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Distribution of leucine 2,3-aminomutase activity in various organs of the rat and in subcellular organelles of rat liver

J. Michael Poston

Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health
Bethesda, Maryland 20205

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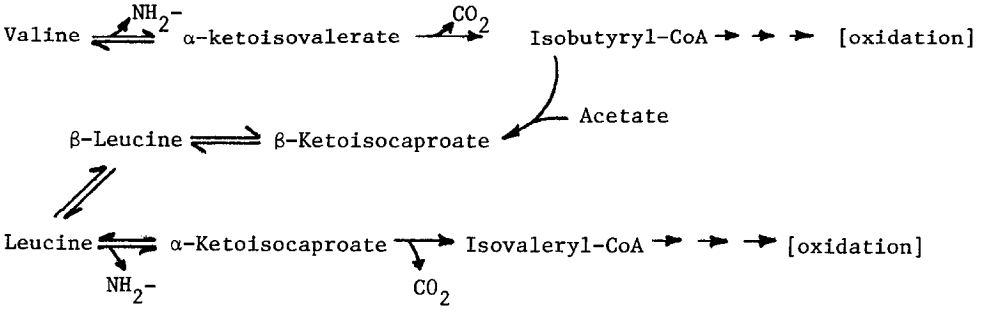
Summary: Leucine 2,3-aminomutase, the cobalamine-dependent enzyme involved in the conversion of β-leucine to leucine, has been shown to be widely distributed in rat organs. The activity is highest in skeletal and cardiac muscle, lower in brain, liver, and kidney, and very low in small intestine. In rat liver, the activity is nearly all in the cytosolic fraction.

The pathways of oxidation of isoleucine, leucine, and valine have the first two steps in common; the amino group is removed and the resulting α-keto acid undergoes oxidative decarboxylation. In addition, leucine has been shown to be synthesized from the catabolites of valine (1) as shown below.



Much of the catabolism of the branched-chain amino acids may take place in the liver, although it has been shown that the oxidation of leucine may also occur in extra-hepatic tissue (2,3,4). In the rat most of the aminotransferase

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activity is found in muscle tissue, whereas most of the activity catalyzing the oxidative decarboxylation, branched-chain α-keto acid dehydrogenase, is found in the liver (5,6).

The synthesis of leucine from valine catabolites has been demonstrated in rat liver and in human leukocytes and hair roots (1). Because these findings suggested that the synthetic pathway was more uniformly distributed than the oxidative activities, a survey of some of the organs of the rat was undertaken. Additionally, since nearly all enzyme preparations had been made in the presence of detergent, it was not known whether there was any subcellular compartmentation of the synthetic activities. Accordingly, the characteristic enzyme of the synthetic pathway, leucine 2,3-aminomutase, an enzyme dependent upon adenosylcobalamin, was assayed in six organs of the rat and in rat liver organelles.

MATERIALS AND METHODS

Rats, 250-300 g, were stunned and killed by exsanguination. The organs were removed, blotted, weighed, and placed in cold medium in an ice bath. For surveying the tissue distribution of leucine 2,3-aminomutase, the organs were suspended in a volume of 0.02 M potassium phosphate buffer, pH 7.0, containing 0.8 percent Triton X-100 equal to twice the tissue weight. The small intestine from just distal to the pylorus to just proximal to the cecum was freed of mesentery and slit longitudinally. The luminal contents were flushed away with 0.9 percent saline. The muscular tissues were minced with scissors and each of the tissues was homogenized in a Potter-Elvehjem teflon homogenizer and centrifuged at 30,000 x g for 30 minutes; the pellet was discarded.

For the preparation of subcellular organelles using the differential centrifugation method of Hogeboom (7), the liver was suspended in cold 0.25 M sucrose (9 ml per g of tissue). The pellets resulting from the differential centrifugation were suspended in the potassium phosphate buffer. The cytosolic fraction was concentrated by adding solid ammonium sulfate to achieve 70 percent saturation. The pellet was taken up in buffer and desalted by passage through a column of Sephadex G-25 equilibrated with the phosphate buffer.

Protein content of the tissue extracts and organelle preparations was measured by the method of Lowry *et al.* (8). The results were not affected by the content of Triton X-100.

Leucine 2,3-aminomutase was measured as described previously using 20 mM DL-β-leucine as the substrate and measuring the formation of leucine (9). When added, adenosylcobalamin was 5.8×10^{-8} M. Leucine was measured gas chromatographically on OV-11 as the trimethylsilyl derivative after the method of Gehrke and Leimer (10).

TABLE I

Leucine 2,3-aminomutase activity in various rat tissues

	Tissue	Leucine formed	
		No addition	Adenosylcobalamin added
		nmol/hr/mg protein	
1.	Brain	43	204
2.	Heart	185	421
3.	Kidney	42	76
4.	Liver	82	100
5.	Skeletal muscle	180	525
6.	Small intestine	159	62

RESULTS AND DISCUSSION

Table 1 shows a typical distribution of specific activity for leucine 2,3-aminomutase in rat organs. It is clear that each of the organs has enzymic activity. The activity in each of the organs, except for small intestine, was stimulated by the addition of exogenous adenosylcobalamin. As might be expected, the activity from the liver was stimulated the least, probably reflecting the relatively large stores of B_{12} factors in that organ. Interestingly, both cardiac and skeletal muscle have high specific activities and both show strong stimulation upon the addition of adenosylcobalamin. It seems reasonable to conclude that skeletal muscle, by virtue of the amount of the tissue in the animal, must play a significant role in the synthesis of leucine from β-leucine. The activity in the brain is the one most stimulated by the addition of adenosylcobalamin. This may reflect a relatively poor transport of the cobalamin across the blood-brain barrier. The activity in small intestine appears to be somewhat decreased by the addition of adenosylcobalamin. This decrease may not be

Table II

Distribution of leucine 2,3-aminomutase in subcellular organelles of rat liver

Subcellular fraction	Volume ml	Protein mg/ml	Activity[a] nmol/hr/mg	Total Activity nmol/hr
Nuclear	6.5	30.8	28.9	5780
Mitochondrial	4.9	18.0	2.5	221
Microsomal	5.5	22.2	10.8	1318
Cytosolic	11.3	12.4	290.0	40647

[a] Activity of leucine 2,3-aminomutase is expressed as nmol of leucine formed from DL-β-leucine per mg protein at 37°C. Reaction mixtures contained 5.8×10^{-8} M adenosylcobalamin.

significant since the organ in extremely rich in free amino acids. The change in concentration of leucine is only about 35 percent of the free leucine pool, whereas in liver it is approximately 700 percent. The values for the small intestine, in addition, represent those of the whole tissue, both smooth muscle and mucosa.

The distribution of activity shown in Table II is typical for subcellular organelles. There is some activity in the nuclear fraction. This fraction contains the nucleus, cellular debris, and unbroken cells. It is also contaminated, to a degree, with mitochondria, microsomal material, and cytosol. Consequently, it is not surprising to see activity in this fraction. Nonetheless, the activity is not high in this fraction and the nucleus can probably be discounted as a locus of the enzyme. The activity in the mitochondria is very low. The preparation of the mitochondria involved resuspension and centrifugation so that most contaminating cytosolic material should be removed. This low activity, therefore, may represent an intrinsic mitochondrial enzyme. The

microsomal fraction shows a somewhat higher activity than does the mitochondrial fraction, but this probably represents contamination with cytosolic enzyme. Because activity in the microsomal fraction is smaller than that in the cytosolic fraction by greater than an order of magnitude, the microsomal fraction also should be discounted as a locus for the enzyme. Because most of the activity of the leucine 2,3-aminomutase is found in the cytosolic fraction, it seems reasonable to conclude that the enzyme is soluble and not associated particularly with any membrane system.

The soluble nature of the enzyme is in marked contrast with the branched-chain α-keto acid dehydrogenase which is responsible for the oxidative decarboxylation step in the catabolism of the branched-chain amino acids. That enzyme is clearly associated with the mitochondrion (11). Odessey and Goldberg (11) reported that the first step in the catabolism is catalyzed by a soluble aminotransferase. These authors also note that the dehydrogenase can be found in muscle mitochondria and suggest that the reason others have not been able to demonstrate the enzyme in muscle stems from the vigorous methods others used in isolating the mitochondria and in the fact that the muscle enzyme has optimal conditions that are quite different from those for the liver enzyme.

The separation of the synthetic enzyme, leucine 2,3-aminomutase, from the probably source of the valine catabolites poses some problems of intracellular logistics. If this pathway is to function, isobutyrate or isobutyryl-CoA must either be transported out of the mitochondrion so that the condensation with acetate may occur to form β-ketoisocaproate in the cytosol or the condensation must occur within the mitochondrion and either the β-keto acid or the β-leucine must be transported to the cytosol where leucine 2,3-aminomutase can act upon it. In view of the apparent preference for transportation of amino acids by the mitochondrion, the probable sequence is the latter, viz. conversion of valine metabolites to β-amino acid to leucine in the cytosol.

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