with 0.02 M ammonium carbonate. The effluent was monitored by measuring the absorbance at 280 nm. The β_2 -microglobulin fraction was determined by using authentic human β_2 -microglobulin as the indicator. The lyophilized protein was rechromatographed on a column of Sephadex G-75 (5×140 cm) using 0.02 M ammonium carbonate. The main fraction was collected and then lyophilized. It was further fractionated on a column of DEAE-cellulose (2×43 cm), equilibrated with 0.01 M phosphate buffer, pH 7.5. The column was run with a 500-ml linear gradient of sodium chloride, from 0 to 0.2 M. The main fraction was collected and then lyophilized. The

material was desalted on a column of Sephadex G-25 (2.8×40 cm) using 0.02M ammonium carbonate. For further purification, the ion exchange chromatography was carried out repeatedly. All operations were performed at 4°C.

Amino acid analysis was undertaken with a JEOL-6AH analyzer on the purified rabbit β_2 -microglobulin hydrolyzed with 6 N HCl for 24 hr. Half cystine was estimated as cysteic acid after performic acid oxidation. Tryptophan was not determined.

SDS disc electrophoresis and isoelectrofocusing with a PAG plate (LKB Produkter AB/Sweden) were carried out according to the methods of Weber and Osborn⁵⁾ and Vesterberg and Svensson⁶⁾, respectively.

RESULTS

The gel filtration pattern of 150 ml urine on a Sephadex G-100 column with 0.02M ammonium carbonate is shown in Fig. 1. The elution position of the rabbit β_2 -microglobulin was estimated from that of authentic human β_2 -microglobulin on the same column of Sephadex G-100. The β_2 -microglobulin fraction is indicated by the arrow in Fig. 1. The crude materials were obtained from several separations. They were gathered and run on a Sephadex G-75 column with 0.02 M ammonium carbonate. The elution pattern is presented in Fig. 2. The main fraction indicated by the arrow was eluted at about 1.51. It was lyophilized and then applied to a DEAE-cellulose column. Fig. 3 indicates the ion exchange chromatogram of crude rabbit β_2 -microglobulin obtained by two gel filtrations. A small peak appeared at the void volume. The main peak indicated by the arrow was eluted at a concentration of about 0.05 M Na^+ . The

Fig. 1. Gel filtration of rabbit urine on a Sephadex G-100 column (10×140 cm).

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Fig. 2. Gel filtration of rabbit β_2 -microglobulin on a Sephadex G-75 column (5 $\times$ 140 cm).

Fig. 3. Ion exchange chromatography of rabbit β_2 -microglobulin on a DEAE-cellulose column (2 $\times$ 42 cm) (linear gradient; 0.01 M phosphate buffer, pH 7.5 (0-0.2 M NaCl)).

protein of the main peak was desalted on a Sephadex G-25 column using 0.02 M ammonium carbonate and then rechromatographed on DEAE-cellulose column by the same procedure as described above. This final chromatographic fractionation was omitted, if the sample did not require further purification. About 30 mg of purified β_2 -microglobulin was recovered from 900 ml of urine obtained from rabbits with proteinuria.

The protein gave a single band on disc electrophoresis and no impurities could be found on isoelectrofocusing, as shown in Fig. 4. The isoelectric point of the rabbit

Fig. 4. Isoelectrofocusing of human, monkey and rabbit β_2 -microglobulins.

β_2 -microglobulin was estimated to be 5.6 on the basis of the position on the isoelectrofocusing plate. This value was identical with that of human β_2 -microglobulin but different from that of monkey β_2 -microglobulin⁷⁾. The molecular weight of the rabbit β_2 -microglobulin was calculated to be 10,500 using SDS disc electrophoresis.

Table 1 shows the amino acid composition of the rabbit β_2 -microglobulin. This amino acid composition is identical with that of the rabbit β_2 -microglobulin prepared by Berggård⁴⁾.

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DISCUSSION

A protein with a molecular weight of 10,500 was prepared from the urine of rabbits injected Na-chromate by a simple method using gel filtration and ion exchange chromatography without ultrafiltration and dialysis. It gave a single band on disc electrophoresis or isoelectrofocusing, and contained no impurities. The amino acid composition of the protein was identical with that the rabbit β_2 -microglobulin reported by Berggård⁴⁾. The present method is thus very effective for preparing rabbit β_2 -microglobulin from urine.

The isoelectric points of both rabbit and human β_2 -microglobulin are 5.6, even through the amino acid compositions are different. However, the isoelectric point of monkey β_2 -microglobulin is 7.5⁷⁾, even though its amino acid composition is similar to that of human β_2 -microglobulin.

Table 1. Amino acid composition of rabbit β_2 -microglobulin*.

Amino acid	Moles of amino acid on the basis of 4 Arg residues	
	Obs.**	Ref. 4
Asx	15.08	15
Thr	3.87	4
Ser	4.98	6
Glx	11.45	11
Pro	8.25	7-8
Gly	4.64	3
Ala	2.68	2
1/2 Cys	1.20	2
Val	9.86	10
Met	0.99	1
Ile	2.89	3
Leu	7.33	7
Tyr	4.39	5
Phe	4.69	5
His	3.89	5
Lys	7.47	8
Arg	4.00***	4
Try	not determined	2

* The rabbit β_2 -microglobulin exhibited a single band on disc electrophoresis.

** Calculated on the basis of 4 Arg residues.

*** 4 Arg residues.

ACKNOWLEDGEMENT

This investigation was supported by NHW's Contract Fund for Food Sanitation Research.

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REFERENCES

- 1) Berggård, I. and Bearn, A. G. (1968) *J. Biol. Chem.* **243**, 4095.
- 2) Smithies, O. and Poulik, M. D. (1972) *Proc. Natl. Acad. Sci.* **69**, 2914.
- 3) Cigen, R., Ziffer, J. A., Berggård, B., Cunningham, B. A. and Berggård, I. (1978) *Biochemistry* **17**, 947.
- 4) Berggård, I. (1974) *Biochem. Biophys. Res. Comm.* **57**, 1159.
- 5) Weber, K. and Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406.
- 6) Vesterberg, O. and Svensson, H. (1966) *Acta Chem. Scand.* **20**, 820.
- 7) Kimura, M. and Otaki, N. In preparation.