SUBCELLULAR LOCALIZATION AND TISSUE DISTRIBUTION OF α-KETOADIPATE REDUCTION AND OXIDATION IN THE RAT

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Summary: Studies using subcellular fractions of rat liver have revealed that α-ketoadipate dehydrogenase is principally localized in the mitochondrial fraction and that α-ketoadipate reductase is principally localized in the cell supernatant fraction. Both enzymes have the highest activity in heart muscle. The ratio of the dehydrogenase activity to the reductase activity is highest in kidney followed by heart, brain, liver, leg muscle, and small intestine. This pattern is consistent with the relative mitochondrial density of the various tissues tested, and suggests that the reductase has a dominant role in α-ketoadipate metabolism in tissues that have a low mitochondrial density.

INTRODUCTION

The degradative route of lysine in mammals has been shown to join that of tryptophan at α-ketoadipate. α-Ketoadipate is then believed to undergo oxidative decarboxylation (1,2,3):

$$\alpha\text{-Ketoadipate} + NAD^+ + CoA \rightarrow \text{glutaryl-CoA} + CO_2 + NADH + H^+$$

The enzyme catalyzing this reaction is only tentatively referred to as α-ketoadipate dehydrogenase because a purified α-ketoglutarate dehydrogenase complex has also been reported to catalyze this reaction (3,4,5). We have recently described the purification and the characteristics of an enzyme, designated α-ketoadipate reductase, that specifically catalyzed the following reaction (6):

$$\alpha\text{-Ketoadipate} + NADH + H^+ \rightarrow \alpha\text{-hydroxyadipate} + NAD^+$$

An individual with a defect in the decarboxylation of α-ketoadipate has been reported to have increased urinary excretion not only of α-ketoadipate but also of α-hydroxyadipate (7,8); this suggests the possibility of an alternate route of α-ketoadipate degradation.

In this report the activities of these two enzymes have been compared in various organ samples of rat and in subcellular fractions of rat liver.

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MATERIALS AND METHODS

Enzyme source Male Sprague-Dawley rats weighing approximately 100 g were used for all experiments. The organs under study were homogenized in 9 vol. of 0.25 M sucrose solution with a Teflon homogenizer. The liver homogenate was fractionated by differential centrifugation (9). Each sample was sonicated in 0.25 M sucrose for 2 min using a Raytheon magnetostrictive osillator, model DF 101.

Enzyme assay α-Ketoadipate reductase was assayed spectrophotometrically by following the oxidation of NADH at 37°C. Each sample was incubated for 5 min in a medium (0.3 ml) containing the following: 2 μmole of KCN; 24 ng Lubrol WX; and 50 mM maleate buffer, pH 6.3. To this was added 0.24 ml of a solution containing 0.06 μmole of NADH and 60 mM maleate buffer, pH 6.3. The reaction was initiated by the addition of 0.06 ml of 0.1 M α-ketoadipate. α-Ketoadipate was omitted from the control cuvette. This concentration of Lubrol WX had no effect on the α-ketoadipate reductase activity and served to eliminate turbidity in the spectrophotometric assay.

α-Ketoadipate dehydrogenase was assayed by measuring the formation of $^{14}CO_2$ with α-[1-^{14}C]ketoadipate as substrate (10). α-[1-^{14}C]Ketoadipate was prepared as described previously (6). The reaction mixture had a final volume of 1.0 ml and contained 5 mM α-[1-^{14}C]ketoadipate (0.12 μCi), 0.8 mM NAD, 0.8 mM CoA, 0.8 mM thiamine pyrophosphate, 0.8 mM $CaCl_2$, 0.8 mM Na_2CO_3, 5 mM cysteine, and 75 mM potassium phosphate buffer, pH 7.2. Calcium ion was reported to stimulate α-ketoglutarate dehydrogenase more strongly than Mg^{2+} (5,11). The activity of α-ketoadipate dehydrogenase was also found to increase by 30-40% when Ca^{2+} was used instead of Mg^{2+}.

α-Ketoglutarate dehydrogenase was determined as described previously (5) with the following exceptions: α-ketoglutarate was omitted from the control incubation, and the liver homogenate and its supernatant fraction were dialyzed against 0.25 M sucrose solution. This was necessary to reduce background ferrocyanide formation.

The cytochrome C oxidase assay was identical to that described previously (12). Protein content was determined by the method of Lowry *et al.* (13).

RESULTS

As shown in Table I, the α-ketoadipate reductase was mainly found in the supernatant fraction of liver, whereas the α-ketoadipate dehydrogenase was principally associated with the mitochondrial fraction. Most of the cytochrome C oxidase and α-ketoglutarate dehydrogenase was recovered in the mitochondrial fraction. The sum of the activities of each of these enzyme in the various subcellular fractions indicated essentially quantitative recovery (Table I).

It was also found that the assay of α-ketoadipate reductase activity in the supernatant fraction did not require the presence of KCN or Lubrol WX. Reaction stoichiometry is shown in Table II.

Table I Subcellular Localization of α-Ketoadipate Reductase and α-Ketoadipate Dehydrogenase in Rat Liver

Fraction	Protein	Cytochrome C oxidase		α-Ketoglutarate dehydrogenase		α-Ketoadipate reductase		α-Ketoadipate dehydrogenase	
	(mg/ml)	Activity	%	Activity	%	Activity	%	Activity	%
Homogenate	16.6	84	100	0.44	100	0.784	100	0.245	100
Nuclei	2.2	10	12	0.09	20	0.053	7	0.059	24
Mitochondria	3.0	62	24	0.32	73	0.041	5	0.189	77
Microsomes	4.1	6	7	0.02	5	0.096	12	0.007	3
Supernatant	6.6	0	0	0.03	7	0.586	75	0	0
(Recovery)	15.9	78	93	0.46	105	0.776	99	0.255	104

The assay method for each enzyme is given in MATERIALS AND METHODS. Each enzyme activity is expressed as μmole/min/g of fresh liver. Percentage values are calculated on the basis of the homogenate activity. The data are average values of 2 or 3 experiments.

Table II

Stoichiometry of the α-Ketoadipate Reductase Reaction in the Rat Liver Supernatant Fraction

Incubation time (hours)	Total NADH added (nmole)	NADH utilized (nmole)	α-Hydroxyadipate produced (nmole)	α-Ketoadipate remaining (nmole)
2.5	120	91	89	492
7	210	152	151	419

A 150-μl mixture containing 4 mM α-[1-^{14}C]ketoadipate (1.5 μCi), 0.2 mM NADH, 0.05 M maleate buffer, and 30 μl of the supernatant fraction of rat liver was incubated at 37°C. Since high concentration of NADH inhibited the enzyme, NADH was added in 30 μmole increments as the reaction rate approached zero. After incubation α-hydroxyadipate and α-ketoadipate were separated for quantitation on an AG1-X8 column as described previously (6). NADH utilization was determined by following the absorption change at 340 nm.

Table III shows the tissue-distribution pattern of the two enzymes tested. Both enzymes have the highest activity in heart muscle. The ratio of the dehydrogenase activity to the reductase activity is highest in kidney followed by heart, brain, liver, leg muscle, and small intestine.

DISCUSSION

Two enzymes active in α-ketoadipate degradation are here shown to be localized in different cellular compartments. An interesting relationship is found when α-ketoadipate reductase activity and α-ketoadipate dehydrogenase activity are compared in the various tissues. The ratio of the dehydrogenase activity to reductase activity varies with the relative mitochondrial density of the tissue; that is, in tissues where there exist few mitochondria, such as mammalian skeletal and smooth muscle, and where mitochondria have low densities of respiratory

Table III Tissue Distribution of α-Ketoadipate Reductase and α-Ketoadipate Dehydrogenase

Tissue	α-Ketoadipate reductase (A)	α-Ketoadipate dehydrogenase (B)	Ratio
	μmole/min/g of protein		(B)/(A)
Heart muscle	9.88 ± 0.71	7.69 ± 0.47	0.78
Kidney	4.69 ± 0.35	5.72 ± 0.24	1.22
Liver	4.59 ± 0.44	1.54 ± 0.09	0.34
Brain	4.60 ± 0.15	2.12 ± 0.22	0.46
Leg muscle	4.81 ± 0.20	1.20 ± 0.08	0.25
Small intestine	7.83 ± 0.26	0.53 ± 0.04	0.07

The assay conditions for both enzyme were as described in MATERIALS AND METHODS. Each result is expressed as the mean ± S.E.M. for 4 or 5 determinations. Both enzymes were also measured in lung, stomach, large intestine, and spleen and found to be low in activity.

assemblies and few cristae (relatively anaerobic tissues), such as liver (14-16), this ratio is smaller. Conversely, in tissues where cells contain abundant mitochondria with large numbers of respiratory assemblies and numerous cristae (relatively aerobic tissues), such as heart muscle and kidney (14-16), this ratio is larger. It is therefore probable that in tissues that are relatively aerobic the major route of α-ketoadipate degradation is through the conversion of α-ketoadipate to glutaryl-CoA as has been previously described. Tissues that are relatively anaerobic would then rely more on the conversion of α-ketoadipate to α-hydroxyadipate, and possibly other unidentified compounds, as a means of degrading this intermediate of lysine and tryptophan metabolism.

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