USE OF THE THERMOSTABLE GLUCOSE-6-PHOSPHATE DEHYDROGENASE AS A LABEL ENZYME IN STEROID ENZYME IMMUNOASSAYS

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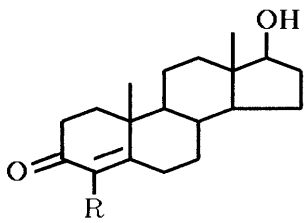
A thermostable glucose-6-phosphate dehydrogenase was assessed as a label enzyme in a testosterone enzyme immunoassay system. Enzyme labeling of a steroid with the enzyme was carried out by the *N*-succinimidyl ester method. A dose-response curve with a satisfactory sensitivity could be obtained by the use of the enzyme-labeled antigen. This enzyme should be useful in hapten enzyme immunoassays because of its relatively high stability.

KEYWORDS thermostable glucose-6-phosphate dehydrogenase; enzyme immunoassay; enzyme labeling; glucose-6-phosphate dehydrogenase-steroid conjugate; testosterone

Enzyme immunoassays of hormones and drugs have been developed using various enzymes as labels. The sensitivity of the assay depends on the enzyme employed. Enzymes most commonly used in the heterogeneous enzyme immunoassay of steroid hormones are alkaline phosphatase, horseradish peroxidase, β-galactosidase, and glucose oxidase. The choice of enzyme is based on various criteria such as activity and stability.[1] Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of D-glucose 6-phosphate to D-glucose-δ-lactone 6-phosphate in the presence of cofactors. The enzyme from baker's yeast or *Leuconostoc mesenteroides* has also been used as an enzyme label in immunoassay systems. In 1985, Okuno *et al.*[2] isolated G6PDH from the thermophilic bacteria, *Bacillus stearothermophilus* ; this enzyme has been reported to show a remarkable thermostability as well as storage stability. The G6PDH is an attractive label, if applicable, in immunoassay systems. In general, it is expected that a new immunoassay system can be developed by utilizing the characteristics of such an enzyme. We report here the first use of the so-called thermostable G6PDH in a heterogeneous testosterone assay system.

Enzyme labeling of testosterone was carried out by the *N*-succinimidyl ester method.[3] A solution of the *N*-succinimidyl ester of 4-(carboxymethylthio)testosterone (T·CMT)(7.3 μg) in dimethylsulfoxide (0.1 ml) was added at 0 °C to a solution of G6PDH (EC 1.1.1.49, >100 U/mg, Unitika)(100 μg) in 0.05 M phosphate buffer (PB), pH 7.3 (0.2 ml), containing 0.22 M glucose 6-phosphate, 8.6 mM NADPH, and 0.01 M ethylenediaminetetraacetic acid as active-site-blocking agents.[4] The mixture was gently stirred at 4 °C for 4 h. After addition of PB (0.5 ml), the resulting solution was dialyzed against cold PB (2 l) for 2 d. A 0.7 ml sample of the dialyzed conjugate solution was transferred to a test tube; the solution was stored at 4 °C at a concentration of 50 μg/ml, adjusted with PB containing 0.1% gelatin, 0.9% NaCl and 0.1% NaN_3 (assay buffer). In the enzyme labeling, the recovery of G6PDH activity was found to be approximately 70%. The result is satisfactory at the present time.

The enzyme immunoassay procedure was carried out in triplicate in glass test tubes (10 ml) as follows, using an anti-testosterone antiserum elicited in a rabbit by immunization with the conjugate of 4-hydroxytestosterone 4-hemiglutarate (T·HG) with bovine serum albumin:[5] solutions of testosterone (0-1000 pg) in assay buffer and G6PDH-labeled antigen (30 ng) in the buffer (0.1 ml) containing 0.5% normal rabbit serum were added to diluted anti-testosterone antiserum (1:15000, 0.1 ml), and the mixture was incubated at 4 °C for 4 h. Goat anti-rabbit immunoglobulin G antiserum (0.1 ml) diluted 1:30 with assay buffer containing 0.3% ethylenediaminetetraacetic acid was added to the incubation mixture, and the solution was vortex-



testosterone: R=H
T·CMT: R=SCH_2COOH
T·HG: R=$OCO(CH_2)_3COOH$

Chart 1

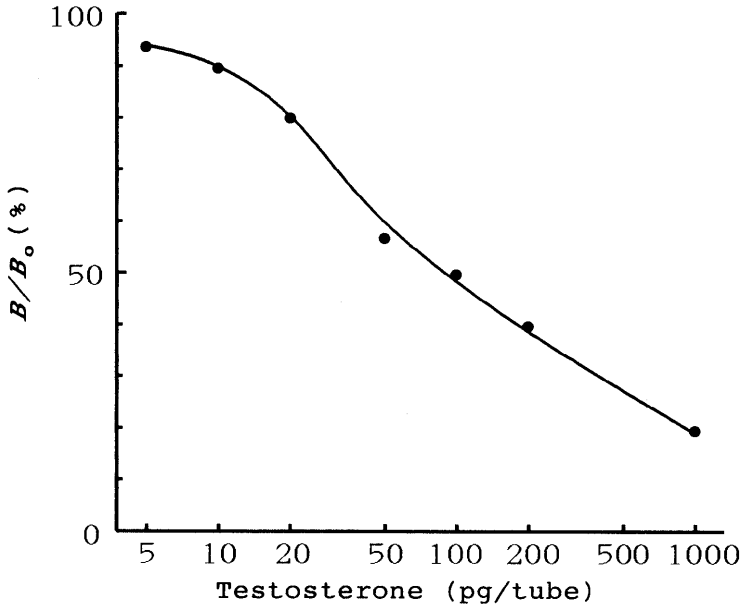


Fig. 1. A Dose-Response Curve for Testosterone Enzyme Immunoassay Using the G6PDH Label

mixed, then allowed to stand at 4 °C for 16 h. The resulting mixture was diluted with assay buffer (1.5 ml) and centrifuged at 3000 rpm for 10 min, and the supernatant was removed by aspiration. The immune precipitate was washed once with assay buffer (1.5 ml) and diluted with 0.08 M Tris-HCl buffer, pH 9.0 (2 ml), containing 1.6 mM glucose 6-phosphate, 0.16 mM $NADP^+$ and 0.02 M $MgCl_2$, vortex-mixed, and incubated at 30 °C for 1 h. The reaction was terminated by the addition of 0.2% sodium dodecyl sulfate (2 ml). The fluorescence intensity was measured at 460 nm with excitation at 340 nm. A typical dose-response curve for the testosterone enzyme immunoassay using the G6PDH-labeled antigen is shown in Fig. 1. It can be seen that a satisfactory sensitivity was obtained: the amount of testosterone needed to displace 50% of the bound label was *ca.* 80 pg. Optimization of assay conditions including the enzyme labeling procedure is being conducted.

Thus, the thermostable G6PDH should be useful as a label in hapten enzyme immunoassays. Systematic studies on the enzyme labeling in comparison with G6PDHs from other sources are in progress in these laboratories.

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