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Pyridoxal 5'-phosphate as an Activator of the Apoenzyme of Alanine Aminotransferase in Human Serum

By *K. Jung* and *E. Egger*

Department of Clinical Biochemistry and Chair of Pathological Biochemistry, Charité, Humboldt University Berlin

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Summary: The relationship between reaction conditions and the stimulation of alanine aminotransferase by pyridoxal 5'-phosphate was investigated. Reaction conditions given for optimized alanine aminotransferase measurements are also optimal for measurements in the presence of pyridoxal 5'-phosphate taking into consideration the inhibitory effect of phosphate buffer. Additon of 150 µmol/l pyridoxal 5'-phosphate to the reaction mixture guarantees a maximal stimulation after a pre-incubation period of 10 min. Factors influencing the magnitude of stimulation rates of both aminotransferases in different patient groups by pyridoxal 5'-phosphate are discussed.

Pyridoxal-5'-phosphat als Aktivator des Apoenzyms der Alaninaminotransferase im Humanserum

Zusammenfassung: Die Abhängigkeit der Stimulierung der Alaninaminotransferase-Aktivität durch Pyridoxal-5'-phosphat von den Reaktionsbedingungen wurde untersucht. Ein Zusatz von 150 µmol/l Pyridoxal-5'-phosphat zum Reaktionsgemisch garantiert unter den zur Zeit bekannten optimalen Reaktionsbedingungen für die Alaninaminotransferase bei einer Vorinkubationszeit von 10 min eine maximale Stimulierung dieses Enzyms. Die Möglichen Ursachen für die unterschiedliche Stimulierung der beiden Aminotransferasen durch Pyridoxal-5'-phosphat bei verschiedenen Patientengruppen werden diskutiert.

Pyridoxal 5'-phosphate is the coenzyme of aspartate aminotransferase (EC 2.6.1.1) and of alanine aminotransferase (EC 2.6.1.2) (1). Addition of pyridoxal 5'-phosphate to the reaction mixture or serum increases considerably the activity of aspartate aminotransferase in many patients (2–7). IFCC recommends the inclusion of pyridoxal 5'-phosphate in the reaction mixture (8). On the other hand, the influence of pyridoxal 5'-phosphate on the alanine aminotransferase activity has received little consideration hitherto (2, 4, 9). In order to obtain further information on the influence of pyridoxal 5'-phosphate upon alanine aminotransferase we have investigated the relationship between pyridoxal 5'-phosphate activation and the other reaction conditions for the determination of alanine aminotransferase.

Materials and Methods

Enzyme reaction rates were measured at 340 nm on the LKB Reaction Rate Analyzer 8600 at 37 °C.

Unless otherwise stated, the final reaction mixture contained, per liter: 400 mmol of *L*-alanine; 100 mmol of tris buffer, pH 7.4; 0.133 mmol of NADH; 12 mmol of 2-oxoglutarate; 1800 U lactate dehydrogenase (EC 1.1.1.37) and 150 µmol of pyridoxal 5'-phosphate. Ratio of sample volume to final volume was 1:7.5.

After pre-incubation of enzyme-substrate for 15 min the reaction was started by addition of 2-oxoglutarate. The nonspecific reaction rate and the apo-aminotransferase activity of lactate dehydrogenase were subtracted. Duplicate assays were performed and the data given are the means of the duplicates. The standard deviation was estimated by duplicates using the following equation $s^2 = \frac{\Sigma R^2}{2m}$ (R = difference between duplicates, m = number of duplicate assays). s was ± 1.4 U/l in the range of activities from 25 to 75 U/l. The within series precision of the method expressed as coefficient of variation was 3.29% (mean test activity 30.4 U/l, a series of 12).

L-alanine, pyridoxal 5'-phosphate, lactate dehydrogenase were obtained from Boehringer Corp., Mannheim, NADH from VEB Arzneimittelwerke Dresden, 2-oxoglutarate from Reanal, Budapest and tris and NaH_2PO_4 from E. Merck, Darmstadt. The serum samples were taken from patients.

Results and Discussion

The role of pyridoxal 5'-phosphate in the activation of alanine aminotransferase was recently demonstrated (2, 4, 9), but no systematic investigations have been published so far. It was shown that the reaction conditions influence markedly the magnitude of stimulation of

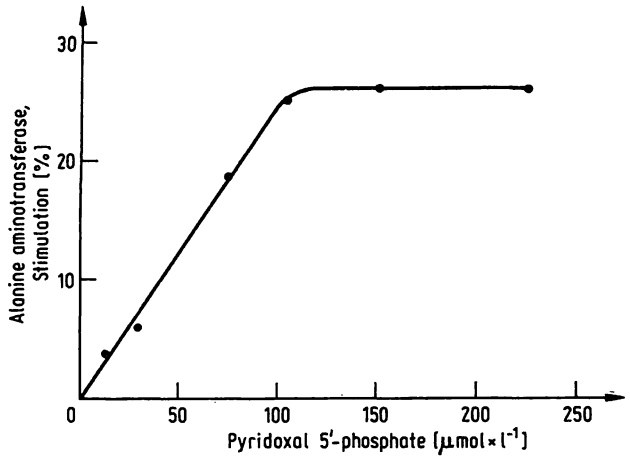

Fig. 1. Influence of increasing concentrations of pyridoxal 5'-phosphate on alanine aminotransferase.

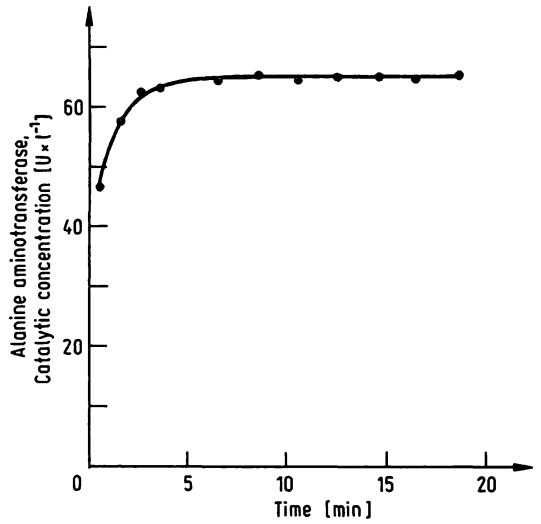

Fig. 2. Influence of pre-incubation time of serum samples in the reaction mixture containing pyridoxal 5'-phosphate on the stimulation of alanine aminotransferase. Before addition of 150 μmol/l pyridoxal 5'-phosphate the serum samples were incubated for 15 min in the reaction mixture (without 2-oxoglutarate). After addition of pyridoxal 5'-phosphate, incubation was continued for the indicated times and the reaction was started by 2-oxoglutarate.

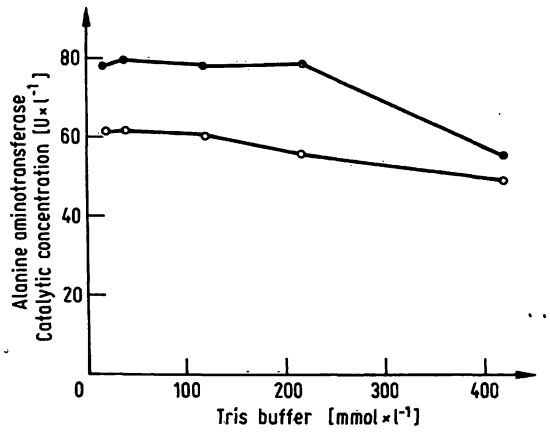

Fig. 3. Influence of tris buffer on the stimulation of alanine aminotransferase by pyridoxal 5'-phosphate.
●—● with pyridoxal 5'-phosphate; o—o without pyridoxal 5'-phosphate.

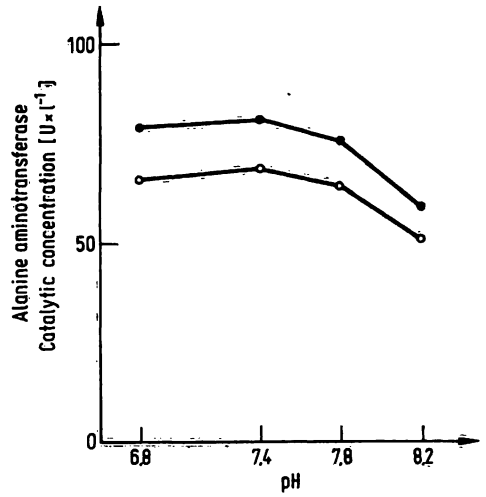

Fig. 4. Influence of pH in the reaction mixture on the stimulation of alanine aminotransferase by pyridoxal 5'-phosphate.
Symbols are given in Fig. 3.

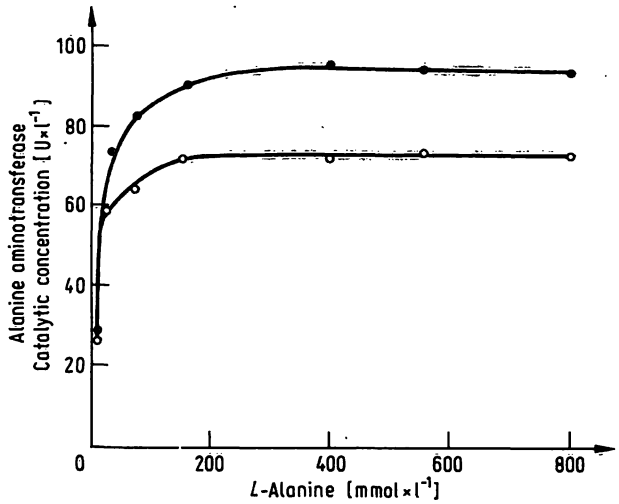

Fig. 5. Influence of increasing concentration of *L*-alanine on the stimulation of alanine aminotransferase by pyridoxal 5'-phosphate.
Symbols are given in Fig. 3.

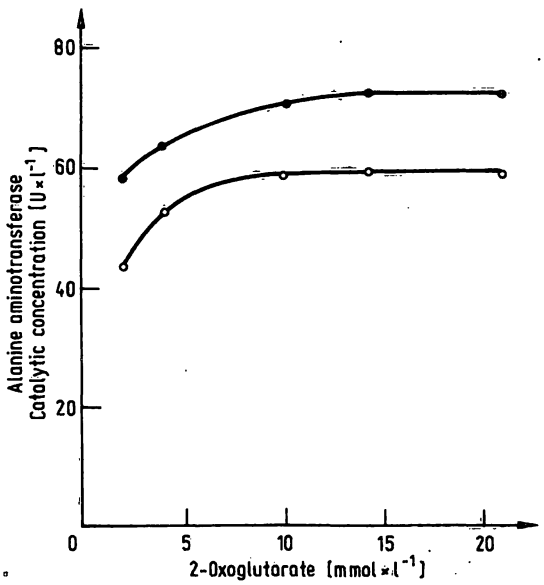

Fig. 6. Influence of increasing concentration of 2-oxoglutarate on the stimulation of alanine aminotransferase.
Symbols are given in Fig. 3.

aspartate aminotransferase by pyridoxal 5'-phosphate (10). Therefore, it is also necessary to investigate these relationships for alanine aminotransferase.

The inhibition of the pyridoxal 5'-phosphate stimulation by phosphate buffer, which has been observed with aspartate aminotransferase (9, 11, 12), has now been shown by the present authors with alanine aminotransferase. By addition of pyridoxal 5'-phosphate the mean stimulation of alanine aminotransferase activity in sera from 20 different patients was 35.3% ± 4.38 in the reaction mixture with 100 mmol/l tris buffer in comparison with 6.73% ± 1.25 in the reaction mixture with 80 mmol/l phosphate buffer (arithmetic mean ± standard deviation of the mean). The poor stimulation of alanine aminotransferase described by other authors (2, 4) can be explained by the buffer used. In further investigations we used only tris buffer.

Maximum activation of alanine aminotransferase was observed in the presence of about 100 µmol/l of pyridoxal 5'-phosphate (fig. 1). For the presented method we propose 150 µmol/l of pyridoxal 5'-phosphate as a sufficient concentration for activation of alanine aminotransferase.

Investigating the dependence of incubation time on the activation effect of pyridoxal 5'-phosphate we obtained the maximum activation after 7 to 10 min pre-incubation of sera in the reaction mixture with pyridoxal 5'-phosphate before the reaction was started (fig. 2). In general use, the preincubation time is 10 to 15 min in alanine aminotransferase measurements. Therefore, the preincubation time does not need to be extended in measurements with pyridoxal 5'-phosphate.

Investigating the other reaction conditions e.g. pH, substrate and buffer concentrations (figs. 3, 4, 5, 6) we found that the reaction conditions given for optimized alanine aminotransferase measurements (13, 14, 15) are also optimal for measurements in the presence of pyridoxal 5'-phosphate. Pyridoxal 5'-phosphate prevents the inhibition by tris buffer up to 200 mmol/l. Therefore, an intended standardization of alanine aminotransferase measurement in the presence of pyridoxal 5'-phosphate can be carried out by the usually optimized methods with the addition of pyridoxal 5'-phosphate.

We measured the activation of the apoenzyme of alanine aminotransferase and of aspartate aminotransferase in three groups: in subjects with normal values of aminotransferases, in patients with chronic liver disease and in renal transplant patients (tab. 1). In all three investigated groups we could not find any significant relations between the extent of stimulation of both aminotransferases by pyridoxal 5'-phosphate ($p > 0.05$). We must also take into account the different stimulation rates of aminotransferases in the same sera of the groups investigated: patients with chronic liver disease show a lower stimulation of aspartate aminotransferase and renal transplant patients show a higher stimulation of alanine aminotransferase than normal subjects. It becomes evident that the factors influencing the magnitude of stimulation of both aminotransferases by pyridoxal 5'-phosphate are very complex. It is possible that the level of pyridoxal 5'-phosphate varies in different tissues and therefore, the aminotransferases released can show a different saturation with pyridoxal 5'-phosphate depending on the kind of disease. The different affinity of both aminotransferases and isoenzymes for pyridoxal 5'-phosphate (16) can be another factor contributing to these differences in stimulation rates. Aspartate aminotransferase binds pyridoxal 5'-phosphate more loosely than alanine aminotransferase (16). On the other hand, pyridoxal 5'-phosphate is more strongly bound to cytoplasmatic isoenzyme than to the mitochondrial isoenzyme of aspartate aminotransferase (16). Besides we must take into consideration a possible change in the properties of aminotransferases in serum after their release from tissues. For instance an activation of aspartate aminotransferase was observed after splitting this enzyme into monomers (16).

Our investigations indicate that under special circumstances a great part of alanine aminotransferase in human serum can exist without pyridoxal 5'-phosphate like aspartate aminotransferase (2–6, 9). Therefore, in order to measure the maximum activities of alanine aminotransferase in serum we recommend, in accordance with *Bergmeyer* et al. (9), the incorporation of pyridoxal 5'-phosphate into the reaction mixture, taking into consideration the above mentioned conditions.

Tab. 1. Stimulation of aminotransferases in human serum by pyridoxal 5'-phosphate. Stimulation rates are given in percent in relation to aminotransferase without addition of pyridoxal 5'-phosphate (arithmetic mean ± standard deviation of the mean). Stimulation values for patients were compared with values for normal subjects. The relationship between the stimulation rates for both aminotransferases are given as correlation coefficients.

\+ $p > 0.05$
++ $p < 0.01$
+++ $p < 0.001$

Group	aspartate aminotransferase $\bar{x}$ (U/l)	aspartate aminotransferase stimulation (%)	alanine aminotransferase $\bar{x}$ (U/l)	alanine aminotransferase stimulation (%)	correlation coefficient (r)
normal subjects (n = 18)	30.7	28.2 ± 5.07	31.2	18.9 ± 3.9	0.41+
patients with chronic liver disease (n = 31)	70.9	10.5 ± 1.74+++	90.3	12.9 ± 1.99+	0.01+
renal transplant patients (n = 20)	47.4	22.5 ± 2.12+	58.3	44.4 ± 5.28++	0.01+

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Dr. K. Jung, Prof. Dr. med. habil. E. Egger
Abteilung Klinische Biochemie des Bereichs Medizin (Charité)
und Lehrstuhl für Pathologische Biochemie
der Humboldt-Universität Berlin
Schumannstraße 20/21
DDR-104 Berlin