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Determination of Serum Glutamate Oxaloacetate Transaminase and Glutamate Pyruvate Transaminase by Using L-Glutamate Oxidase

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Serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were determined by using L-glutamate oxidase from *Streptomyces violascens*. GOT and GPT produce L-glutamic acid as a common product, and L-glutamic acid can be easily determined by using L-glutamate oxidase. Hydrogen peroxide produced from L-glutamic acid by L-glutamate oxidase was consumed to couple two mol of homovanillic acid to yield the fluorophore in the presence of peroxidase. The resulting fluorophore was determined with a spectrofluorometer at an excitation wavelength of 315 nm and an emission wavelength of 425 nm. The GOT and GPT activities obtained by our method were well correlated with those obtained by the Centrifichem System 400 method.

Keywords——GOT; GPT; L-glutamate oxidase; fluorometric determination

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) catalyze the following reactions: L-aspartic acid + α-ketoglutaric acid → L-glutamic acid + oxaloacetic acid, and L-alanine + α-ketoglutaric acid → L-glutamic acid + pyruvic acid, respectively. Both enzymes produce L-glutamic acid as a common product. We have found a new enzyme L-glutamate oxidase, from culture broth filtrate of *Streptomyces violascens*,[1] and L-glutamate oxidase catalyzes the following reaction: L-glutamic acid + O_2 + H_2O → α-ketoglutaric acid + NH_3 + H_2O_2. Furthermore, we have reported a simple method to determine L-glutamic acid.[2] However, this method was not sensitive enough to determine L-glutamic acid produced in an assay mixture of GOT and GPT. Snyder and Hendley[3] reported a sensitive method to assay monoamine oxidase and diamine oxidase by fluorometry. In their method, hydrogen peroxide formed was consumed quantitatively to couple 2 mol of homovanillic acid oxidatively in the presence of peroxidase. In this work, sensitive assay methods to determine GOT and GPT were devised by using L-glutamate oxidase in conjunction with fluorometry.

Materials and Methods

L-Glutamate oxidase was prepared from the culture filtrate of *Streptomyces violascens*, as previously described.[1] Horseradish peroxidase (90 units/mg) was purchased from Sigma Chemical Co. Moni-Trol II which contained GOT (88 units/l) and GPT (100 units/l), a product of American Dade, was used as the control serum. All other reagents were purchased from commercial sources and were of analytical grade. The activity of L-glutamate oxidase was measured as previously described.[1] Optimization studies were performed with Moni-Trol II for each of the constituents contained in the assay mixture. L-Glutamic acid produced by GOT and GPT was oxidized to α-ketoglutaric acid, NH_3 and hydrogen peroxide by L-glutamate oxidase. Hydrogen peroxide generated was measured in terms of the formation of a fluorophore from 2 mol of homovanillic acid by oxidative coupling in the presence of peroxidase. Fluorescence intensity was measured with a spectrofluorometer at an excitation wavelength of 315 nm and an emission wavelength of 425 nm.

The final assay procedure is as follows: 20 μl of serum or plasma was incubated with 2.4 ml of 0.05 M potassium-

phosphate buffer (pH 7.4) containing 0.24 unit of L-glutamate oxidase, 1.15 units of peroxidase, 24 μmol of α-ketoglutaric acid and 2.4 mg of homovanillic acid for 3 min at 37 °C, then 0.5 ml of aqueous L-aspartic acid solution (100 μmol/ml) for GOT or 0.5 ml of aqueous L-alanine solution (2.5 μmol/ml) for GPT was added, and the mixture was incubated for 30 min at 37 °C. The enzyme reaction was terminated by adding 0.1 ml of 0.5N NaOH. The fluorescence intensity was read with excitation at 315 nm and emission at 415 nm. One hundred % intensity was adjusted using quinine sulfate solution (2.5 μg/ml). Activities were defined using Moni-Trol II as a standard.

Results

Determination of L-Glutamic Acid

The amount of L-glutamic acid was determined by measuring the hydrogen peroxide generated during the oxidation of L-glutamic acid. The resulting hydrogen peroxide was consumed to couple 2 mol of homovanillic acid in the presence of peroxidase. The reaction was accomplished within 30 min at 37 °C. Under the assay conditions, L-glutamic acid concentration was a linear function of fluorescence intensity (Fig. 1).

Assay Conditions for GOT and GPT

Optimization studies were performed, and the final optimized conditions are summarized in Table I. The resulting fluorophore was stable for at least 60 min at room temperature.

Relationship between Enzyme Concentration (GOT and GPT) and Activity

Figure 2 shows the relationship between the fluorescence intensity and enzyme concentration of GOT and GPT in Moni-Trol II. Linear plots for GOT and GPT were observed up to at least 88 units/l and 100 units/l, respectively.

Effects of Anticoagulants and Biological Materials on the Determination of GOT and GPT

As shown in Table II, one anticoagulant, citric acid, interfered with the determination of

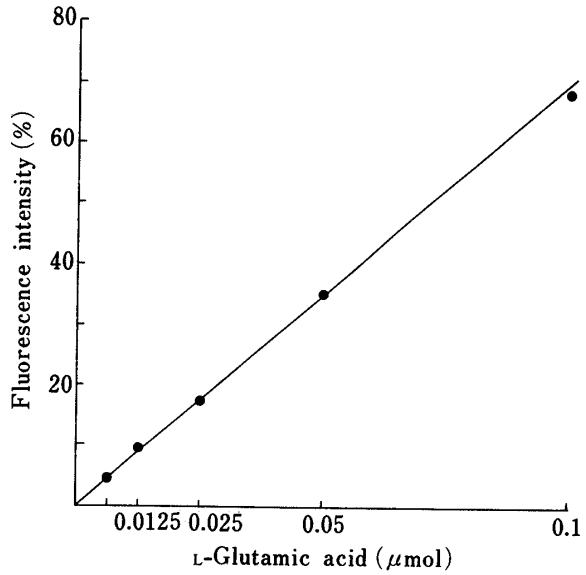

Fig. 1. Proportionality of Fluorescence Intensity to L-Glutamic Acid Concentration

L-Glutamic acid solution was incubated with 2.4 ml of 0.05 M K-phosphate buffer (pH 7.4) containing 0.24 unit of L-glutamate oxidase, 1.15 units of peroxidase and 2.4 mg of homovanillic acid for 30 min at 37 °C. The enzyme reaction was terminated by adding 0.1 ml of 0.5 N NaOH. The fluorescence intensity was read with excitation at 315 nm and emission at 415 nm; 100% intensity was adjusted with a quinine sulfate solution (5 μg/ml).

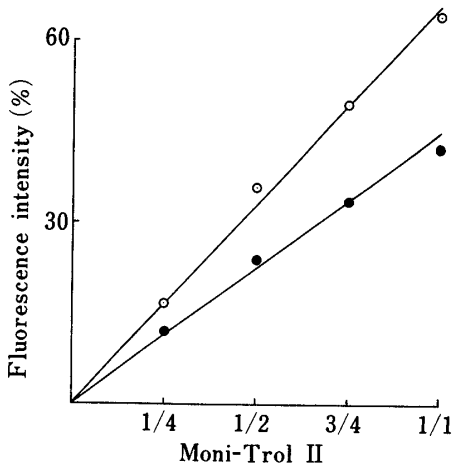

Fig. 2. Relationship between Enzyme Concentration and Activity Determined by the Fluorometric Method

GOT and GPT activities were measured by the methods described in Table I; 100% intensity was adjusted with a quinine sulfate solution (2.5 μg/ml). ○, GOT 88 units/l; ●, GPT 100 units/l.

TABLE I. Assay System for the Determination of GOT and GPT

Constituent	Concentration
α-Ketoglutaric acid	10 mM
Homovanillic acid	1 g/l
Horseradish peroxidase	480 units/l
L-Glutamate oxidase	100 units/l
In 0.05 M potassium phosphate buffer (pH 7.4)	

A 20 μl aliquot of serum or plasma was incubated with 2.4 ml of assay solution for 3 min at 37 °C, then 0.5 ml of aqueous L-aspartic acid solution (100 μmol/ml) for GOT or 0.5 ml of aqueous L-alanine solution (2.5 μmol/ml) for GTP was added, the mixture was incubated for 30 min at 37 °C. The enzyme reaction was terminated by adding 0.1 ml of 0.5 N NaOH. The fluorescence intensity was read with excitation at 315 nm and emission at 415 nm.

TABLE II. Effects of Anticoagulants and Biological Materials on the Determination of GOT and GPT

Compound	Concn.	Relative activity	
		GOT	GPT
None	—	100	100
Heparin	100 units/ml	100.3	99.5
Citric acid	10 mg/ml	68.9	66.8
EDTA	10 mg/ml	98.4	99.7
Creatinine	10 mg/dl	99.3	95.3
Ascorbic acid	4 mg/dl	55.4	42.9
Uric acid	10 mg/dl	98.3	84.2
Glucose	600 mg/dl	96.3	91.3
Bilirubin	10 mg/dl	64.8	64.7
Glutathione	5 mg/dl	98.0	94.7
Hemoglobin	75 mg/dl	82.9	77.4
Catalase	100 units/ml	105.7	102.9

EDTA: ethylenediaminetetraacetic acid.

GOT and GPT. Biological materials, ascorbic acid, bilirubin, uric acid and hemoglobin; also interfered with the determination of both enzymes.

Correlation

The results obtained by the present method were compared with those obtained by the Centrifichem System 400 method. The calculated correlation for GOT was 0.96 and the linear regression equation was $Y=1.02X-2.6$ ($n=19$). The calculated correlation for GPT was 0.93 and the linear regression equation was $Y=0.99X-4.5$ ($n=19$). The values of coefficient variation for GOT and GPT were 2.86 and 1.58%, respectively.

Discussion

GOT and GPT activities in serum can be determined by using L-glutamate oxidase. These new methods are simpler and more sensitive than the Reitman–Frankel method[4] or the Karmen method.[5] Further, the Reitman–Frankel method requires 0.2—0.3 ml of serum or plasma, whereas our methods require only 20 μl. GOT and GPT activities obtained by our methods were well correlated to those obtained by the Centrifichem System 400 method.

Recently, Okawa *et al.*[6] have developed the pyruvate oxidase method[6] to determine

GOT and GPT. In the GPT reaction, pyruvic acid is produced from L-alanine, while oxaloacetic acid is produced from L-aspartic acid in the GOT reaction. Oxaloacetic acid is converted to pyruvic acid by using oxaloacetic acid decarboxylase and thiamine pyrophosphate. They determined this pyruvic acid by using pyruvate oxidase. However, pyruvate oxidase is unstable, whereas L-glutamate oxidase is relatively stable, so that the latter is preferable as a reagent to determine GOT and GPT activities in serum. Further, in our methods, only L-glutamate oxidase is necessary to produce hydrogen peroxide, and no cofactor is required.

Ascorbic acid, bilirubin, uric acid and hemoglobin interfered with the determination of both enzymes in our methods, though the concentrations of these compounds in normal serum are lower than those employed in this experiment. However, further work is necessary to exclude these effects. The sensitivities of our methods are equal to those of the pyruvate oxidase method, while our methods are superior to the pyruvate oxidase method in terms of cost and simplicity.

References and Notes

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