SYNCATALYTIC SULFHYDRYL GROUP MODIFICATION IN MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE FROM CHICKEN AND PIG HEART

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Received January 22,1975

SUMMARY. One sulfhydryl group of the mitochondrial isoenzyme of aspartate aminotransferase from both chicken and pig heart exhibits syncatalytic reactivity changes similar to those found previously in the cytosolic isoenzyme from pig heart (Birchmeier, W., Wilson, K.J., and Christen, P. (1973) J. Biol. Chem. 248, 1751-1759). The reactivity of the only titratable sulfhydryl group toward 5,5'-dithiobis-(2-nitrobenzoate) is at a minimum in the free pyridoxal and pyridoxamine form of the enzyme and is increased by approximately one order of magnitude when covalent enzyme-substrate intermediates are formed. The modification of the sulfhydryl group does not affect enzymatic activity. This finding supports the earlier conclusion that the syncatalytic reactivity changes are not due to a direct participation of this group in the active site but rather to conformational adaptations of the enzyme-coenzyme-substrate compound occurring in the catalytic mechanism of aspartate aminotransferases.

Recent studies on chemical modification of the cytosolic isoenzyme of aspartate aminotransferase from pig heart have shown that the reactivity of one particular cysteinyl residue toward sulfhydryl reagents is increased by 2 orders of magnitude in certain covalent enzyme-substrate intermediates. These syncatalytic changes in the susceptibility of a side chain group toward chemical modification are thought to reflect conformational adaptations of the enzyme-coenzyme-substrate compound concomitant with the catalytic reaction (1).

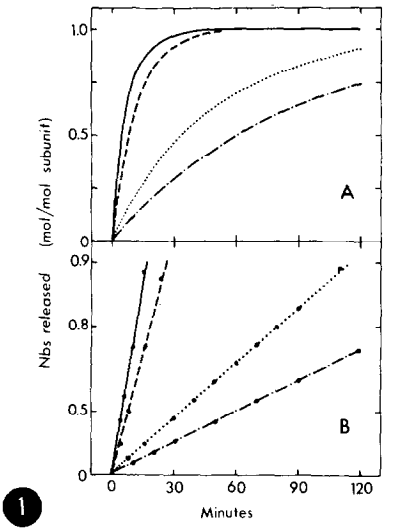
The present communication reports similar syncatalytic reactivity changes of a sulfhydryl group in the mitochondrial isoenzymes from both chicken and pig heart.

MATERIALS AND METHODS. The mitochondrial isoenzymes of aspartate aminotransferase were isolated from chicken and pig heart according to previously described methods (2,3,4). Protein concentrations were determined spectrophotometrically using a molar absorptivity of the subunit $\varepsilon_{280} = 7.0 \times 10^4\ M^{-1}\ cm^{-1}$. The specific activity of the chicken enzyme at 25° was 140 U/mg and that of

Abbreviations: Nbs_2, 5,5'-dithiobis-(2-nitrobenzoate); Nbs, 5-thio(2-nitrobenzoate).

the pig heart enzyme 240 U/mg. Both preparations were homogenous as judged by starch gel electrophoresis (pH 8.7), by sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.5 per cent), and by isoelectric focusing in polyacrylamide gel (Schlegel, H., and Christen, P., in preparation). Enzymatic activity was measured in the coupled assay with malate dehydrogenase (E.C. 1.1.1.37, obtained from Boehringer) as described previously (1). The pyridoxamine form of the enzymes was prepared by addition of 20 mM cysteine sulfinate (5) and subsequent gel filtration on a column of Sephadex G-25 equilibrated with 50 mM sodium phosphate, pH 7.5. L-Glutamic acid, L-aspartic acid, oxalacetic acid, 2-mercaptoethanol and Nbs_2 were obtained from Fluka. L-Cysteine sulfinic acid and α-methyl-DL-aspartic acid were from Sigma, and DL-erythro-β-hydroxy-aspartic acid from Calbiochem. Pyridoxal-5'-P and pyridoxamine-5'-P were from Merck. The thiol group content of the enzymes was determined according to the procedure of Ellman (6) with 0.5 mM Nbs_2 in 0.5 per cent sodium dodecyl sulfate - 50 mM sodium phosphate, pH 7.5, using a molar absorptivity of Nbs $\varepsilon_{412} = 13,600\ M^{-1}\ cm^{-1}$ (1).

RESULTS. The reaction of Nbs_2 with mitochondrial aspartate aminotransferase from chicken heart results in the modification of one sulfhydryl group per enzyme subunit. The rate of the reaction depends markedly on the functional state of the enzyme; it is slowest with the free pyridoxal form of the enzyme, is somewhat higher with the pyridoxamine form and is increased by approximately one order of magnitude when covalent enzyme-substrate intermediates are formed in the presence of either the substrate pair aspartate and oxalacetate or of the substrate analog β-hydroxyaspartate (Fig. 1A). The extent of the reaction, however, remains unaltered in the different functional states of the enzyme. Under all conditions, only 1 sulfhydryl group reacts out of a total of 5.0 (SD $\pm$ 0.2) per enzyme subunit (cf. Methods) following pseudo-first order kinetics over 90 per cent of the total range of the reaction (Fig. 1B). The same substrate-dependent chemical modification is found with the mitochondrial isoenzyme from pig heart where 1 sulfhydryl group out of a total of 6.9 (SD $\pm$ 0.4) per subunit is modified by Nbs_2 (Fig. 2A and B). The absolute rates of modification and their modulation from one enzyme-substrate intermediate to the other are virtually identical in the avian and the porcine enzyme (Table I). With both enzymes the modification rates of the free pyridoxal and pyridoxamine form



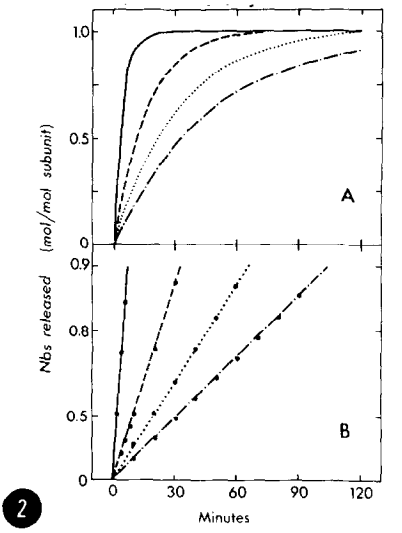


Fig. 1 Modification of mitochondrial aspartate aminotransferase from chicken heart with Nbs_2. Effect of substrates. The reaction was started by the addition of Nbs_2 (0.5 mM) to an enzyme solution (0.013 mM subunit concentration) containing the indicated substrates and 0.2 mM pyridoxal-5'-P or 0.4 mM pyridoxamine-5'-P (for the experiment with the pyridoxamine form of the enzyme) in 50 mM sodium phosphate (pH 7.5, 25°). A, the release of Nbs was followed spectrophotometrically at 412 nm (ϵ_{412} = 13,600 M^{-1} cm^{-1}). Pyridoxal form of the enzyme in the presence of 20 mM DL-erythro-β-hydroxyaspartate (——), in the presence of the substrate pair 11 mM aspartate and 0.4 mM oxalacetate (----), and in the absence of substrate (-·-·-); pyridoxamine form of the enzyme in the absence of substrate (····). B, semi-log plot of the data from part A.

Fig. 2 Modification of mitochondrial aspartate aminotransferase from pig heart with Nbs_2. Effect of Substrates. The concentration of Nbs_2 was 1 mM and the enzyme subunit concentration was 0.02 mM. Other conditions are those of Fig. 1. A, the release of Nbs was followed spectrophotometrically at 412 nm. Pyridoxal form in the presence of 20 mM DL-erythro-β-hydroxyaspartate (——), in the presence of the substrate pair 55 mM glutamate and 4 mM α-ketoglutarate (----), and in the absence of substrate (-·-·-); pyridoxamine form in the absence of substrates (····). B, semi-log plot of the data from part A.

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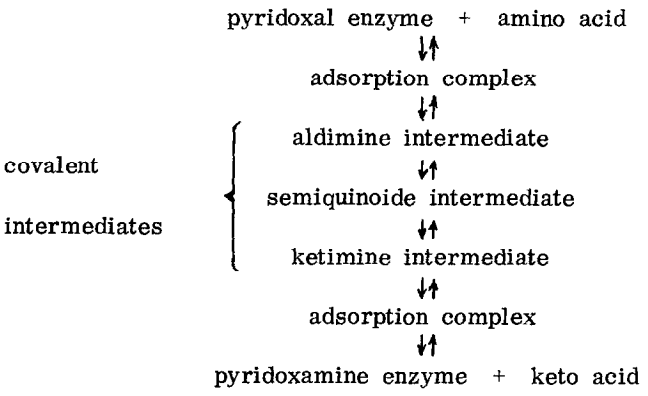
and their unproductive adsorption complexes with keto acid or amino acid, respectively, (7) are slower than those of the covalent enzyme-substrate intermediates formed in the presence of the keto acid amino acid substrate pairs (Table I). The maximum rate of modification is attained, however, in the presence of substrate analogs that form only a part of the covalent enzyme-substrate intermediates of the transamination cycle (cf. Scheme).



With α-methyl aspartate and the pyridoxal form only the first of the covalent intermediates, the aldimine, is formed (8). Addition of erythro-β-hydroxy-aspartate to the pyridoxal form results in an accumulation of the semiquinoide intermediate (9,10). The proportionality of the rate enhancement and the concentration of the semiquinoide enzyme-substrate intermediate formed with β-hydroxyaspartate (Fig. 3) supports the conclusion that the increase in the rate of modification depends on the formation of covalent enzyme-substrate intermediates.

The modification of 1 sulfhydryl group per subunit does not alter the enzymatic activity of either the chicken or the pig enzyme. The incorporation of 1 mol Nbs per mol of subunit was verified by the absorption spectrum of the modified enzyme (cf. 1) and by the release of 1 mol Nbs per mol of subunit on addition of 100 mM 2-mercaptoethanol.

DISCUSSION. The reactions of the mitochondrial isoenzymes of aspartate aminotransferase with Nbs_2 are similar though not identical in all respects to the corresponding reaction of the cytosolic isoenzyme from pig heart reported previously (1). Again, the susceptibility of one particular sulfhydryl group toward chemical modification changes synchronously with catalysis from one functional form of the enzyme to the other, maximum reactivity being reached in the covalent enzyme substrate intermediates (cf. Scheme). However, the syncatalytically modifiable sulfhydryl group of the mitochondrial isoenzyme in the pyridoxal form reacts 2 orders of magnitude faster with Nbs_2 than that of the cytosolic isoenzyme. Its maximum reactivity is attained in the presence of the substrate analogs α-methylaspartate and β-hydroxyaspartate (Table I) whereas in the cytosolic iso-

Table I

Reactivity of the titratable thiol group of mitochondrial aspartate aminotransferase in different functional states of the enzyme.
The rates of modification with Nbs_2 are taken from experiments corresponding to those of Fig. 1.

Conditions for modification	Relative rate of modification	
	Chicken enzyme	Pig enzyme
Pyridoxal enzyme		
No substrate	1.0[a]	1.0[a]
+ α-Ketoglutarate (6 mM)	1.6	1.0
+ α-Ketoglutarate (4 mM) + glutamate (55 mM)	4.6	3.0
+ Oxalacetate (0.4 mM) + aspartate (11 mM)	8.7	11.9
+ α-Methylaspartate (35 mM)	10.5	11.8
+ β-Hydroxyaspartate (20 mM)	13.3	13.2
Pyridoxamine enzyme		
+ Glutamate (55 mM)	2.3	1.8
+ Aspartate (11 mM)	2.8	1.6
No substrate	1.9	1.5

[a]The absolute values of the second order rate constants are 20 M^{-1} min^{-1} for the chicken heart and 23 M^{-1} min^{-1} for the pig heart enzyme.

enzyme the substrate pair is most effective in promoting the susceptibility toward modification (1,12). Further, the isoenzymes differ with regard to the effect of the syncatalytic modification on enzymatic activity. Nbs_2 does not affect the enzymatic activity of mitochondrial aspartate aminotransferases while it reduces that of the cytosolic isoenzyme to ~3 per cent of its initial value (1).

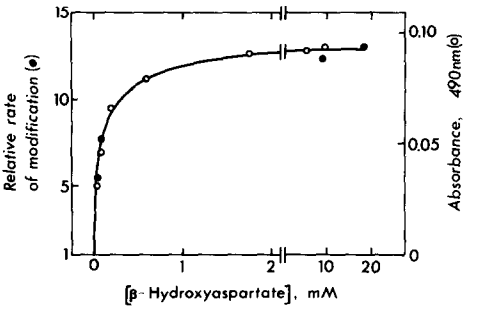


Fig. 3 Rate of modification with Nbs_2 and concentration of the semiquinoide enzyme β-hydroxyaspartate intermediate as a function of the β-hydroxyaspartate concentration. The rates of modification of the chicken heart enzyme were determined under the conditions of Fig. 1. The concentration of the semiquinoide enzyme β-hydroxyaspartate intermediate was measured spectrophotometrically at 490 nm (11).

These differences between the syncatalytic modification of the isoenzymes would appear to be due to a different microenvironment and/or structural relationship of the modified sulfhydryl group to the active site. In the mitochondrial isoenzymes both from pig and chicken heart the syncatalytically modifiable group is the only reactive sulfhydryl group of the enzyme; the residual groups, 4 in the porcine and 6 in the avian enzyme, are not modified with Nbs_2 under the conditions employed. In the cytosolic isoenzyme from pig heart 3 thiol groups out of a total of 5 per subunit react with Nbs_2 under non-denaturing conditions, the syncatalytically modifiable group being the least reactive of the three (1,13). Clearly, the identification of homologies of particular sulfhydryl groups in the cytosolic and mitochondrial isoenzymes will require the determination of their position in the primary structure (cf. 1,14).

The failure of the charged and relatively bulky Nbs substituent to impair the catalytic activity of the mitochondrial isoenzymes corresponds with previous data on the cytosolic isoenzyme which also showed that the syncatalytically modifiable cysteinyl residue is not part of the enzyme's active site (1,15). Hence, the syncatalytic changes in reactivity of this residue are not due to a direct involvement in the catalytic processes at the active site but rather to conformational alterations of the enzyme-coenzyme-substrate compound concomitant with the transamination reaction. The present results demonstrate that such syncatalytic conformational changes are not an isolated phenomenon in the

cytosolic aspartate aminotransferase from pig heart but may represent an integral feature of the mechanism of action of transaminases in general.

ACKNOWLEDGMENT. We thank Katy Brunschweiler for her technical and Genia de Vallier for her secretarial assistance. This work was supported by SNSF-grant 3.1570.73.

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