Immunochemical Quantitation of Prostatic Phosphatase

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A monkey antiserum to a fraction of human urine precipitated by concentrations of ammonium sulfate between 1.5 and 2.5 mol/liter was shown to contain antibodies specific for prostatic acid phosphatase. Using a modification of Laurell’s immunodiffusion method with a phosphatase stain, we devised a quantitative immunoassay for this enzyme.

Additional Keyphrases: Laurell’s "rocket" immunodiffusion method - Ouchterlonny double-diffusion technique - diagnostic aid: prostatic carcinoma - enzyme measurement specificity

As in most forms of cancer, early diagnosis of prostatic cancer is crucial for a high curability rate. A number of biochemical tests are now available to help identify suspected prostatic cancer in the patient by measuring the activity of acid phosphatase (EC 3.1.3.2) in the patient’s serum.

Multiple forms of human acid phosphatase have been described in different organs, but the richest source of this enzyme is the prostate (1-3). Acid phosphatase is mainly localized in the lysosomes and is released, when the cell is damaged, into serum and urine. Although various organs possess characteristic patterns of acid phosphatase isoenzymes, as can be seen on zonal electrophoresis, most phosphatases have similar biochemical features, hydrolyzing a wide variety of organic phosphate esters and thus defying differentiation (4). The biochemical tests presently used to measure the activity of phosphatase in serum depend upon the ability of the enzyme to split different substrates at various pH’s. However, this property is not organ-specific, failing to pinpoint the prostatic phosphatase. Thus there has been a problem in interpreting the activities of serum acid phosphatase and the test has not always been reliable in detecting unsuspected cases of prostatic carcinoma (5-8). Tartrate inhibition of prostatic phosphatase increases the specificity considerably; however, even this method gives a number of false-positive results (8). The renal acid phosphatase shares the property of inhibitability by L-tartrate, contributing to the confusion (9). Bodansky’s method involving β-glycerophosphate, a substrate that is believed to be more specific for prostatic acid phosphatase, has been the most successful procedure so far.

A number of investigators (8, 10, 11) have produced antisera in goats and rabbits to the human prostate. These antisera contained antibodies to several acid phosphatases, as demonstrated by the double-diffusion test. They potentially provide a highly specific reagent for the prostatic acid phosphatase, but so far their application has been limited because of their possible reactions with nonprostatic sources of tartrate-inhibitable acid phosphatase. Furthermore, in diagnosis of prostatic carcinoma, the emphasis must also be placed upon the quantitative activity and not the qualitative presence of the prostatic acid phosphatase in the serum.

Methods and Results

We have produced a specific antiserum to the prostatic acid phosphatase by injecting a rhesus monkey with the fraction of male human urine that precipitates between the concentrations 1.5 and 2.5 moles of ammonium sulfate per liter. Although the urine fraction contained prostatic and nonprostatic acid phosphatases, the monkey evidently produced antibodies only to the prostatic acid phosphatase. A cow, injected with a human fetal lung cell line, also produced antibodies to acid phosphatase. The specificity of the antisera were established by Ouchterlony’s double-diffusion test and subsequent enzyme staining of the precipitate with naphthol AS-MX phosphoric acid as a substrate and diazonium salts as a coupling reagent. The monkey anti-phosphatase and the cow anti-phosphatase were added to wells adjacent to human urine fraction, where they produced a reaction of non-identity (Figure 1). The specificity of the monkey anti-prostatic acid phosphatase serum was then defined by the L-tartrate inhibition and by absorption. When L-tartrate (2 × 10⁻² mol/liter) was incorporated in the histochemical stain for acid phosphatase, the precipitate produced by the monkey antiserum was no longer stained. The precipitate produced by the cow antiserum was not inhibited. The absorption of monkey anti-prostatic acid phosphatase serum with a homogenate of human prostatic tissue removed the

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precipitin band of acid phosphatase observed with the unabsorbed serum. Similar homogenates of other human tissues (liver, kidney, lung, and spleen) did not remove the antibody to prostatic acid phosphatase. The cow antibody was absorbed by all of these homogenates, but not by that of prostate.

The monkey antiserum was further investigated by the immunoelectrophoretic method. The prostatic acid phosphatase reacted with the monkey antiserum near the anode, producing a single arc of reaction, while the tissue acid phosphatase produced a single line of reaction with the cow antiserum in the cathodal region (Figure 2). Figure 3 shows the reaction of immunologic identity of the acid phosphatase present in human urine and human prostate when tested with the monkey antiserum.

The monkey antiserum was then used in the quantitative estimation of the relative amounts of the prostatic acid phosphatase. We used the modified Laurell method (12), which involves electrophoresis of an antigen into an antibody-containing gel, thereby inducing precipitating zones in the shape of a cone. A positive linear relationship exists between the concentration of the antigen and the height of the precipitation peak.

Electrophoresis was performed using agarose (10 g/liter of 25 millimolar barbitol buffer, pH 8.2) to which the monkey antiserum was added at a final concentration of 3 ml/100 ml. Twelve milliliters of this agar-antiserum mixture was poured onto an \( 8 \times 10 \) cm glass plate. A row of holes, 3 mm in diameter, was cut parallel to one of the longer edges of the plate and filled with 10 \( \mu l \) of antigen solution. Electrophoresis was at 4°C, 4 V/cm, for 4 h. The plates were washed in phosphate-buffered saline overnight, then dried and stained with the histochemical stains described above (Figure 4). The ammonium sulfate-precipitated urine fraction was used as a standard to which the relative amount of the enzyme was referred.

After processing the slides as described above, the resulting peaks were measured to the nearest 0.01 mm, and, on semi-logarithmic paper, these values
were plotted against the concentration of the sample. The urine fraction was assigned an arbitrary value of 100 units. A linear relationship was thus obtained. The enzyme concentration in urine samples was interpolated graphically from the calibration curve. The relative amounts of the enzyme in each unknown sample is then expressed in terms of units per milligram of protein in the sample.

Discussion

More work needs to be done before this test can be introduced into the hospital clinical laboratory. For instance, the prostatic acid phosphatase must be isolated and its specific activity determined in order to provide a proper standard. However, the assay described above presents great possibilities for the much-needed specific test for the detection of prostatic carcinoma. This novel approach permits isoenzyme quantitation, which until now has been accomplished only by more laborious biochemical methods. Immunochemical assay can measure a particular isoenzyme, in this case specific for the prostate, in mixtures of isoenzymes and quantitate it by means of a specific antiserum. It permits the study of immunochemical relations between various molecular forms of enzymes, and distinguishes enzymes having closely similar substrate specificities.

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References