REACTION OF ASPARTATE AMINOTRANSFERASE APOISOZYMES WITH GLYCERALDEHYDE-3-P: EFFECT OF $NaBH_4$ TREATMENT AND SUBSEQUENT REACTIVATION BY PYRIDOXAL-5′-P

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1. Introduction

We have shown recently that the anionic and cationic isozymes of aspartate aminotransferase (L-aspartate:2 oxoglutarate aminotransferase, EC 2.6.1.1) are both inhibited by glyceraldehyde-3-P [1]. It was further shown that this inhibition was potentiated by aspartate [1] and that the K_i values obtained were lower for the pyridoxamine forms of each isozyme than for the pyridoxal forms [2]. On the basis of our observations, we proposed [1, 2] that glyceraldehyde-3-P inhibits aspartate aminotransferase by forming a Schiff base with one of the ε-amino groups of lysine at the enzymically active site. Aspartate would potentiate the inhibition by converting the enzyme to the pyridoxamine form, thereby exposing a second ε-amino lysyl group which could react with the inhibitor. This communication shows that sodium borohydride reduction of apoisozymes in the presence of glyceraldehyde-3-P prevented the restoration of activity by pyridoxal-5′-P. This finding supports our proposed mechanism of inhibition.

2. Experimental procedure

The isozymes of aspartate aminotransferase were purified from rat liver as described by Nisselbaum and Bodansky [3]. Enzyme activity was measured by the coupled reaction described by Karmen [4], as modified by Kopelovich et al. [1]. In general accordance with the procedure of Scardi et al. [5], each holoisozyme was resolved to apoisozyme by incubation for 15 min at room temperature in the presence of 25 mM L-aspartate at pH 7.5, followed by incubation in 0.5 M KH_2PO_4, pH 5.5, for one hour at room temperature. Resolved isozymes were dialyzed agianst 2 changes of 0.1 M phosphate buffer, pH 6.0, and then against 2 changes of 4 mM barbital-HCl-34 mM NaCl, pH 7.4. Restoration of activity was measured after incubation of apoisozyme at 37° with 0.4 mM pyridoxal-5′-P (Pal-5-P). Although reactivation of the apoisozyme was virtually complete within 15 min, incubation times of 90 min were used in some experiments.

3. Results and discussion

Table 1 shows that resolution of the anionic isozyme was complete. After dialysis against 4 mM barbital-HCl-34 mM NaCl, pH 7.4, incubation of anionic apoisozyme with Pal-5-P prior to assay, resulted in the recovery of 60% of the original activity. Resolution of the cationic isozyme was complete to the extent of 91%, and incubation with Pal-5-P resulted in the recovery of 66% of the original activity.

Each sample of apoisozyme was divided into two aliquots, one of which was incubated with glyceraldehyde-3-P (Ga-3-P) at a final concentration of 4 mM, and the other served as control. This concentration of Ga-3-P in the presence of aspartate

Table 1
Resolution of aspartate aminotransferase isozymes.

Treatment	% of original activity	
	Anionic isozyme	Cationic isozyme
25 mM aspartate, 0.5 M phosphate, pH 5.5	1.1 ± 0.1	13 ± 5
Restoration of activity after 15 min incubation with 0.4 mM Pal-5-P	69 ± 7	80 ± 5
Dialysis vs 0.1 M phosphate, pH 6.0, then vs 4.0 mM barbital-HCl-34 mM NaCl, pH 7.4	1.4 ± 0.5	9 ± 4
Restoration of activity after 15 min incubation with 0.4 mM Pal-5-P	60 ± 4	66 ± 2

Table 2
Recovery of aspartate aminotransferase activity from treated apoisozymes upon incubation with pyridoxal-5'-P[a].

Treatment	Apoisozyme	
	Anionic	Cationic
	%	%
Control	100	100
$NaBH_4$ reduction	49 ± 6	64 ± 5
Ga-3-P incubation	75 ± 11	64 ± 8
Ga-3-P followed by $NaBH_4$	13 ± 1	15 ± 3
$NaBH_4$/Ga-3-P + $NaBH_4$	3.8	4.3

[a] Recoveries of activity are expressed as % of control values and are the averages of 2 experiments.

inhibited the anionic and cationic holoisozymes by 70% and 90%, respectively [1]. After 30 min at 37° the samples were cooled in an ice-water bath and each was further divided into two portions. One was reduced with 0.1 ml of an ice-cold solution of $NaBH_4$ (25 mg/ml) per 1.0 ml of sample; 0.01 ml of 2 N HCl was added to maintain the pH at 7.4. This solution was centrifuged at low speed for 15 min while the reaction proceeded in order to minimize denaturation due to foaming. The other portion was treated with barbital-HCl buffer, pH 7.4. The procedure was repeated twice. All samples were then dialyzed over a 24 hr period against 2 to 3 changes of 100 volumes of 4 mM barbital-HCl-34 mM NaCl to remove unreacted Ga-3-P and $NaBH_4$. The activity of each sample was measured after incubation with Pal-5-P. The recovery of activity after each treatment was expressed as percentage of the activity of the control samples that were not treated with either Ga-3-P or $NaBH_4$.

Table 2 shows that addition of Pal-5-P resulted in substantial reactivation of both apoisozymes that had been treated with either $NaBH_4$ or Ga-3-P, 50–75%. In contrast, apoisozymes that had been treated first with Ga-3-P, then reduced with $NaBH_4$ showed only slight reactivation, about 14%. The ratios of the activity of the reactivated apoisozyme that had been treated with $NaBH_4$ to that which had been treated with both Ga-3-P and $NaBH_4$ were approximately 4 for each isozyme.

These results are consistent with the proposal that Ga-3-P inhibits the isozymes of aspartate aminotransferase by reacting with free amino groups at the enzymically active site, probably ε-amino lysyl residues, to form Schiff base. Reduction by $NaBH_4$ would convert the Schiff base to a secondary amine and prevent the recombination of the coenzyme with apoisozyme and the formation of the internal aldimine on the holoenzyme. The latter structure has been reported to be an intermediary stage in the expression of the activity of aspartate aminotransferase [6]. Indeed, Ivanov and Karpeisky [6] have postulated that both of the ε-amino lysyl residues in the active site are necessary for transamination.

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