ISOLATION AND CHARACTERISTICS OF A RABBIT β_2-MICROGLOBULIN: COMPARISON WITH HUMAN β_2-MICROGLOBULIN*

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SUMMARY: A low molecular weight β_2-globulin has been isolated from urine of rabbits treated with sodium chromate. Several findings indicate that this protein is the rabbit counterpart of human β_2-microglobulin which is known to occur linked to the major histocompatibility (HL-A) antigens of man. The rabbit β_2-globulin and human β_2-microglobulin are very similar in amino acid composition, molecular size, molecular weight, electrophoretic mobility, and also in the presence of apparently analogous disulfide loops. More than half of the amino acids in the two proteins seem to be present in identical numbers. The urinary excretion of both proteins is increased by agents which induce renal tubular damage.

β_2-microglobulin is a small protein originally found in human biological fluids (1). Analyses of the amino acid sequence of human β_2-microglobulin have shown that this protein is related to the immunoglobulins (2-4) and homologous to the constant domains of IgG (3,4). There is no correlation between synthesis of β_2-microglobulin and immunoglobulins (5,6) and β_2-microglobulin is manufactured not only by lymphoid cells (5,6,7) but also by a variety of mesenchymal and epithelial cells (5). This supports the idea that the β_2-microglobulin gene has evolved directly from the immunoglobulin precursor gene (3) and indicates that β_2-microglobulin has a genetic control system independent of that of the immunoglobulins (5). β_2-microglobulin has been found on the surface of leucocytes (3) and recent work (8,9,10) has shown that it is identical with a small polypeptide that is bound to HL-A alloantigens of different specificities.

In order to investigate the connexions of β_2-microglobulin with immunoglobulins and histocompatibility antigens in detail it is important to define similar proteins in species other than man. The isolation of a dog β_2-microglobulin was recently reported (11). In this communication, I describe the isolation and some characteristics of a rabbit β_2-microglobulin. Urine from patients with low molecular weight proteinuria due

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to various disorders of the renal tubules has been the source for purification of human β_2-microglobulin (1). I have induced proteinuria in rabbits by treating them with sodium chromate. This agent is known to induce tubular damage and lysozymuria in rats (12).

MATERIALS AND METHODS: Rabbits were given single injections of sodium chromate (10 mg/kg) subcutaneously and kept in metabolism cages. 24 h urine specimens were collected in bottles containing 1 ml of 5 % sodium azide as preservative. Tests for protein with Uristix strips (Ames Company, England) were usually negative in normal rabbit urine. Upon sodium chromate administration, the animals responded with proteinuria, which most often was maximum (3+ to 4+) during the 3rd to 5th day after injections. The urine samples were centrifuged, dialyzed exhaustively at +4° against distilled water, and lyophilized. Yields of lyophilized material from each 24 h volume varied from 0.1 - 0.2 g in the normal urines to 1 - 2 g at the height of proteinuria.

Gel chromatography on Sephadex G-100, zone electrophoresis in sodium borate buffer, and chromatography on DEAE-cellulose were carried out as described (1). Agarose gel electrophoresis in 0.075 M sodium barbital buffer, pH 8.6, was performed according to Johansson (13). Half-cystine was estimated as cysteic acid after performic acid oxidation (14). The other analytical methods have been reported previously (1,15).

RESULTS AND DISCUSSION: Non-dialyzable urinary substances produced after sodium chromate injections were separated on small columns of 'Sephadex' G-100. Patterns indicating increased excretion of low molecular weight proteins were only observed during the first week. Accordingly, materials collected during the first week were used for preparative separations on 'Sephadex' G-100. Fig. 1 shows a comparison of chromatograms of urinary macromolecules from injected rabbits and from humans with low molecular weight proteinuria. The curve of rabbit proteins displays a peak which corresponds to that of human β_2-microglobulin. This peak from several 'Sephadex' G-100 separations was pooled and concentrated by ultrafiltration. In one of the preparations, 21.6 g lyophilized material gave a fraction containing 320 mg protein. This material was subjected to zone electrophoresis in 0.1 M sodium borate buffer, pH 8.9. After concentration, the single distinct protein fraction obtained (69 mg) was again separated on a 'Sephadex' G-100 column. It emerged as a practically symmetrical peak. The main part of the peak (48 mg) was chromatographed on DEAE-cellulose. The column, equilibrated with 0.01 M Tris-HCl buffer, pH 7.85, was eluted with a linear gradient of sodium chloride, from 0 to 0.2 M. The rabbit

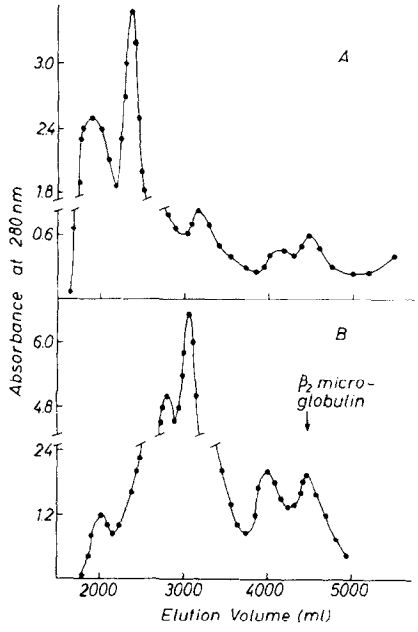

Fig. 1. Chromatography on 'Sephadex' G-100 of concentrated urinary proteins (3200 mg) from rabbits injected with sodium chromate (A), and of concentrated urinary proteins (5600 mg) from human patients with chronic cadmium poisoning and low molecular weight proteinuria (B). The column (118x8 cm) was equilibrated with 0.1 M Tris-HCl + 1.0 M sodium chloride, pH 8.0. The flow rate was 72 ml/h. The peak of human β_2-microglobulin is indicated.

protein was eluted at a sodium chloride concentration similar to human β_2-microglobulin. Small amounts of impurities were removed, and 40 mg of protein was recovered after dialysis against distilled water and lyophilization.

On agarose gel electrophoresis at pH 8.6 the rabbit protein had the mobility of a β_2-globulin. When examined by polyacrylamide gel electrophoresis at pH 8.9, it gave one intense zone which migrated only slightly slower than an intense zone given by purified human β_2-microglobulin. Analysis by sedimentation equilibrium ultracentrifugation indicated that the rabbit β_2-globulin is homogeneous and has a molecular weight of 12,400. This is close to the value of 11,800 estimated for human β_2-microglobulin (1).

Table 1 gives the amino acid contents of the rabbit β_2-globulin together with that of human β_2-microglobulin. An almost even number of residues was obtained for most amino acids when calculations were made on basis of 4.0 residues of arginine per molecule. Obviously the two proteins have quite similar compositions. More than half of the amino acids seemed to be present in identical numbers.

Table 1. Amino acid composition of rabbit β_2-globulin and human β_2-microglobulin

Amino acid	Rabbit β_2-globulin*	Human β_2-microglobulin+	Difference‡
	residues/molecule§		
Aspartic acid	14.9 (15)	12	+ 3
Threonine	4.1 (4)	5	- 1
Serine	6.1 (6)	10	- 4
Glutamic acid	11.3 (11)	11	0
Proline	7.5 (7-8)	5	+2 or +3
Glycine	3.2 (3)	3	0
Alanine	2.2 (2)	2	0
Half-cystine	2.2 (2)	2	0
Valine	9.8 (10)	7	+ 3
Methionine	0.9 (1)	1	0
Isoleucine	3.0 (3)	5	- 2
Leucine	6.7 (7)	7	0
Tyrosine	5.1 (5)	6	- 1
Phenylalanine	4.9 (5)	5	0
Lysine	7.8 (8)	8	0
Histidine	4.0 (4)	4	0
Arginine	4.0 (4)	5	- 1
Tryptophan	2.1 (2)	2	0
Total	99 or 100	100	-1 or 0

* The figures for all amino acids, except half-cystine and tryptophan, are average values from analysis of three different preparations. On each specimen, one 24-hour and one 72-hour hydrolysis were performed. Two preparations were analysed for half-cystine, which was determined as cysteic acid after performic acid oxidation. Tryptophan was estimated spectrophotometrically on one of the specimens.

+ Data from reference 1.

‡ Given as deviation from the composition of human β_2-microglobulin.

§ Calculated on the basis of 4.0 residues of arginine per molecule. The nearest integer values for the rabbit β_2-globulin are given within parentheses.

A characteristic feature of both human β_2-microglobulin and the immunoglobulin domains is that they possess an intrachain disulfide loop of the same size and location (3). On starch gel electrophoresis in 8 M urea at acid pH, the rabbit β_2-globulin and human β_2-microglobulin had similar

mobilities whether untreated or reduced and alkylated in the absence of urea. The two proteins, reduced and alkylated in the presence of urea, also had similar, but lower, mobilities (see Fig. 2). The decreased mobility after reduction in urea is most probably due to unfolding of the mole-

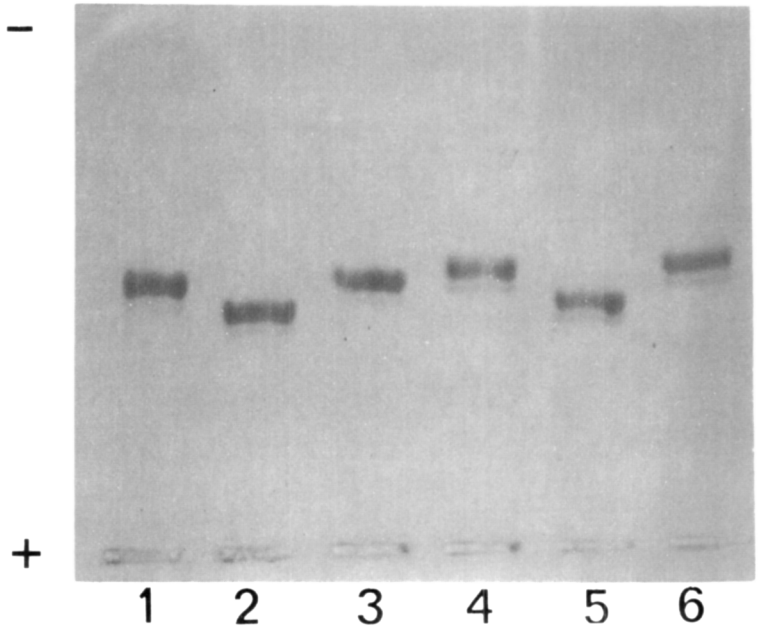

Fig. 2. Starch gel electrophoresis in 8 M urea and formate buffer, pH 3.0, of: rabbit β_2-globulin (1-3) and human β_2-microglobulin (4-6); 1, 4 untreated; 2, 5 reduced and alkylated in the presence of 8 M urea; and 3, 6 reduced and alkylated in the absence of urea

cules resulting from split of a disulfide bond. These results indicate that the rabbit β_2-globulin consists of a single polypeptide chain and that its two half-cystine residues (Table 1) are involved in a disulfide bond. As the rabbit and human proteins were alike in electrophoretic mobility after reduction in urea, they seem to have disulfide loops of similar size.

The amino acid sequence of dog β_2-microglobulin has been partially determined (11). Dog and human β_2-microglobulins were demonstrably different at only 6 of the 41 positions compared. All differences can be explained by single base-pair substitutions (11). The amino acid composition of the dog protein has not been reported. But it is interesting to note that the partial sequence of the dog protein includes two proline residues instead of a threonine and a serine residue, which are present

in the human protein. As shown here, the rabbit β_2-globulin contains more of proline and less of threonine and serine than human β_2-microglobulin.

Recently we have obtained a goat antiserum against the rabbit β_2-globulin. On Ouchterlony immunodiffusion analyses, human β_2-microglobulin did not precipitate with this antiserum but it partly inhibited the formation of a precipitation line between the rabbit β_2-globulin and the antiserum. Accordingly, the antigenic structure of the rabbit protein appears to resemble that of human β_2-microglobulin.

To summarize: the rabbit protein and human β_2-microglobulin are similar in amino acid composition, molecular size, molecular weight, and electrophoretic mobility, and also in the presence of apparently analogous disulfide loops. Their antigenic structures seem to be related. Furthermore, increased amounts of both proteins are found in urine in conjunction with renal tubular damage. These results strongly suggest that the above-described protein is the rabbit counterpart of human β_2-microglobulin.

As human β_2-microglobulin occurs linked to HL-A antigens (8,9,10) it will be important to find out if the rabbit β_2-globulin is present on the surface of cells and if it is bound to rabbit transplantation antigens. It appears also to be of considerable interest to obtain information about the amino acid sequence of this rabbit protein. Human β_2-microglobulin has been shown to be homologous in sequences to the constant homology regions of IgG (3,4) and knowledge of the covalent structure of the rabbit β_2-globulin may help to clarify the evolutionary relations between β_2-microglobulins and immunoglobulins.

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