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Structural comparison between the mitochondrial aralkyl-CoA and arylacetyl-CoA *N*-acyltransferases

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The aralkyl and arylacetyl transferases were purified to homogeneity from bovine kidney by a slight modification of a previous procedure. The M_r of the arylacetyl transferase was estimated to be 33500 by SDS/PAGE and that of the aralkyl transferase to be 33750 by a combination of SDS/PAGE and gel-filtration analysis. *N*-Terminal-sequence analysis indicated a blocked *N*-terminus for the arylacetyl transferase and gave the following sequence for the aralkyl transferase: M-F-L-L-Q-G-A-Q-M-L-Q-M-L-E-K. Amino acid analysis revealed differences in composition between the two enzymes. Most notable was the fact that the aralkyl transferase had more methionine and leucine. This difference could be partially accounted for by assuming that the methionine-and-leucine-rich *N*-terminus was missing from the arylacetyl transferase. Chemical cleavage of the two enzymes at methionine residues using CNBr gave rise to several peptides for each enzyme. *N*-Terminal-sequence analysis of the 8000-M_r peptide from the arylacetyl transferase gave a sequence with 69% similarity to the 9000-M_r peptide from the aralkyl transferase. This was taken to indicate a common origin for the two enzymes.

INTRODUCTION

Many carboxylic acid xenobiotics are conjugated with an amino acid, primarily glycine, before excretion. Prominent compounds that are conjugated in this way are benzoic acid, salicylic acid, valproic acid and phenylacetic acid. Amino acid conjugations occur in both kidney and liver, and the enzyme systems catalysing these reactions are located in the matrix space of mitochondria [1,2]. Amino acid conjugation involves a two-step pathway in which the carboxylic acid is first activated to the CoA thioester by the action of a ligase; this acyl group is then transferred to the amino group of glycine (or another amino acid) by the action of an *N*-acyltransferase.

Two distinct *N*-acyltransferases have been isolated and characterized from bovine and primate liver mitochondria [3–6]. The predominant transferase has glycine-conjugating activity towards the CoA thioesters of substituted benzoic acids, including salicylic acid, and was originally termed a benzoyltransferase. However, the enzyme also catalyses the glycination of medium-chain-length fatty-acyl-CoAs and is now referred to as aralkyl-CoA:glycine *N*-acyltransferase [6] or in shortened form as the aralkyl transferase. The other transferase catalyses the conjugation of arylacetic acids with glycine, glutamine or arginine (depending on the species) and does not have activity towards any alkyl-CoA. This enzyme is an arylacetyl-CoA:amino acid *N*-acyltransferase and is referred to in shortened form as arylacetyl transferase.

The two transferases are thought to be quite similar, since they catalyse similar reactions and have similar M_r values [3–6]. However, there is no structural information available to serve as a basis for this supposition. We have therefore undertaken the structural comparison of these two enzymes. We use kidney as the source of the two enzymes because, unlike the liver where the level of arylacetyl transferase is low [6], kidney has about an equal titre of both enzymes [7].

MATERIALS AND METHODS

Materials

Benzoyl-CoA, phenylacetyl-CoA, leupeptin, chymostatin and CNBr were obtained from Sigma, St. Louis, MO, U.S.A. Sequenal-grade trifluoroacetic acid was obtained from Pierce, Rockford, IL, U.S.A. Westran blotting membrane was obtained from Schleicher and Schuell, Keene, NH, U.S.A.

Purification of transferases

The aralkyltransferase and arylacetyltransferase were both purified from bovine kidney mitochondria. Kidneys were taken immediately upon removal from the animal and cut into sections to increase the rate of cooling, placed in bags, and packed in ice for the 45 min trip to the laboratory. The kidneys were then homogenized in a medium containing 0.5 μM each of the proteinase inhibitors chymostatin and leupeptin, and the mitochondrial fraction was isolated by centrifugation [6]. Mitochondria were submitted to several freeze–thaw cycles, and a particle-free fraction was obtained by ultracentrifugation. The purification of the two transferases was carried out as previously described for liver mitochondria [6], i.e., DEAE-cellulose chromatography, Bio-Gel P100 gel-filtration chromatography, chromatofocusing chromatography and a second Bio-Gel P100 step. The only change was in the initial DEAE-cellulose column-chromatography step, where the gradient used for elution was 0–0.1 M-KCl.

Preparation of CNBr peptides

The cleavage of the transferases at methionine residues was accomplished by the CNBr method [8]. The enzymes were first dialysed exhaustively against 50 mM-$(NH_4)_2SO_4$ and then freeze-dried. Approx. 10 μg of protein in 70% trifluoroacetic acid was placed in a stoppered acid-washed glass tube along with 2–5 μg of CNBr and allowed to react at 30 °C for 20 h. The reaction

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mixture was then diluted with 10 vol. of water and freeze-dried. The resulting peptides were separated by SDS/PAGE using the gel system for peptides developed by Schagger & von Jagow [9]. The separated peptides were then electroblotted on to Westran blotting membrane, from which they could be cut out and sequenced directly off of the membrane.

Amino acid analysis

Amino acid analysis of the transferases was conducted on the Beckman model-6300 amino acid analyser. The purified enzymes were dialysed exhaustively against 0.1 M-$(NH_4)_2SO_4$ and then freeze-dried in acid-washed pyrolysed tubes. Half of each sample was treated with performic acid by Moore's procedure [10] to allow for quantification of cysteine and methionine. All samples were then hydrolysed in 6 M-HCl under vacuum at 110 °C for 24 h. The samples were then blown dry and dissolved in formic acid for analysis.

Sequencing

N-Terminal sequencing of the transferases and the CNBr-generated peptides was conducted by using Edman-degradation chemistry on an Applied Biosystems 470A gas-phase sequenator. Both the transferases and the peptides were sequenced directly from Westran membrane after SDS/PAGE and blotting.

RESULTS AND DISCUSSION

The lysate of bovine kidney mitochondria routinely contains a higher specific activity of arylacetyl transferase as compared with liver lysate, and kidney has an equivalent aralkyl transferase activity [7]. Thus the kidney serves as a better source of the two transferases. The purified kidney arylacetyl transferase was eluted from a chromatofocusing column at a pH 7.46, whereas the aralkyl transferase eluted at pH values of 7.57–7.63.

The M_r of the arylacetyl transferase as determined by SDS/PAGE was 33500; that obtained by gel filtration was decidedly lower (32000) but, as discussed previously for the liver enzyme [6], this value is likely to be unreliable, owing to the tendency of this enzyme to bind to gel-filtration supports. The M_r of the aralkyl transferase from kidney was 33500–33750 by SDS/PAGE and 34000 by gel-filtration analysis on Bio-Gel P-100. These data are consistent with those of Nandi *et al.* [4], which indicates that the aralkyl transferase is slightly larger than the arylacetyl transferase by SDS/PAGE.

The two purified transferases were subjected to *N*-terminal sequence analysis. The *N*-terminal sequence obtained for the aralkyl transferase was:

Met-Phe-Leu-Leu-Gln-Gly-Ala-Gln-Met-Leu-Gln-Met-Leu-Glu-Lys

No minor sequences were obtained. *N*-Terminal-sequence analysis of the arylacetyl transferase gave no sequence, indicating that the *N*-terminus is blocked.

The purified transferases were subjected to amino acid analysis. Performic acid oxidation was used to quantify the cysteine and methionine residues. The results are shown in Table 1. The amino acid compositions are clearly distinct for the two transferases. It is noteworthy that the aralkyl transferase has six more methionine residues, nine more leucine residues and five more lysine residues. This is of interest, because in the first 15 residues of the *N*-terminus of the aralkyl transferase there are four methionine residues, three leucines and one lysine residue. A great deal of the difference in amino acid composition between the two enzymes could be eliminated by hypothesizing that the arylacetyl transferase has had this sequence deleted. The fact that the *N*-terminus is blocked on the arylacetyl transferase clearly indicates that the *N*-termini are different and is consistent with this hypothesis.

The two enzymes were next subjected to chemical cleavage in order to generate a number of peptides that could be sequenced. CNBr cleavage at methionine residues was used. The cleavage patterns of the two enzymes might be expected to be considerably different because of the large number of methionine residues in the aralkyl transferase. However, the *N*-terminal sequence of the aralkyl transferase reveals that several of these cleavages give rise to very small peptides which could not be detected in our system. In fact, the CNBr cleavage patterns had similarities (Fig. 1). On

Table 1. Amino acid composition of the aralkyl and arylacetyl transferases

	Composition			
	Aralkyl transferase		Arylacetyl transferase	
Amino acid	Mol %	No. of residues*	Mol %	No. of residues†
Asn/Asp	11.2	29	9.8	26
Thr	4.6	12	4.6	12
Ser	7.4	19	8.0	21
Gln/Glu	12.3	32	15.8	42
Gly	5.5	14	10.4	25
Ala	5.0	13	10.4	25
Val	6.4	17	4.9	13
Met	4.1	11	2.0	5
Ile	3.2	8	3.1	8
Leu	11.4	30	7.9	21
Tyr	2.7	7	2.5	7
Phe	4.1	11	3.1	8
Pro	6.4	17	4.8	13
Lys	7.0	18	5.0	13
His	3.0	8	3.1	8
Arg	3.4	9	3.1	8
Cys	1.8	5	1.4	4

* Based on an M_r of 33750 and ignoring tryptophan.
† Based on an M_r of 33500 and ignoring tryptophan.

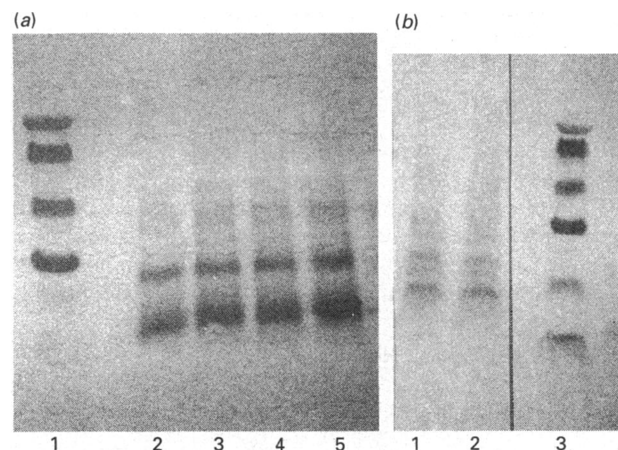

Fig. 1. CNBr cleavage patterns of the arylacetyl and aralkyl transferases

The aralkyl (*a*) and arylacetyl (*b*) transferases were cleaved with CNBr, subjected to SDS/PAGE, blotted on to Westran membranes and then stained and photographed. In (*a*), lanes 2, 3, 4 and 5 contain the CNBr digest of the aralkyltransferase and lane 1 contains the M_r standards (46000, 30000, 21500 and 14300). In (*b*) lanes 1 and 2 contain the CNBr digest of arylacetyl transferase and lane 3 contains M_r standards (46000, 30000, 21500, 14300, 6500 and 3400).

Table 2. *N*-Terminal sequences of the 9 kDa cyanogen bromide peptides obtained from the aralkyl and arylacetyl transferases

The aralkyl and arylacetyl transferases were cleaved by treatment with CNBr. The resulting mixture of peptides was separated by SDS/PAGE and electroblotted on to Westran membrane. The 8000–9000-M_r band from each enzyme was cut out and sequenced.

Aralkyl transferase		Arylacetyl transferase	
Sequence	Yield (pmol)	Sequence	Yield (pmol)
(M)		(M)	
K	25	A	108
D	21	D	95
D	28	D	170
L	24	F	90
D	16	D	100
H	5	H	27
Y	12	Y	50
T	5	T	38
N	8	N	40
T	6	T	50
Y	8	Y	40
H	3	Q	20
V	9	I	35
Y	8	Y	40
S	3	S	20
E	4	K	15
D	5	D	40
L	7	L	30
K	4	N	20
N	3	N	40
G	3	X	—
Q	<3	Q	6
E	<3	E	<3
F	3	S	<3
L	4	L	<3

SDS/PAGE both had bands in regions roughly corresponding to M_r values of 6000–7000, 8000–9000 and 13000. Furthermore, the sequence of the 9000-M_r band from the aralkyl transferase was extremely similar to the 8000-M_r band from the arylacetyl transferase (Table 2). The two sequences are 69 % similar, and all but one of the differences can be accounted for on the basis of a single-base-pair substitution. The sequences of the 13000-M_r bands were heterogeneous for both enzymes and the 6000–7000-M_r bands did not show any apparent similarity.

Looking at the yield of amino acid phenythiohydantoin derivatives for the sequences in Table 2, it is noteworthy that, for the arylacetyl transferase, there is a large decrease in yield after the residue 21 (residue X). This suggests that residue 21 may have a post-translational modification such as acylated serine or cysteine residue. It is also a possibility, though, that the decrease in yield occurred as a result of sequencing through a glycosylation site.

The data indicate clearly that the two transferases are quite distinct. They differ at the *N*-terminus, they have clearly distinct amino acid compositions, and chemical cleavage at methionine gives rise to peptides with unique sequences. However, the two enzymes are of approximately equivalent size and contain at least one region of apparent sequence identity. Thus it seems likely that they arose from a common vestigial gene.

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