CONSPICUOUS DEGREE OF HOMOLOGY BETWEEN THE MITOCHONDRIAL ASPARTATE AMINOTRANSFERASES FROM CHICKEN AND PIG HEART

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SUMMARY. The sequence of 40 amino acid residues at the amino terminus of mitochondrial aspartate aminotransferase from chicken heart differs in only 2 positions from the sequence of mitochondrial aminotransferase of pig heart. Close structural similarity had been suggested by previous data on syncatalytic sulfhydryl modifications (Gehring H., and Christen P. (1975) Biochem. Biophys. Res. Commun. 63, 441-447). The cytosolic aspartate aminotransferases from the same two species have now been found to differ considerably in the mode of their syncatalytic modifications. The data suggest that the cytosolic and mitochondrial aspartate aminotransferases might have evolved at different organelle-specific rates.

Two distinct isoenzymes of aspartate aminotransferase, one located in the cytosol and one in the mitochondria, have been found in animal cells and possibly occur in all eucaryotic cells (for review cf. 1). Both the cytosolic and the mitochondrial isoenzyme are generally assumed to be coded by nuclear DNA (2). The total amino acid sequence of the cytosolic aminotransferase from pig heart has been reported (3, 4). The partial sequence data presently available on the mitochondrial enzyme from the same source (5, 6, 7) indicate a considerable degree of homology between these two isoenzymes.

The present study was prompted by the results of a comparison of the syncatalytic modifications of the mitochondrial isoenzymes from pig heart and from chicken heart. In both enzymes one sulfhydryl group showed nearly identical reactivity changes in

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Abbreviations: Nbs_2, 5,5'-dithiobis-(2-nitrobenzoate); Nbs, 5-thio (2-nitrobenzoate); TNM, tetranitromethane; NEM, N-ethylmaleimide.

the presence of substrates or substrate analogs (8). This finding was unexpected since syncatalytic modifications of the cytosolic isoenzymes from the same two species had indicated considerable differences (9, 10). Therefore, we initiated an examination of the degree of similarity between the two mitochondrial enzymes and between the two cytosolic enzymes.

MATERIALS AND METHODS. The cytosolic and mitochondrial isoenzyme of aspartate aminotransferase were isolated from chicken heart according to previously described methods (11, 12). Protein concentrations were determined spectrophotometrically using a molar absorptivity of the subunit $\epsilon_{280} = 7.0 \times 10^4$ M^{-1} cm^{-1}. The specific activity of the mitochondrial chicken enzyme at 25° was 140 U/mg, and that of cytosolic chicken enzyme was 220 U/mg. The preparations were homogenous as judged by starch gel electrophoresis (pH 8.7), 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 (9). L-Glutamic acid, 2-ketoglutaric acid, NEM, TNM, and Nbs_2 were obtained from Fluka. CNBr was from Pierce. The thiol group content of the enzymes was determined according to the procedure of Ellman (13) 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 ϵ_{412} = 13,600 M^{-1} cm^{-1} (9).

Automatic protein sequencing was performed with a Beckman Sequencer model 890 B (updated) using the dimethylbenzylamine buffer system (14) and the Beckman peptide program (111374). Propanol and dimethylbenzylamine were purchased from Pierce; all other chemicals used in the sequencer were purchased from Beckman. Per run 240 nmol of protein (subunit concentration) were applied. The repetitive yields in all experiments were 92 - 95%. Direct identification of the phenylthiohydantoin amino acid derivatives was carried out by gas chromatography on a Beckman gas chromatograph, Model GC-65, equipped with 10% SP-400 columns. Indirect identification of the derivatives was made by amino acid analyses of the free amino acids following HI hydrolysis (15). Amino acid analyses were performed on a Durrum D-500 analyzer.

RESULTS AND DISCUSSION. The sequence of the 40 amino terminal residues of mitochondrial aspartate aminotransferase from chicken heart is shown in Figure 1. Just two residues, Ser^5 and Ala^{28}, were found to differ from the sequence of the mitochondrial enzyme from pig heart reported previously (5). The substitutions correspond to single base changes. Further possible differences might exist in the amidation of the Asx residues in positions 32

Fig. 1 The Amino-Terminal Sequence of Mitochondrial Aspartate Aminotransferase from Chicken Heart.

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                                        5                   10
m-chicken            Ser-Ser-Trp-Trp-Ser-His-Val-Glu-Met-Gly-
m-pig                                   Ala
c-pig        Ala Pro Pro     Val Phe Ala Glu     Pro Gln Ala

                                       15                   20
m-chicken            Pro-Pro-Asp-Pro-Ile-Leu-Gly-Val-Thr-Glu-
m-pig
c-pig                Gln     Val Leu Val Phe Lys Leu Ile Ala

                                       25                   30
m-chicken            Ala-Phe-Lys-Arg-Asp-Thr-Asn-Ala-Lys-Lys-
m-pig                                           Ser
c-pig                Asp     Arg Glu     Pro Asp Pro Arg

                                       35                   40
m-chicken            Met-Asn-Leu-Gly-Val-Gly-Ala-Tyr-Arg-Asx-
m-pig                    Asx                                Asp
c-pig                Val                                    Thr
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Enzyme, carboxymethylated according to Hirs (16), was subjected to automatic sequence degradation and the phenylthiohydantoin derivatives were identified as described in Methods. The results represent a composite of 3 degradations performed on 3 different enzyme preparations. For comparison, residues which differ in the mitochondrial (5) and the cytosolic (3, 4) enzymes from pig are given on the second and third lines, respectively.

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and 40. The degree of inter-species homology between the mitochondrial enzymes clearly exceeds the intra-species homology found previously between the cytoplasmic and mitochondrial isoenzymes from pig heart (5, 6, 7). The occurrence of nearly identical N-terminal amino acid sequences in the two mitochondrial enzymes is remarkable since comparison of the sequences of the mitochondrial and the cytosolic enzyme from pig heart has revealed a degree of homology in this part of the polypeptide chain which is markedly below average when compared with the rest of the protein (5, 6). In the syncatalytic modifications reported previously one sulfhydryl group shows virtually identical reactivity changes in both mitochondrial enzymes in all their different functional states, i.e. in the presence or absence of different substrates or substrate analogs (8). Recent

Table I Comparison of the Syncatalytic Reactions of Cytosolic Aspartate Aminotransferase from Chicken Heart with Nbs_2, NEM, and TNM

Reagent	Enzymatic activity (Per cent of initial value) -Substrate	+Substrate[a]
Nbs_2[b]	100	100
NEM[c]	86	55
TNM[d]	90	65

[a] In the presence of 70 mM glutamate plus 2 mM 2-ketoglutarate.

[b] Pyridoxal form of the enzyme (0.016 mM subunit concentration) in 1.0 mM Nbs_2 — 50 mM sodium phosphate (pH 7.5, 25°, 2 hours).

[c] Pyridoxal form of the enzyme (0.01 mM subunit concentration) in 50 mM NEM — 100 mM sodium phosphate (pH 7.5, 25°, 3 hours).

[d] Data from (10).

experimental evidence has shown that the syncatalytic reactivity changes reflect spatially and temporally coordinated conformational alterations involving several if not many different groups of the enzyme-coenzyme-substrate system (K. Pfister, and P. Christen, in preparation). According to these findings close similarity with respect to syncatalytic modifications would in fact presuppose a close structural similarity such as indicated by the present sequence data.

Sequence comparisons with the cytosolic isoenzyme from chicken heart were not possible since both the carboxymethylated and the

oxidized enzyme resisted Edman degradation. Similarly, the largest CNBr fragment of the cytosolic chicken enzyme, the fragment possibly corresponding to residues 1 - 212 of the cytosolic enzyme from pig heart (17), could not be degraded. The comparison between the structures of the two cytosolic enzymes is to date confined to a comparison of the properties of their sulfhydryl groups and the mode of their syncatalytic modification. In the native cytosolic pig heart enzyme 3 sulfhydryl groups are susceptible toward chemical modification. One of these thiol groups (Cys^{390}(17)) exhibits marked syncatalytic reactivity changes and its modification impairs enzymatic activity (9). The cytosolic chicken enzyme, however, possesses only 1 sulfhydryl group reactive toward Nbs_2. Its reactivity is not changed syncatalytically (second order rate constant with Nbs_2 $k' = 730\ M^{-1}\ min^{-1}$ in 50 mM sodium phosphate at pH 7.5, 25°) and its chemical modification with Nbs_2 does not affect the enzymatic activity (Table I). However, syncatalytic inactivation does occur with NEM and TNM (Table I). Apparently, the inactivation is due to the modification of additional residues, e.g. tyrosyl residues have been found to be nitrated in the syncatalytic modification with TNM (cf. 10).

The differences between the cytosolic enzymes with respect to syncatalytic modification possibly reflect a variance in structure greater than that between the two mitochondrial enzymes. The type of constraints operative on the structure of mitochondrial aspartate aminotransferase is open to conjecture; they might relate to the uptake of the protein from its place of synthesis into the mitochondrial matrix. Studies currently in progress in this laboratory aim at delineating the probable difference in the rate of evolution of the cytosolic and the mitochondrial aspartate aminotransferases.

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