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Biochemical studies on branched-chain oxoacid oxidases

The catabolism of valine, leucine, and isoleucine is of importance in relation to the genetically determined metabolic disorder "branched-chain oxoacid aciduria"(or "maple syrup urine disease"[1]), and the "isovaleric acidemia"[2]. The first-mentioned disease is caused by a mutative alteration of the genetic information for the synthesis of those enzymes which normally catalyze the oxidative decarboxylation of the α-oxoacids, derived from the branched-chain amino acids by transamination or by oxidative deamination[3,4]. Few data are available on the oxidative decarboxylation reactions of the branched-chain amino acids in mammals.

In children, homozygous for the mutated allele of the autosomal gene which normally controls the synthesis of these oxoacid oxidases, there is no enzyme activity in leucocytes; death will ensue after a short time if they are not treated immediately with a special diet[5,6] containing small amounts of valine, leucine and isoleucine. Heterozygotes and normal homozygotes can be differentiated by direct measurement of the activity of the oxoacid oxidases in white blood cells[7,8]. Until recently there has been no exact knowledge as to whether three distinct specific enzymes are responsible for the oxidative decarboxylations mentioned above, or if one enzyme catalyzes these reactions. Also, there are no available data in relation to their differentiation with respect to pyruvate and oxoglutarate oxidases.

TABLE I

SUBSTRATE SPECIFICITY AND K_i VALUES

Substrate	*mμmoles converted substrate per 10 mg protein*			*Substrate : α-oxoisocaproic acid*	
	Homogenate	*Enriched α-oxoisocaproic acid oxidase*	*Enriched α-oxoisovaleric acid oxidase*	*$10^5 \times K_i$ (M)*	*Mode of inhibition*
α-Oxoisocaproate	20	400	39	—	—
α-Oxoisovalerate	68	18	440	0.71	competitive
α-Oxo-β-methylvalerate	5	21	4	0.6	competitive
α-Oxo-*n*-valerate	—	—	—	1.0	competitive
α-Oxoglutarate	33	6	18	228.0	competitive
Oxalic acid acetate	—	—	—	66.0	competitive
α-Oxobutyrate	—	—	—	5.2	non-competitive
Pyruvate	2	5	5	17.0	non-competitive

Our studies on human leucocytes, leucocytes from cattle, and on cattle liver, demonstrate three specific proteins for the three different reactions; they are not identical with pyruvate and oxoglutarate oxidases. Table I presents inhibitor constants and the results of investigations on substrate specificity performed with homogenate, enriched α-oxoisocaproic acid oxidase, and enriched α-oxoisovaleric acid oxidase, using different substrates. The pH optimum of α-oxoisocaproic acid oxidase is at pH 6.3, and the Michaelis–Menten constant for α-oxoisocaproate is $2.1 \cdot 10^{-5}$ M.

The Michaelis–Menten constant of α-oxoisovaleric acid oxidase with α-oxoisovalerate is $1.0 \cdot 10^{-4}$ M; the pH optimum for this enzyme is at pH 7.4.

In order to measure the enzyme activity, the ^{14}C-labelled α-oxoacids were incubated with the enzymes. The $^{14}CO_2$ liberated was absorbed on filter paper soaked with KOH, and then quantitatively estimated in the liquid scintillation spectrometer. The oxoacids which are not available were prepared by incubation of 1-^{14}C-labelled amino acids with amino acid oxidase from snake venom and isolated by thin-layer chromatographic techniques, and ion-exchange column chromatography, respectively. Exact details on the applied methods, determination of the kinetics of the reactions, purification of the enzymes, and the characterization of the α-oxo-β-methyl-valeric acid oxidase, will be published elsewhere[9].

Results on the investigation of five families in which diminished activity or total lack of α-oxoacid oxidase activity (these children died) were found, have been published[10,11]. In the liver of a child who died from this disease, there was a highly reduced activity of all the three oxoacid oxidases in comparison to a control.

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1 J. MENKES, P. HURST AND J. CRAIG, *Pediatrics*, 14 (1954) 462.
2 K. TANAKA, M. A. BUDD, M. L. EFRON AND K. J. ISSELBACHER, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 236.
3 J. DANCIS, J. HUTZLER AND M. LEVITZ, *Biochim. Biophys. Acta*, 43 (1960) 342.
4 J. DANCIS, J. HUTZLER AND M. LEVITZ, *Biochim. Biophys. Acta*, 78 (1963) 85.
5 L. E. HOLT, JR., S. E. SNYDERMAN, J. DANCIS AND P. M. NORTON, *Federation Proc.*, 19 (1960) 10.
6 D. LONSDALE AND D. H. BARBER, *New Engl. J. Med.*, 271 (1964) 1338.
7 H. W. GOEDDE, E. RICHTER, C. STAHLMANN, B. SIXEL, *Klin. Wochschr.*, 41 (1963) 953.
8 H. W. GOEDDE, E. RICHTER, M. HÜFNER AND B. SIXEL, *Klin. Wochschr.*, 42 (1964) 818.
9 H. W. GOEDDE *Biochemical Genetics*, Plenum Publ. Corp., New York, N.Y., in preparation.
10 H. W. GOEDDE, E. RICHTER, M. HÜFNER AND A. V. Z. MÜHLEN, *Humangenetik*, 1 (1964) 163.
11 H. W. GOEDDE, F. MÖHLENBECK AND K. -G.BLUME, *Med. Klinik*, 61 (1966) 2063.

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Cofactor requirements of thymine 7-hydroxylase

In the conversion of thymine to the pyrimidines of RNA by Neurospora, 5-hydroxymethyluracil appears to be an intermediate[1,2]. Cell-free extracts of this mold have been found to contain an enzyme, thymine 7-hydroxylase, which effects the formation of 5-hydroxymethyluracil by catalyzing the hydroxylation of the methyl group of thymine[3]. Thymine 7-hydroxylase activity was demonstrable in these extracts when they were assayed in the presence of NADPH and GSH. The loss of