Vol. 90, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 27, 1979 Pages 447-452

CYSTEINE SULFINATE TRANSAMINATION ACTIVITY OF ASPARTATE AMINOTRANSFERASES

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Received August 6,1979

SUMMARY. Aspartate aminotransferases from pig heart cytosol and mitochondria, Escherichia coli B and Pseudomonas striata accepted L-cysteine sulfinate as a good substrate. The mitochondrial isoenzyme and the Escherichia enzyme showed higher activity toward L-cysteine sulfinate than toward the natural substrates, L-glutamate and L-aspartate. The cytosolic isoenzyme catalyzed the L-cysteine sulfinate transamination at 50% the rate of L-glutamate transamination. The Pseudomonas enzyme had the same reactivity toward the three substrates. Antisera against the two isoenzymes and the Escherichia enzyme inactivated almost completely cysteine sulfinate transamination activity in the crude extracts of pig heart muscle and Escherichia coli B, respectively. These results indicate that cysteine sulfinate transamination is catalyzed by aspartate aminotransferase in these cells.

INTRODUCTION

Aspartate aminotransferase (AAT, EC 2.6.1.1) catalyzes the transamination between L-aspartate and α-ketoglutarate and between L-glutamate and oxaloacetate as a reverse reaction. The enzyme is widely distributed in various organisms and microorganisms as summarized by Braunstein (1). It has been shown that AATs catalyze the transamination of various natural or synthetic amino acids besides the natural substrates, L-glutamate and L-aspartate (1).

L-Cysteine sulfinate, which is a structural analog of L-aspartate and a key intermediate in taurine biosynthesis, has been shown to be a good substrate for AAT from Proteus vulgaris and the cytosolic isoenzyme from pig heart muscle with a crude extract and a highly purified preparation, respectively (2,3).

0006-291X/79/180447-06$01.00/0

Thus, it has been assumed that transamination of L-cysteine sulfinate in the cells is catalyzed by AAT. On the other hand, the presence of cysteine sulfinate aminotransferases besides AAT was recently reported in the mouse and rat nervous systems (4,5). In order to clarify the role of AAT in cysteine sulfinate transamination, we have carried out immunochemical studies on the reactivity of the enzyme toward L-cysteine sulfinate.

The present communication shows that homogeneous AATs from Escherichia coli B, Pseudomonas striata and pig heart muscle cytosol and mitochondria have high and characteristic cysteine sulfinate transamination activities and that in the cells of Escherichia coli B and pig heart muscle, the transamination between L-cysteine sulfinate and α-ketoglutarate is catalyzed almost exclusively by AAT.

MATERIALS AND METHODS

AATs, from Escherichia coli B and Pseudomonas striata were purified as reported previously (6,7). Pig heart muscle cytosolic and mitochondrial AATs were gifts from Drs. Tanase and Morino, Kumamoto University Medical School. Antisera against both the isoenzymes were gifts from Drs. Sakakibara and Wada, Osaka University Medical School. Antiserum against Escherichia AAT was prepared as follows. Adult female rabbits were immunized with the homogeneous enzyme by three weekly subcutaneous injections on the back. All the injections were of 200 μg of the enzyme in 0.01 M potassium phosphate buffer (pH 7.0) containing 0.85% NaCl. The solution was mixed with an equal volume of Freund's complete adjuvant before each injection. One week after the last injection bleeding was performed. Antisera were stored at -20°C. Malate dehydrogenase was purchased from Boehringer, L-cysteine sulfinate from Sigma and α-[^{14}C(U)]-ketoglutarate from New England Nuclear. L-Glutamate and L-aspartate were obtained from Kyowa Hakko Kogyo, Tokyo.

AAT activity was determined by two methods. Assay A was a modification of the coupled malate dehydrogenase method as described previously (7). Radioassay (assay B) was performed by the method of Novogrodosky and Meister (8) with a modification as follows. The reaction mixture consisted of 2.5 μmol of α-[^{14}C(U)]-ketoglutarate, 25 μmol of L-amino acid, 5 nmol of pyridoxal 5'-phosphate, 50 μmol of Tris-HCl buffer (pH 8.5) or potassium phosphate buffer (pH 7.4) and the enzyme in a final volume of 0.5 ml. Aliquots of 0.1 ml were removed at 3- or 4-min intervals and mixed with 0.1 ml of 10% trichloroacetate. To the mixture was added 0.1 ml of 0.2 M sodium phosphate buffer (pH 5.0) containing 0.5 N NaOH. The radioactivity of L-[^{14}C(U)]-

glutamate separated by ion exchange chromatography with Bio-Rad AG 50Wx2 was determined with a Tracor Analytic liquid scintillation spectrometer Mark III-6880. One unit of enzyme was defined as the amount of enzyme that catalyzed the formation of 1.0 μmol L-glutamate. Cysteine sulfinate transaminase activity of the crude preparation was determined by measuring the formation of sulfur dioxide as described previously (10) except that 50 mM and 5 mM of L-cysteine sulfinate and α-ketoglutarate, respectively, were used.

RESULTS AND DISCUSSION

AATs from various sources showed high reactivity toward L-cysteine sulfinate, suggesting that AAT acts as a cysteine sulfinate transaminase (Table I). Specific activities of AATs toward L-glutamate were taken as 100%. The relative activity of each AAT was almost independent of the reaction pH (pH 7.4 and 8.5). All the AATs had pH optima at pH 8.0-9.0 determined with the three substrates in Table I. L-Cysteine sulfinate was a substrate for pig heart cytosolic AAT with a relative rate of about 50% of that of L-glutamate. The mitochondrial isoenzyme resembled Escherichia AAT in the reactivities toward the three substrates: L-cysteine sulfinate was the best substrate and reactivity with L-aspartate was about 30% of that of L-glutamate. Pseudomonas AAT had nearly equal reactivity toward each of the three substrates. In all cases, the concentrations of the three substrates (50 mM) were in saturation (1,7) : Km values of L-cysteine sulfinate were generally lower than those of L-aspartate, although accurate values were not determined.

To examine the presence of cysteine sulfinate transaminases besides AAT in the cells, immunochemical experiments were performed (Figure 1). The crude extract of Escherichia coli B incubated with the antiserum against AAT from E. coli B showed parallel and almost complete inactivation of cysteine sulfinate and aspartate transaminase activities (Figure 1,A). Control serum did not inactivate these activities. Antisera against cytosolic and mitochondrial

Table I. Cysteine sulfinate transamination activities of aspartate aminotransferases. Activities were determined by assay B as described in Materials and Methods. Specific activities of Escherichia AAT, Pseudomonas AAT and pig heart cytosolic and mitochondrial AATs determined by assay A were 152, 98, 200 and 140 unit/mg, respectively. The concentration of substrates was 50 mM.

Amino Acids	Relative Activities (%)							
	Cytosolic AAT		Mitochondrial AAT		Escherichia AAT		Pseudomonas AAT	
	pH 7.4*	pH 8.5*	pH 7.4	pH 8.5	pH 7.4	pH 8.5	pH 7.4	pH 8.5
L-Cysteine sulfinate	49	54	110	115	119	129	90	103
L-Aspartate	43	40	36	33	27	35	94	101
L-Glutamate	100	100	100	100	100	100	100	100

*0.1 M potassium phosphate buffer (pH 7.4) and 0.1 M Tris-HCl (pH 8.5).

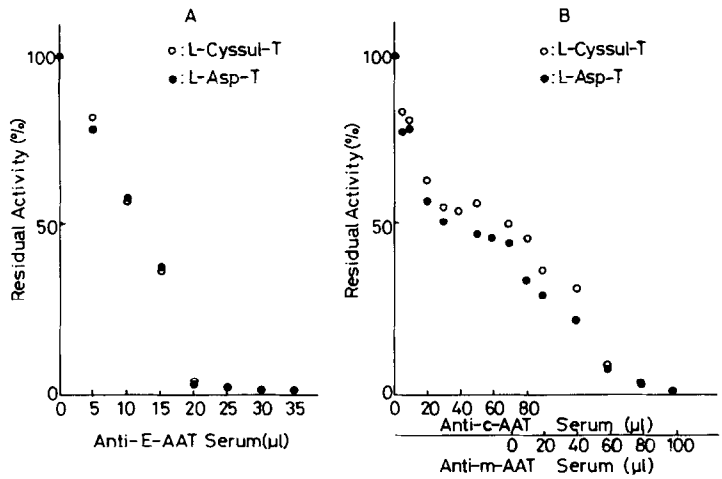

Figure 1. Inhibition of aspartate and cysteine sulfinate transamination by antisera. A: crude extract of Escherichia coli B was incubated with the antiserum against Escherichia AAT. B: crude extract of pig heart muscle was incubated with antisera against pig heart cytosolic and mitochondrial AATs. After 70 μl of antiserum against cytosolic AAT was added, by which cytosolic AAT in the crude extract was precipitated almost completely, anti-mitochondrial AAT serum was then added. After addition of antisera the crude extracts were allowed to stand for 40 min at 25°C, and the precipitate was centrifuged off. Aspartate (●, L-Asp-T) and cysteine sulfinate (o, L-Cyssul-T) transaminase activities were determined as described in Materials and Methods. Anti E-AAT, anti-c-AAT and anti-m-AAT serum represent antiserum against Escherichia AAT and pig heart muscle cytosolic and mitochondrial AATs, respectively.

AATs of pig heart muscle were added separately to the crude extract of the tissue; by the addition of anti-cytosolic AAT serum (70 μl) AAT activity was reduced to about one-half the initial value, and subsequent addition of anti-mitochondrial AAT serum caused almost complete inactivation of AAT (Figure 1,B). Parallel inactivation of cysteine sulfinate transamination activity was observed in the two cases of antiserum addition. As mitochondrial AAT showed higher activity toward L-cysteine sulfinate than L-aspartate (Table I), a slightly higher cysteine sulfinate transamination activity remained after cytosolic AAT was precipitated with the antibody. The pH optimum profiles of the extracts were the same as those obtained with the homogeneous AATs. Although the existence of cysteine sulfinate transaminase has been suggested in the mouse

and rat brain (4,5), our results strongly indicate that a cysteine sulfinate transaminase besides AAT is absent in pig heart muscle and Escherichia coli B and that the cysteine sulfinate transamination observed in these cells is catalyzed by AAT.

ACKNOWLEDGEMENTS. We wish to thank Dr. K. Soda, Kyoto University for his helpful advice, Drs. S. Tanase and Y. Morino, Kumamoto University for the gift of pig heart AATs, and Drs. R. Sakakibara and H. Wada, Osaka University for the gift of antisera against pig heart AATs.

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