

[Chem. Pharm. Bull.]
30(8)2870-2873(1982)

The Determination of Human Urinary γ -Esterase Activity using Antibody-Conjugated Paper Disk¹⁾

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(Received February 1, 1982)

A new and specific method for the determination of human urinary γ -esterase activity, which migrates to the γ -globulin region on cellulose acetate electrophoresis, was developed using an antibody-conjugated paper disk. The method was applied experimentally, and the results indicate that an increase of γ -esterase activity in the urine reflects renal damage, that is, urinary γ -esterase activity was increased in patients with nephrotic syndrome (20.3 ± 15.0 units/g of creatinine) and chronic nephritis (8.00 ± 5.23 units/g of creatinine) as compared with that of healthy subjects (3.08 ± 1.90 units/g of creatinine). γ -Esterase was localized at the renal tubules of a patient with renal disease, and it was considered that γ -esterase was excreted in the urine when the renal tubules were damaged. Therefore, the determination of urinary γ -esterase activity by this method should be an effective aid in the diagnosis of renal disease.

Keywords— γ -esterase; urine; human kidney; renal disease; antibody-conjugated paper disk

Introduction

Esterases existing in sera of animals can be classified into three main types,²⁻⁴⁾ aryl-esterase, carboxyesterase and cholinesterase. Two types of protein with esterase activity were found on starch gel electrophoresis of human urine by Terrien *et al.*⁵⁾ We had previously reported that an esterase which migrated to the γ -globulin region (γ -esterase) on cellulose acetate electrophoresis was excreted in the urine of patients with renal disease. This phenomenon was of great interest and clinical value.

In the previous work,⁶⁾ the γ -esterase from human kidney was purified, and its enzymic and immunological properties were clarified. These data suggested that kidney γ -esterase and urinary γ -esterase were identical and that urinary enzyme originated from the kidney.

Large amounts of proteins are excreted in the urine of patients with renal disease. The detection of these proteins, such as albumin, IgG, β_2 -microglobulin, retinol-binding protein and lysozyme, are important for clinical renal diagnosis.⁷⁻¹²⁾

Another approach to the diagnosis of renal disease has been to determine the urinary γ -esterase activity by using antibody-conjugated paper disks. We have used this approach to investigate a large group of patients with renal disease, and it appears to be suitable for routine use under clinical conditions.

Experimental

Materials—Filter paper (No. 51A) for the preparation of the paper disks was purchased from Toyo Roshi Co., Ltd. Fluorescein isothiocyanate was obtained from Baltimore Biochemical Laboratory, Inc. Other reagents used were of analytical reagent grade.

Purification of γ -Esterase from Human Kidney— γ -Esterase from human kidney was purified according to the method described in the previous paper.⁶⁾

Preparation of Antibody—Antibody was prepared according to the method described in the previous paper.⁶⁾

Preparation of Antibody-Conjugated Paper Disks—Antibody-conjugated paper disks were prepared according to the method of Ceska and Lundkvist.¹³⁾ Filter paper disks with a diameter of 6 mm were punched out. The paper disks were soaked in distilled water, and then mixed with 80 ml of CNBr solution (2.7 g of CNBr in 80 ml of distilled water). The pH was brought to 10.5 with 1 M NaOH for 30 min. The liquid was aspirated and the disks were washed with 100 ml of 5 mM NaHCO₃. This washing procedure was repeated 12 times. These disks were then washed twice with 100 ml of distilled water. Ten mg of antibody was mixed with 2 g of the activated paper disks, and the mixture was stirred at 4°C for 3 h. The disks were then washed with 200 ml of 0.1 M NaHCO₃. The whole washing procedure was performed at room temperature. The remaining reactive groups on the paper disks were blocked with 40 ml of 50 mM ethanolamine in 0.1 M NaHCO₃ for 3 h at room temperature. The disks were again washed twice with 100 ml of 0.5 M NaHCO₃ and then with 0.1 M acetate buffer (pH 4.0). The latter washing procedure was repeated once more with an incubation period of 30 min. The disks were then washed twice with 100 ml of incubation buffer (see below). They were stored at 4°C in a small volume of incubation buffer.

Incubation Buffer—Fifty mM Tris-HCl buffer (pH 7.5) containing 0.9% NaCl, 0.05% sodium azide and 0.6% Triton X-405.

Standard Assay—One paper disk conjugated with antibody was used per tube. To each tube was added 100 μ l of incubation buffer and 50 μ l of urine or standard enzyme solution. The tubes were covered with parafilm and the whole rack was kept on a horizontal shaker at 4°C overnight. The solution in the tube was aspirated and the paper disk was then washed three times with 2 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 0.9% NaCl. The amount of γ -esterase activity on the paper disks was determined by the method described in the previous paper.⁶⁾

Creatinine—Urinary creatinine concentration was determined by the method of Bonsnes and Taissky.¹⁴⁾

Photomicrography of Human Kidney Specimens—Photomicrography of kidney specimens from patients with tubular disease was performed by the method of Hintz and Goldberg.¹⁵⁾ Each specimen was stained with fluorescein isothiocyanate-labelled anti-human kidney γ -esterase IgG.

Results

Incubation Time for Immunoreaction

The incubation time required for the immunoreaction was examined. As can be seen in Fig. 1, the plateau value in the immunoreaction was reached at about 10 h. Therefore, overnight incubation, *e.g.* 18 h, was selected as a suitable period.

Calibration Curve and Time Course of γ -Esterase Activity

The calibration curve of γ -esterase activity was prepared by the proposed method. The plot was linear up to 20 mU and passed through the origin. The time course of γ -esterase activity in the proposed method was linear during a reaction period of 120 min.

Reproducibility and Recovery of the Proposed Method

By using urine from patients with renal diseases, the within-day and day-to-day reproducibilities were investigated. The values obtained were $3.2 \pm 0.2\%$ ($n=20$) and $5.0 \pm 1.0\%$ ($n=7$) (mean \pm S.D.) respectively. The recovery of the proposed method was $95.0 \pm 3.5\%$ ($n=5$) (mean \pm S.D.).

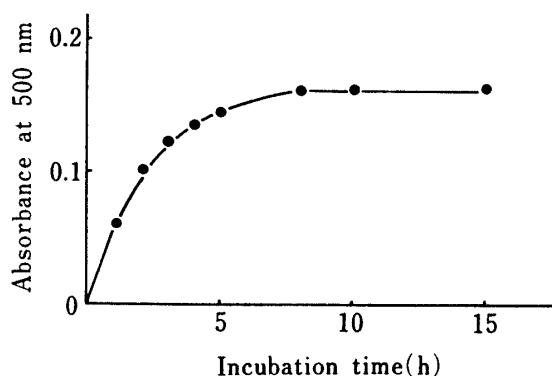


Fig. 1. Effect of Incubation Time on the Proposed Method

Nine mU of the purified human kidney γ -esterase was added to each tube. After incubation at 4°C for the indicated time, the activity was determined as described in the text.

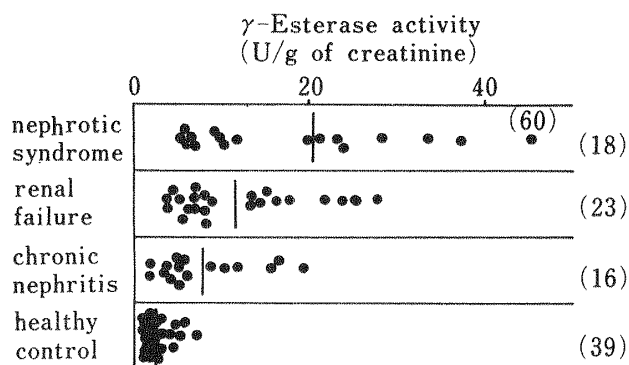


Fig. 2. γ -Esterase Activity in the Urine from Patients with Various Renal Diseases

Bars represent the mean values of γ -esterase activity in the urine from patients with various renal diseases. Figures in parenthesis are the numbers of patients.

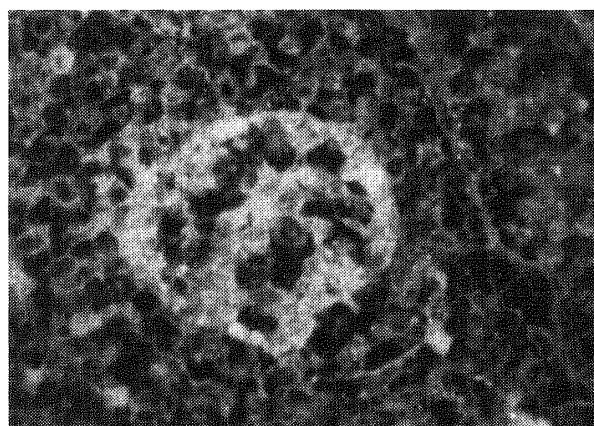


Fig. 3. Photomicrograph of a Biopsy Specimen from the Kidney of a Patient with Pyelonephritis

The specimen was stained with fluorescein isothiocyanate-labelled anti-human kidney γ -esterase IgG.

γ -Esterase Activity in the Urine from Patients with Renal Diseases

γ -Esterase activity in the urine from healthy subjects and patients with renal diseases was determined by the proposed method. As shown in Fig. 2, γ -esterase activity increased in the urine of patients with nephrotic syndrome (20.3 ± 15.0 units/g of creatinine), chronic nephritis (8.00 ± 5.23 units/g of creatinine) and chronic renal failure (12.0 ± 7.19 units/g of creatinine) as compared with that of healthy subjects (3.08 ± 1.90 units/g of creatinine) (mean \pm S.D.).

Photomicrograph of the Kidney Tissue of a Patient with Pyelonephritis

A specimen from the kidney of a patient with pyelonephritis was investigated photomicrographically using fluorescein isothiocyanate-labelled anti-human kidney γ -esterase IgG. As shown in Fig. 3, γ -esterase was localized at the renal tubule of the human kidney. In the case of healthy subjects, the enzyme was not detected at that site.

Relation between Urinary γ -Esterase Activity and Renal Tubular Change for Patients with Renal Diseases

The relation between urinary γ -esterase activity and renal tubular change was investigated in patients with various renal diseases. The urinary γ -esterase activity was determined by the proposed method. To investigate the renal tubular change, biopsy specimens from the kidneys of patients were investigated photomicrographically using fluorescein isothiocyanate-

TABLE I. Relation between Urinary γ -Esterase Activity and Pathological Diagnosis for Patients with Renal Diseases

Name	Age	Sex	γ -Esterase activity ^{a)}	Clinical diagnosis	Pathological diagnosis	Renal tubular change ^{c)}
Y.I.	16	F	33.4	Nephrotic syndrome	Segmental and focal GN ^{b)}	++
N.H.	12	M	9.5	Nephrotic syndrome	Segmental and focal GN	+
T.N.	14	M	15.9	Nephrotic syndrome	Mild diffuse mesangic proliferative GN	+
M.S.	16	F	37.5	Acute GN	Mild diffuse mesangic proliferative GN	++
Y.H.	28	M	19.5	Chronic nephritis	Mild diffuse mesangic proliferative GN	+

a) γ -Esterase activity is expressed in U/g of creatinine.

b) Glomerulonephritis.

c) The renal tubular change was observed by photomicrography of a kidney specimen. +; mild change, ++; marked change.

labelled anti-human kidney γ -esterase IgG. As shown in Table I, γ -esterase activity was increased in the urine of patients with various renal diseases with renal tubular change. These results (Fig. 3 and Table I) suggest that γ -esterase was excreted in the urine of patients with damaged renal tubules.

Discussion

We have discovered that large quantities of γ -esterase are excreted in the urine of patients with renal disease. In the previous paper,⁶⁾ we reported that the enzyme in the urine was identical with that from the human kidney by biochemical, immunological and histochemical criteria. Therefore, it should be possible to utilize γ -esterase activity in the urine as an aid for the clinical diagnosis of various renal diseases.

In view of the substrate specificity of other esterases in the urine,⁵⁾ it is necessary to determine γ -esterase activity specifically. Thus, γ -esterase was removed from the urine by the antibody-conjugated paper disk method and then the esterase activity was determined. The results were of great interest and clinical value. γ -Esterase activity was increased in the urine of patients with nephrotic syndrome, chronic nephritis and chronic renal failure. From the results of the histochemical investigation, the enzyme is localized at the renal tubules of patients with various renal diseases, and renal tubule damage was observed specifically (Fig. 2, 3 and Table I). Thus, the excretion of γ -esterase into the urine implies damage to the renal tubules.

Albumin, alkaline phosphatase, IgG, β_2 -microglobulin, retinol-binding protein and lysozyme are excreted into the urine of patients with renal disease, and the excretion of β_2 -microglobulin, retinol-binding protein and lysozyme into the urine is known to reflect damage to the renal tubules.⁷⁻¹²⁾ From this point of view, retinol-binding protein and lysozyme in the urine from patients were determined by the methods of Kanai and Smolelis respectively.^{16,17)} However, the correlation between γ -esterase and these proteins, which were localized at the renal tubule, is not good. A possible explanation for the discrepancy is as follows. A small quantity of retinol-binding protein could not be determined precisely by the immunological method (single radial immunodiffusional method), and lysozyme could not be determined precisely by the method of *Micrococcus lysodeikticus* lysis. Thus, there may be significant errors in the determinations of these proteins. We are continuing to work on the clarification of the correlation between γ -esterase and other marker of renal tubule damage. This method has wide application for the specific determination of various enzyme activities (γ -glutamyl-transferase, alkaline phosphatase, kallikrein, etc.) in body fluids.

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