Increased Activity of Serum Aspartate Aminotransferase in the Presence of Added Pyridoxal-5'-Phosphate

To the Editor:

O’Kane and Gunsalus (1) showed that pyridoxal-5’-phosphate (a phosphorylated derivative of vitamin B6) is essential for serum aspartate transaminase (AST) activity. This coenzyme is bound to an amino group of the enzyme during transfer, becoming pyridoxamine phosphate (2). In 1954, Karmen et al. (3) described a practical method of measuring AST activity. Although they gave no data, they did add buffered pyridoxal phosphate to the reaction mixture and found it had no significant effect. Amador and Wacker (4), in their analytical assessment of current assay techniques, make no mention of pyridoxal phosphate and apparently assume that it is present in adequate amounts. Producers of commercial AST assay kits assume the same. We show here that added pyridoxal phosphate significantly affects AST activity of serum, leading in some cases to falsely low values if it is not added.

A Gilford Model 2000 temperature-stabilized (25 ± 0.5°C) recording spectrophotometer was used. Monobasic phosphate buffer (0.1 mol/liter), previously adjusted with NaOH to pH 7.4, was used throughout. The pyridoxal-5’-phosphoric acid (Sigma, No. P-9255) was diluted daily to 8.01 mmol/liter with the phosphate buffer. The commercial control serum was “Q-Pak” (Hyland, lot 3697 m023 al).

Each serum sample was assayed simultaneously in two 1-cm cuvettes, one containing pyridoxal phosphate and the other being a control. The reaction solution was prepared by mixing the enzyme solution, 0.9 ml of NADH-MD [0.37 mmol of NADH per liter, 300 units of malate dehydrogenase (EC 1.1.1.37), and 0.15 g of bovine albumin (fraction V) diluted with phosphate buffer to 100 ml] with 2.0 ml of substrate solution (0.55 mol of L-aspartic acid and 0.072 mol of α-ketoglutric acid per liter of distilled water). Both enzyme and substrate solutions were obtained from Worthington “Statzyme GOT” kits. The pre-incubation was performed by adding to two separate test tubes 50 μl of serum, 50 μl of phosphate buffer, and either 10 μl of pyridoxal phosphate solution (20 mg of pyridoxal phosphate) to the experimental tube or 10 μl of additional phosphate buffer to the control tube. After pre-incubation at 25 ± 0.5°C for 15 min, the enzyme-substrate and the serum mixtures were combined and poured taken at 340 mm, and the first 5-min interval was used in slope calculations.

Sera from 41 different patients were assayed, usually within 9 h, and always within 33 h (sera at 4°C overnight). The activities of these samples were either above or in the upper part of the normal range, which is 10–65 U/liter. The coefficient of variation for 12 replicates of control serum was 4.0%. The mean for the pyridoxal phosphate treated sera was 131 ± 65 U/liter. The mean for their respective controls was 119 ± 61 U/liter (P < .0005, t = 6.36, df = 40, matched pair, 1-tail t-test used). The mean increase was 12 U/liter.

Of the 41 patients’ sera, 15 (36.6%) showed significant (at least 15% or 20 U/liter) increases in AST activity after pyridoxal phosphate treatment. Although these increases could not be correlated with the patients’ conditions—which included myocardial infarction, hepatic cirrhosis, and peptic ulcer—the results do imply that these sera were deficient in pyridoxal phosphate insofar as this assay is concerned. Nutritional deficiency is a possibility. Marsh et al. (5) found increases in AST activity averaging 40% in humans given large supplements of vitamin B6 (pyridoxine) for four weeks.

Our data on pyridoxal phosphate treatment of serum assayed for AST activity imply that such treatment is important in increasing the accuracy of the assay. The inconsistency of our findings with those of Karmen et al. (3) is probably the result of our pre-incubation of sera with pyridoxal phosphate, since they do not appear to have included this step. O’Kane and Gunsalus (1) showed clearly that aspartate and α-ketoglutarate interfere substantially with the binding of pyridoxal phosphate to the enzyme at about the same concentration of pyridoxal phosphate that we used. Therefore, pre-incubating the enzyme with pyridoxal phosphate before adding substrate is essential to accuracy.

Eleven vials of Q-Pak were analyzed (13 determinations) within 9 h of reconstitution. Results for those vials pre-incubated with pyridoxal phosphate averaged 189 ± 10 U/liter. Matched controls averaged 123 ± 10 U/liter (P < .0005, t = 17.52, df = 24). This 54% increase in AST activity of a commercial control serum illustrates that such preparations cannot be validly used as primary standards because their activity may be falsely low.

Refrigerated, pyridoxal phosphate is stable in solution for at least two weeks. When Q-Pak solution is refrigerated and run daily, the percent increase caused by pyridoxal phosphate treatment does not change significantly over four days.

A similar study of serum alanine aminotransferase (EC 2.6.1.2) might prove interesting, as pyridoxal phosphate is also a coenzyme for this enzyme.

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References


Andrew G. Ury
Joel R. Chassy
Department of Pathology and Laboratory Medicine
Mount Zion Hospital and Medical Center
1600 Divisadero St.
San Francisco, Calif. 94115

Use of Gelatin-Coated Charcoal in Digoxin Immunoassay

To the Editor:

Since the first method for immunoassay of plasma digoxin was published (1), dextran-coated charcoal (2) has been the preferred reagent for separating antibody-bound and unbound digoxin (3–13). In the immunoassay techniques it is a well-known phe-