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Formation of 2-Oxoisovalerate Dehydrogenase in *Pseudomonas fluorescens*

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Decarboxylation of branched chain 2-oxo acids, 2-oxoisovalerate, 2-oxoisocaproate, and 2-oxo-3-methylvalerate has been detected in animal tissues,[1,2] and in *Pseudomonas aeruginosa*,[3] *Bacillus subtilis*,[4] and *Pseudomonas putida*.[5] 2-Oxoisovalerate dehydrogenase from *Bacillus subtilis* has been purified about 40-fold and it catalyses oxidative decarboxylation of all the three branched chain 2-oxo acids, thus yielding the related acyl coenzyme A esters.[4]

The enzyme is highly stereospecific and the L-isomer is the active substrate. Sulphhydryl reactants inhibit the activity of 2-oxoisovalerate dehydrogenase.[4] The enzyme was induced during growth on valine on *Pseudomonas putida*.[5]

The present investigation shows that 2-oxoisovalerate dehydrogenase is formed in the presence of valine, isoleucine, leucine, and 2-oxo acids derived from these amino acids in *Pseudomonas fluorescens* (strains P-2 and UK-1).

Materials. L-Amino acid oxidase and catalase were purchased from Calbiochem, Los Angeles, and 1-^{14}C-L-valine from the New England Nuclear Corporation, Boston. 1-^{14}C-2-Oxoisovalerate was prepared as described earlier by Meister[6] and purified by ion exchange chromatography.

Cultures. Pseudomonas fluorescens P-2 and UK-1 were used as test organisms. *Ps. fluorescens* P-2 was grown with aeration in the salt solution described by Goodhue and Snell[7] with 10 mM of various carbon sources. The strain UK-1 was cultured in the basal salt solution containing 0.817 g of KH_2PO_4, 0.247 g of $MgSO_4.7H_2O$, and 2.8 mg of $FeSO_4.7H_2O$ per litre.

Growth was estimated from turbidity measurements made with a Klett-Summerson colorimeter, employing filter 62. Cultures were grown as described elsewhere, with some modifications.[8]

Enzyme preparation and assay. The samples (about 4 mg dry weight) withdrawn from the

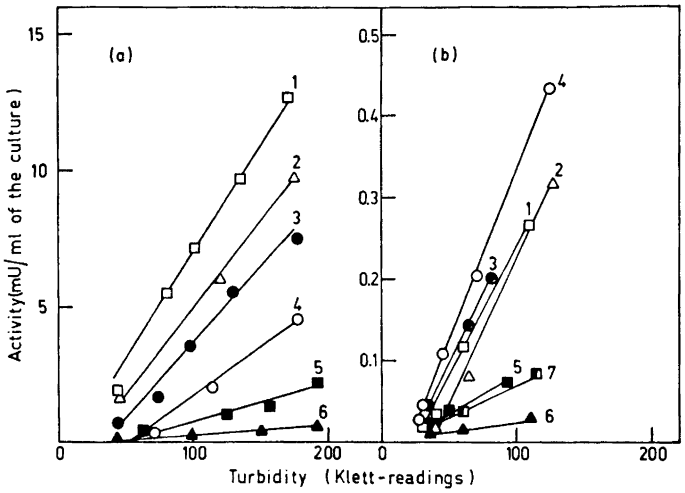

Fig. 1. The formation of 2-oxoisovalerate dehydrogenase on various carbon sources in *Ps. fluorescens*.
2-Oxoisovalerate dehydrogenase was released from the cells by freezing and thawing and by disintegrating them in an MSE sonic oscillator. Enzyme activity was determined by the method of Namba *et al.*[4]
A. Formation of 2-oxoisovalerate dehydrogenase in *Ps. fluorescens* P-2. 1 = leucine, 2 = isoleucine, 3 = 2-oxoisovalerate, 4 = 2-oxobutyrate, 5 = pantothenate, 6 = isobutyrate.
B. Formation of 2-oxoisovalerate dehydrogenase in *Ps. fluorescens* UK-1. 1 = leucine, 2 = isoleucine, 3 = 2-oxoisovalerate, 4 = valine, 5 = propionate, 6 = isobutyrate, 7 = 3-hydroxyisobutyrate.

cultures at intervals of 2 – 6 h were centrifuged at 5000 × *g* for 10 minutes and the cells were washed with 0.05 M phosphate buffer (pH 7.5). The enzyme was released from the cells by freezing and thawing and by disintegrating them in an MSE sonic oscillator.

2-Oxoisovalerate dehydrogenase activity was measured as described earlier by Namba *et al.*[4] Samples for radioactivity measurements were pipetted onto Whatman No. 1 paper. The papers were dried and radioactivity was estimated in a Wallac liquid scintillation spectrometer, with toluene-based scintillation fluid.

Results and discussion. As can be seen from Fig. 1, 2-oxoisovalerate dehydrogenase was formed most effectively by growth on leucine, isoleucine, and 2-oxoisovalerate in *Ps. fluorescens* P-2 and on valine, 2-oxoisovalerate, isoleucine, and leucine in *Ps. fluorescens* UK-1. Marshall and Sokatch [5] have reported induction of 2-oxoisovalerate dehydrogenase in *Ps. putida* by growth on valine, leucine, and isoleucine, too. They explained that the real inducers of 2-oxoisovalerate dehydrogenase were 2-oxo acids derived from the branched chain amino acids, because a mutant of *Ps. putida* which had lost the ability to grow on valine, leucine, or isoleucine formed 2-oxo acid dehydrogenase only in the presence of 2-oxoisovalerate, 2-oxoisocaproate, and 2-oxo-3-methylvalerate. In our experiments, too, the real inducers may be 2-oxo acids, because in *Ps. fluorescens* P-2 precultured on β-alanine, glutamate, or glucose, the generation times were much longer on valine than on 2-oxoisovalerate and the rise in 2-oxoisovalerate dehydrogenase activity followed the growth curves in both media. Furthermore, 2-oxobutyrate had an inducing effect on the enzyme, whereas butyrate and isobutyrate were almost completely inactive.

We have previously shown that a 2-oxo group is essential for the induction of L-valine: 2-oxoisovalerate aminotransferase, and that a branched carbon chain enhances the ability of the compound to serve as

inducer in *Ps. fluorescens*.[9] In the present investigation, too, the 2-oxo group may be essential for the induction of the enzyme, the length of carbon chain being probably less important in the induction process.

The strains of *Ps. fluorescens*, P-2 and UK-1, differ from each other in ability to utilize pantothenate. A diauxin of growth was observed in the strain UK-1 on pantothenate, because β-alanine was utilized very rapidly and pantoate after a lag of few hours. On the other hand,

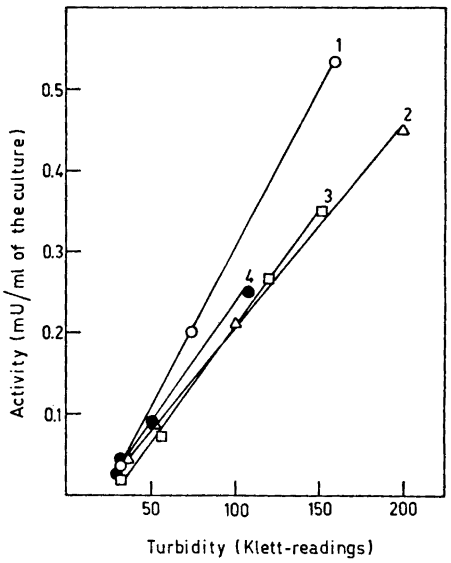

Fig. 2. The effect of mixtures of branched chain amino acids on the formation of 2-oxoisovalerate dehydrogenase in *Ps. fluorescens* UK-1. Other conditions were as described in the legend to Fig. 1.
1 = valine + isoleucine, 2 = leucine + isoleucine, 3 = valine + leucine, 4 = valine + leucine + isoleucine.

the generation times on valine were equal in the two strains, suggesting that the induction patterns of the utilization of valine closely resemble each other.

Fig. 2 shows the results of experiments in which the effects of the mixtures of branched chain amino acids were tested in the induction process in the strain UK-1. Although in *Salmonella typhimurium* and *E. coli* transaminase B was repressed in the presence of valine, leucine, and isoleucine,[10] no multivalent repression of 2-oxoisovalerate dehydrogenase was observed in *Ps. fluorescens*. The rates of enzyme synthesis were to some extent faster on valine + isoleucine, valine + leucine, and valine + isoleucine + leucine than on isoleucine + leucine.

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Reaction of 4,4-Diphenyl-2-aryl-1,3-oxathiolan-5-ones with Grignard Reagents

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The reaction of 1,3-oxathiolanones of type I with Grignard reagents presents an interesting problem because the reagent

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