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ISOLATION AND CHARACTERIZATION OF POST GAMMA GLOBULIN IN MOUSE

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A protein of about 13,000 daltons was isolated from mouse CBA urine after inducing a tubular renal dysfunction. This protein was demonstrated similar to human Post Gamma globulin by electrophoresis, aminoacid content and immunochemical criteria.

Post gamma globulin (PGG) is a low molecular weight protein of about 12,000 daltons, characteristic of the normal C.S.F. (1) and urine from patients with tubular reabsorption disorders (2). It is also found in many other biological fluids (3,4).

In man there are three different PGG's electrophoresis patterns (6) corresponding to a loss of certain basic aminoacids (5). PGG was detected in the urine of monkeys with experimental nephropathy (7). Post gamma globulin was also demonstrated in the urine of dogs (8).

The purpose of this study was to show the presence of PGG in the mouse and to compare its characteristics to other PGG's described previously.

MATERIAL AND METHODS

The mice (CBA) weighed 20 g. They were divided into groups of 4 and placed in metabolic cages. Each mouse received one subcutaneous injection of sodium chromate (1 mg diluted in 2 ml of sterile saline solution).

Urine samples were collected daily from day 2 to day 6 after intoxication. Fifty µl of 4g/l solution of sodium azide were added to each urine sample. Urine from normal mice was collected similarly.

Urine samples were pooled, centrifuged, concentreated to 50 mg/ml by ultrafiltration on UM Amicon membranes, and filtered on AcA54 Ultrogel with a phosphate buffer (0.15M, pH 7.2).

The preparative liquid phase electrophoresis was performed with a buffer Tris-EDTA/boric acid at pH 8.3 (Tris : 0.26 M ;

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EDTA : 0.007 M ; boric acid : 0.24 M) as described before (9). Electrophoresis was performed according to technique of Uriel (10). Immunoelectrophoresis followed the technique described by Scheidegger (11) and gel double diffusion was performed using the technique of Ouchterlony (12).

Molecular weights were determined on SDS-PAGE at 7.5 % (13), after a 125 I labeling using the T chloramine technique (14). Standards for M.W. were labeled using the same technique with 131 I.

Mouse anti-post gamma immunserums were obtained following a technique already described for human anti-post-gamma immunserums (5,15).

The different techniques of oxidation, reduction and alkylation as well as the total determination of aminoacids have been described elsewhere (6).

RESULTS AND DISCUSSION

Physiological proteinuria in the CBA mouse is 1 mg/day and is composed primarily of an anodic protein which is the main urinary protein in the mouse (16). Sodium chromate, injected subcutaneously at 1 mg/mouse, induces a large increase in protein excretion which can vary from 1 mg/day to 15 mg/day/mouse.

The dose of sodium chromate (1 mg/mouse) is very close to the DL 50. In the urine of intoxicated mice, the AcA54 gel filtration profile shows 2 additional peaks, compared to normal urine. These 2 additional peaks, when analyzed on a polyacrylamid gel, are mainly composed of serum proteins (Fig. 1).

Peak 3 represents the main protein found usually in mouse urine. Peak 4 is composed of urinary pigments but also some proteins with a M.W. inferior to 20,000 daltons. The fraction 8 of the gel filtration was recycled on AcA54 in order to eliminate all proteins with M.W. higher than 20,000 daltons. After preparative liquid phase electrophoresis a curve of the O.D. of the 48 tubes (Fig. 2) shows a major peak that was strongly cathodic. The electrophoretic analysis of this peak demonstrates 3 fractions which are similar to the differend PGC described in man (5). The protein isolated in fraction VII of the preparative liquid electrophoresis, and labeled with 125 I, shows after SDS PAGE at 7.5. % an apparent M.W. of 13,400 daltons.

Aminoacid content comparison shows an important difference at the isoleucine level, a few differences concerning the neutral AA, a little less aromatic AA in the mouse PGG, almost a similar number of basic AA and the same number of Cysteines (Table 1). One can conclude, therefore, that both proteins are relatively similar.

Both immune serums obtained, n° 178 and 282, had only one fraction in the urine of mice intoxicated with Na chromate and none in normal serum of CBA mice (Fig. 3).

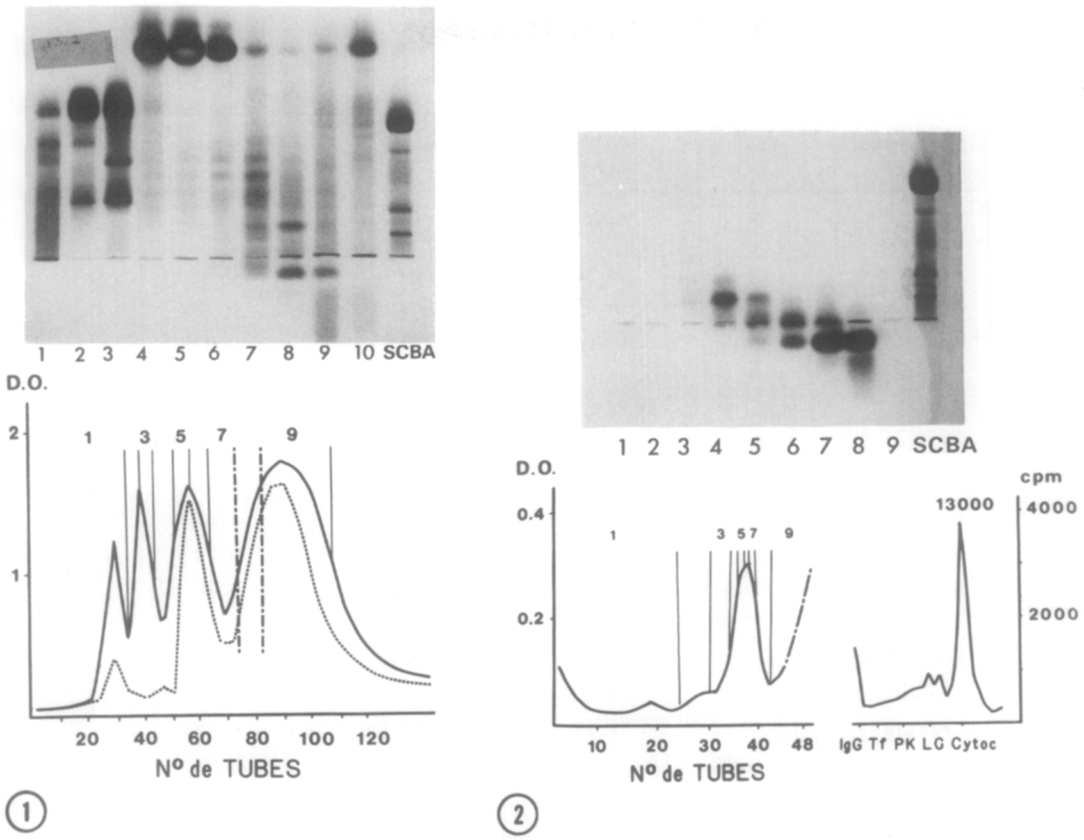

Fig. 1 - Gel filtration on AcA54 of concentrated Mouse Tubular Urine
Analysis of each fraction by Agarose Polyacrylamide Gel Electrophoresis.
In fraction 4, 5, 6, the main component is the Main Urinary Protein
In fraction 8, the main component is PGG

Fig. 2 - Liquide phase electrophoresis (VAP 1) of fraction 8 obtained after gel filtration on AcA54. On the right the fraction is analysed by SDS PAGE in comparison with different M.W. markers

Immunoelectrophoresis performed on the same urines showed that the observed protein is strongly more cathodic than the light chains of IgG detected by a mouse antikappa light chains (Fig. 3).

The immunserum 282 demonstrates perfectly that human and urine PGG both seem to have a antigenic similarity. On the other hand, the human anti-post-gamma N° 20 (5) does not easily detect murine post-gamma. This PGG seems, therefore, to have only a partial antigenic similarity with one of the human PGG.

CONCLUSION

The protein we have isolated is truly the murine equivalent of human PGG.As in man, it is characteristic of urine from

Table 1 : AMINO ACID COMPOSITION OF THE HUMAN "FAST" AND MOUSE PGG
(mol/100mol Aminoacid)

Aminoacid	PGG"fast" (1)	PGG"fast" (2)	Mouse PGG	Difference
Lysine	5.43	5.5	5.15	- 0.35
Histidine	2.52	2.55	2.53	- 0.2
Arginine	4.56	4.61	5.55	0.93
Aspartic Ac	11.53	11.66	11.69	0.03
Threonine	6.48	6.6	5.41	- 1.15
Serine	6.1	6.17	8.75	2.58
Glutamic Ac	11.63	11.77	10.04	- 1.73
Proline	4.5	4.55	6.09	1.54
Glycine	6.75	6.83	8.48	1.65
Alanine	9.38	9.49	9.27	- 0.22
Half Cyst	3.64	3.67	3.23	- 0.44
Valine	7.92	8.01	6.51	- 1.5
Methionine	2.93	2.96	2.42	- 0.55
Isoleucine	1.63	0.78	2.91	2.11
Leucine	7.11	7.19	6.83	- 0.36
Tyrosine	3.14	3.17	2.28	- 0.89
Phenylalanine	4.45	4.50	2.87	- 1.63

(1) **Tonnelle et al** (2) Present study

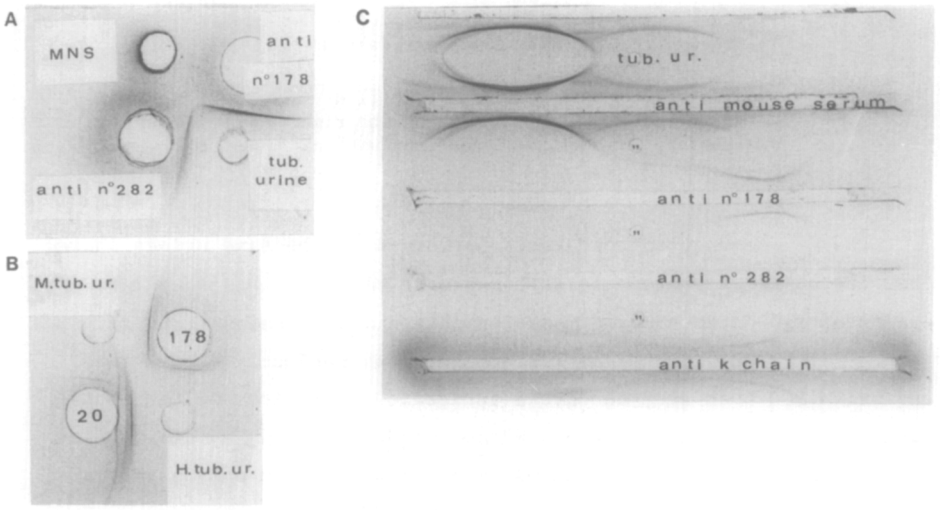

Fig. 3 - A) : Ouchterlony double diffusion of both immunserums anti PGG 178 and 282 against Mouse NOrmal Serum and Mouse Tubular Urine.

B) : Immunoelectrophoresis of Mouse Tubular Urine using Polyclonal Tltal anti Mouse Serum, anti PGG 178, anti PGG 238 and anti Mouse Kappa chains

C) : Ouchterlony double diffusion of anti Mouse PGG 178 and anti Human PGG 20 against Mouse and Human tubular Urine.

patients with tubular reabsorption problems. As in man, the murine PGG seems to show different mobilities, but only one form could be isolated.

Finally, PGG structures seems particularly well conserved between mouse and man, much more than the bêta 2 microglobulin, which has a similar M.W. This apparent conservation through evolution suggests that it has a precise function. The mouse model may be a more practical was to try to find the role of this protein.

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