DETERMINATION OF URINARY $\beta_2$-MICROGLOBULIN IN THE URINE OF THE CADMIUM-POISONED RABBIT

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Abstract: A protein of low molecular weight (MW 12,000) was detected in the urine of a rabbit given cadmium for 17 months, by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. This protein was identified as $\beta_2$-microglobulin by electrosyneresis using standard $\beta_2$-microglobulin, a urine sample and anti rabbit $\beta_2$-microglobulin mouse serum. The urinary concentration of $\beta_2$-microglobulin in the rabbit fed a diet containing cadmium for 17 months was estimated to be 8-40 mg/l by staining the gel with silver and to be 10-20 mg/l (3.6-7.2 mg/day) by electrosyneresis.

Key words: $\beta_2$-microglobulin antibody—Rabbit—Urinary—Cadmium

INTRODUCTION

High excretion of $\beta_2$-microglobulin ($\beta_2$-MG) has been observed in the urine of cadmium-poisoned patients with renal tubular damage1,2). $\beta_2$-MG has been isolated from human3, monkey4), rabbit5) and dog6) urine specimens. The levels of $\beta_2$-MG in mammalian fluids have been measured7,8). Nomiyama et al9) have found proteinuria in rabbits treated with cadmium for a long time and detected a protein of low molecular weight (12,000) in the urine by polyacrylamide gel electrophoresis using sodium dodecyl sulfate (SDS-PAGE).

In this report, we demonstrate that the low molecular weight protein in rabbit urine is identical with $\beta_2$-MG. We also report the semiquantitative analysis of rabbit $\beta_2$-MG in urine by electrosyneresis using anti rabbit $\beta_2$-MG mouse serum5,10) and the highly sensitive determination of the urinary $\beta_2$-MG by SDS-PAGE using silver staining.
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MATERIALS AND METHODS

Administration of cadmium and collection of urine

A male New Zealand White rabbit, aged 4 months and weighing 2 kg, was fed a diets (CLEA, CR-2) containing cadmium chloride (300 µg/g) for 18 months. Urine specimens were collected for 24 hr at intervals. The volume of urine, concentrations of cadmium, protein and glucose, and pH of the urine were measured occasionally by the urinalysis method of Nomiyama et al.9). Urine sample for analysis by SDS-PAGE and electrosyneresis was collected on 17 months.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SDS-PAGE of urine samples from the cadmium-poisoned rabbit was performed according to the method of Weber and Osborn11). Samples for electrophoresis were prepared by addition of an equal volume of 2% SDS solution to the urine. The mixture was heated at 100°C for 1 hr, and then incubated at room temperature overnight. An aliquot of the sample solution was placed on polyacrylamide gel (0.5 × 8 cm). Electrophoresis was performed at 1 mA per tube for the first 30 min, then at a constant current of 8 mA per tube until the Bromphenol blue marker dye had moved a distance of 7~8 cm. Albumin (250 µg/ml), ovalbumin (275 µg/ml), chymotrypsinogen (125 µg/ml), myoglobin (125 µg/ml) and cytochrome c (125 µg/ml) were used as standard proteins for the determination of molecular weight. Protein bands were detected by staining with Amido black 10B or with silver according to the procedure of Switzer et al.12).

Electrosyneresis

Rabbit β2-MG was purified from the urine of a chromium-poisoned rabbit by the procedure of Otaki and Kimura5). Antiserum for rabbit β2-MG was prepared in a CBA/StMs mouse according to the method of Ogata et al.10). Urine from the cadmium-poisoned rabbit was concentrated 5 times by the precipitation method with cold ethanol. The precipitate was finally dissolved in a known volume of barbital buffer, pH 8.6, µ 0.025, and used as the antigen in electrosyneresis.

Urinary protein of low molecular weight from the cadmium-poisoned rabbit was identified as β2-MG by electrosyneresis according to the method of Kohn13). Semiquantitative analysis of the urinary β2-MG by electrosyneresis was carried out by the usual method10). After the electrophoresis, unreacted protein in the gel was washed out by dipping the gel in saline. Coomassie brilliant blue or nigrosine was used to stain the protein.

RESULTS

Urine sample

Urine of the rabbit fed a diet containig cadmium at 300 µg/g for 17 months amounted
Fig. 1. Electrophoretogram of urinary protein of cadmium-poisoned rabbit by SDS-polyacrylamide gel electrophoresis. Urine samples of 25 µl were charged on gels 1 and 6, 10 µl on gels 2 and 7, 5 µl on gels 3 and 8, 2.5 µl on gels 4 and 9, and 0.5 µl on gels 5 and 10. Cytochrome c, myoglobin, chymotrypsinogen, ovalbumin and albumin were used as standard proteins with molecular weights of 12,000, 17,800, 25,000, 45,000 and 67,000, respectively.
to 355 ml/day; it had a total protein concentration of 2.9 mg/ml, no glucose and a pH value of 8. This urine sample was analyzed by SDS–PAGE and electrosyneresis.

**Measurement of molecular weight of urinary protein by SDS–PAGE**

The urine of the cadmium-poisoned rabbit showed several protein bands on staining with Amido black 10B, as shown in Fig. 1. The molecular weights of these proteins were estimated to be 24,500, 33,100, 58,200, 69,200 and 89,100. Two proteins of molecular weight larger than 100,000 were also noted. No protein band of low molecular weight (12,000) was observed, though any protein present in an amount of more than 2 μg could be detected by this staining procedure. Silver staining was highly sensitive and its detection limit for protein was 0.02 μg. If 10 μl of urine sample was subjected to SDS–PAGE, protein at a level of 2 μg/ml was detectable. Silver staining visualized the same protein bands as Amido black staining. Two proteins with molecular weights of 12,000 and 17,400, which were not observed by Amido black staining, could be found by the silver staining. The amount of 12,000–dalton protein was estimated to be 8–40 mg/l, semiquantitatively.

**Semiquantitation of β2-MG in rabbit urine by electrosyneresis**

A urine sample obtained from the cadmium-poisoned rabbit and purified rabbit β2-MG were analyzed by electrosyneresis using anti rabbit β2-MG mouse serum (Fig. 2). The rabbit urine reacted with specific antiserum for rabbit β2-MG and the precipitin line fused with that of rabbit β2-MG. The urine sample and rabbit β2-MG of known concentration were subjected to electrosyneresis (Fig. 3). The detection limit of rabbit β2-MG was 0.01 μg/ml.
MG was 50 mg/l by this method. The concentration of urinary $\beta_2$-MG in the chronically cadmium-poisoned rabbit was estimated to be 10–20 mg/l.

**DISCUSSION**

Low molecular protein (MW 12,000) in urine from a chronically cadmium-poisoned rabbit was detected by SDS-PAGE followed by silver staining. This low molecular protein in the urine was identified as rabbit $\beta_2$-MG by the immunological technique of electrosyneresis with anti rabbit $\beta_2$-MG serum. The amount of $\beta_2$-MG in the urine of the rabbit administered cadmium for 17 months was 10–20 mg/l (3.6–7.2 mg/day) as determined semiquantitatively by electrosyneresis. This concentration of $\beta_2$-MG corresponded to 1/300–1/500 of the concentration of the total protein found in the rabbit urine.

Although the detection limit of $\beta_2$-MG by electrosyneresis using anti rabbit $\beta_2$-MG serum with nigrosine stain was 50 $\mu$g/ml, that of SDS–PAGE with silver stain was greatly superior at 2 $\mu$g/ml. Further improvements of specific antibody production should permit the development of a highly sensitive immunological microassay for $\beta_2$-MG.
REFERENCES