IMMUNOFLUORESCENT STUDY ON THE ORIGIN OF TAMM-HORSFALL GLYCOPROTEIN

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Received for publication December 18, 1974, and in revised form January 16, 1975

Tamm-Horsfall glycoprotein was isolated and characterized, and then was studied using the fluorescent antibody technique. The cytoplasm of the tubule cell was well stained and the glomeruli were remained completely unstained. The fluorescent materials were observed also in tubular lumina.

Tamm-Horsfall glycoprotein was well characterized biochemically (2,6). At present, however, little is known about the origin of this substance. The present paper deals with a demonstration of the source of Tamm-Horsfall glycoprotein in the body, using the fluorescent antibody technique.

MATERIALS AND METHODS

Preparation of Tamm-Horsfall glycoprotein and saline-soluble substances from several human organs.

Tamm-Horsfall glycoprotein was isolated by the method of Tamm-Horsfall (7, 8), from ten human urines of both sexes aged between 18 to 59 years. Six human kidneys, livers, hearts and brains were obtained at autopsy of both sexes aged between 10 and 55 years. Samples were cut into small pieces and homogenized in five volumes of 1/15 M phosphate-buffered saline solution, pH 7.6 saturated with 0.1% sodium azide. The kidneys, however, were separated into bits of cortex, medulla and renal pelvis before the treatment mentioned above. The supernatant obtained by centrifugation at 10,000 rpm for 30 minutes was dialyzed against several changes of deionized water for 3 days and air-dried.

Chemical analyses.

For hexosamine analysis, samples were hydrolysed by heating in an evacuated tube with 4 N HCl at 100°C for 8 hr. After hydrolysis, excess of acid was removed under vacuum. Quantitative determinations of glucosamine and galactosamine were carried out with a Hitachi KLA-3 Amino Acid Analyzer, using a 50 cm column in citrate buffer of pH 5.28 (Na: 0.35 N).

Hexose content was determined by the orcinol method of Winzler (9).

Individual hexose were identified by paper chromatography and determined by gas-liquid chromatography. Samples were hydrolyzed with 0.5 N H₂SO₄ at 100°C for 2 hr. After removal of the sulfate ion with Ba(OH)₂, it was chromatographed on paper with n-butanol: pyridine: water (5 : 3 : 2, v/v) as a solvent

and silver-stained. For gas-liquid chromatography, the hydrolysate was lyophilized after removal of the sulfate ion and heated in dry 0.5 N methanolic HCl at 85°C for 16 hr. It was evaporated to the dryness, then dissolved in distilled water and passed through a Dowex-50 (H⁺-form) column. The eluate was lyophilized and dissolved in dry pyridine, and then converted to trimethylsilyl derivatives. A Shimadzu GC-1C Gas Chromatograph, equipped with a hydrogen flame detector, was used. The column was 2 m long and was packed with 5% SE-30 on Chromosorb W.

Sialic acid was determined by the thiobarbituric acid method of Warren on samples hydrolyzed with 0.1 N H₂SO₄ at 80°C for 1 hr, with N-acetylneuraminic acid as a standard.

Amino acids were analysed by the standard method in the Hitachi KLA-3 Amino Acid Analyzer after hydrosis in 6 N HCl for 15 hr.

Immunological studies and immunofluorescent study.

Rabbits, weighing approximately 3 kg, were immunized by four footpad injections of isolated Tamm-Horsfall glycoprotein (20 mg in each injection) which had previously been emulsified by adding the complete Freund's adjuvant over 5 weeks. Against the obtained antiserum, the antigenic activities of the isolated Tamm-Horsfall glycoprotein, various organ extracts and blood sera were examined by the Ouchterlony double diffusion test and immunoelectrophoresis as employed in the routine works.

The γ -globulin isolated from the antiserum was conjugated to fluorescein isothiocyanate (FITC) as employed in routine work (3). The FITC-conjugated γ -globulin was purified by passing through a Sephadex G-25 and DEAE-cellulose column. Ten cases of the kidney were used for the immunohistological studies. The specimens were fixed in cold ethanol-aceton and embedded in paraffin. The tissue sections were submitted for the direct immunofluorescence staining. The immunofluorescence preparations were inspected under a fluorescence microscope (Nikon, SAR-F, Japan). Photos were taken with a Kodak highspeed Ektachrome film, EH 135 (U. S. A.).

RESULTS

The chemical compositions and amino acid constituents of the Tamm-Horsfall glycoprotein are shown in Table I.

Paper chromatogram of the constituent hexoses of the Tamm-Horsfall glycoprotein revealed silver-stainable spots corresponding to galactose, mannose and fucose. This was confirmed also by gas-liquid chromatography. These data well agreed with Odin (6) and Maxfield (5).

Aqueous suspensions of Tamm-Horsfall glycoprotein antigen in 0.1 to 10 mg per ml gave a single distinct zone of precipitation in the gel diffusion patterns with the rabbit anti-Tamm-Horsfall glycoprotein antibody (Fig. 1). And in the immunoelectrophoresis, it made a similar single distinct precipitation zone corresponding to the region albumin and α -globulin, which well agreed with the results of Keutel et al. (4) (Fig. 2).

All renal tissue preparations including three parts of the cortex, medulla and renal pelvis were consistently led to a positive precipitin reaction with the rabbit anti-Tamm-Horsfall glycoprotein antibody, but no evidence of precipitation was

TABLE 1. *Amino acid and carbohydrate composition of Tamm-Horsfall glycoprotein*

Component	Subject		
	28 years Male	36 years Male	58 years Male
Hexose	8.1	8.4	8.5
Sialic acid	9.2	9.3	9.3
Glucosamine	6.8	6.4	6.5
Galactosamine	1.3	1.6	1.5
Alanine	3.6	3.3	3.5
Arginine	4.5	4.8	4.9
Aspartic acid	8.8	9.0	9.4
Glycine	3.4	3.2	3.0
Histidine	2.2	2.3	2.5
Leucine	5.7	5.6	5.3
Isoleucine	1.8	1.6	2.0
Lysine	2.1	2.0	2.3
Methionine	1.9	2.4	2.1
Phenylalanine	3.1	3.0	3.3
Proline	3.3	3.5	3.2
Serine	4.8	4.9	4.5
Threonine	5.2	5.0	4.9
Tryptophan	3.5	3.8	3.4
Tyrosine	2.9	2.8	3.1
Valine	4.2	4.6	4.3

obtained from the preparations of livers, hearts, brains and sera.

From the present immunohistological findings of the kidney, the tubules were well stained by fluorescent antibody technique and the glomeruli were remained completely unstained (Photo 1). The former was well stained, selectively on the cytoplasm of the tubular epithelial cell with a dark point suggesting the nucleus to be unstainable. The fluorescent materials were observed also in tubular lumina and the cell membranes were remained completely unstained.

DISCUSSION

Boyce et al. (1) have demonstrated that the uromucoid antigen was contained in the kidney with a titre decreasing in the order of the cortex, medulla and pelvis. They found it also in the skin, subcutaneous fat and striated muscle, but not in the

FIG. 1.

FIG. 2.

PHOTO. 1. Photomicrograph of kidney, stained with fluorescent antibody technique.

erythrocyte membrane, saliva, bone matrix and seminal plasma.

Maxfield (1966) thought that the Tamm-Horsfall glycoprotein was formed at the external glycoprotein layer of the tubule cell membranes and was then cast off into the urine, according to the facts that the cell membrane was consisted of three layers, an inner layer of protein, a center layer of lipid and an outer layer of sialic acid-containing glycoprotein, and that the specific receptor substance for influenza virus occurred on the external surface of cell and on the Tamm-Horsfall glycoprotein. But, upon these data obtained by the authors, it may be a reasonable assumption that the Tamm-Horsfall glycoprotein is formed at the cytoplasm of the tubule cell and is then cast off into the urine.

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FIG. 1. Ouchterlony double diffusion tests.

(1)-(10); Aqueous suspensions of Tamm-Horsfall glycoprotein antigen in 1.0 mg per ml (Subject 1-10), Central wells; Anti-Tamm-Horsfall glycoprotein antibody (rabbit).

FIG. 2. Immunelectrophoretic patterns.

(1) and (2); Tamm-Horsfall glycoprotein (Subject 1 and 2), Central troughs; Anti-Tamm-Horsfall glycoprotein Antibody (rabbit).

FIG. 3. Photomicrograph of kidney, stained with fluorescent antibody technique.

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