Regulation of Valine Degradation in *Pseudomonas fluorescens* UK-1. Induction of Enoyl Coenzyme A Hydratase

MATTI PUUKKA

Department of Biochemistry, University of Turku, SF-20500 Turku 50, Finland

Recent studies concerning regulation of valine catabolism in *Pseudomonas* have shown that D-amino acid dehydrogenase, branched chain 2-oxo acid dehydrogenase, 3-hydroxyisobutyryl CoA hydrolase, 3-hydroxyisobutyrate dehydrogenase, and methylmalonate semialdehyde dehydrogenase are inducible enzymes.[1-5] In *P. fluorescens* branched chain amino acid transaminase is induced by branched chain amino acids and by branched chain 2-oxo acids,[1] but in *P. putida* and *P. aeruginosa* this transaminase seems to be a constitutive enzyme.[5] Furthermore, Marshall and Sokatch [5] have established that the catabolic pathway for valine in *P. putida* and *P. aeruginosa* is induced in three separate segments: (1) D-amino acid dehydrogenase, (2) branched chain 2-oxo acid dehydrogenase, and (3) 3-hydroxyisobutyrate dehydrogenase and methylmalonate semialdehyde dehydrogenase. The regulation of enoyl CoA hydratase (EC 4. 2. 1. 17) has not yet been studied.

The data presented in this paper demonstrate that enoyl CoA hydratase in *P. fluorescens* UK-1 is induced by growth on branched chain amino acids, branched chain 2-oxo acids, and branched short chain fatty acids.

Materials. Methacrylyl coenzyme A was synthesized by the method of Simon and Shemin as described by Stadtman,[6] and sodium DL-3-hydroxyisobutyrate was prepared as described elsewhere.[7] All other reagents were commercial preparations.

Organism and cultural conditions. Pseudomonas fluorescens strain UK-1 was grown in a mineral medium containing (per litre) KH_2PO_4, 0.82 g; $MgSO_4.7H_2O$, 0.25 g; $FeSO_4.7H_2O$, 2.8 mg. The pH was adjusted to pH 6.8 with potassium hydroxide. The concentration of the carbon source was 10 mM, and ammonium sulphate (10 mM) was used as nitrogen source. The organism was precultivated in a glutamate (20 mM) medium. The cultures were aerated with an aquarium pump and incubated at 30°. Growth was estimated from turbidity measurements in a Klett-Summerson colorimeter fitted with filter 62.

Preparation and assay of the enzyme. The samples (about 4 mg dry weight) were withdrawn and cell extracts were prepared as described earlier.[1] The enzyme activity was assayed in freshly prepared cell extracts. The rate of enzymatic hydration of methacrylyl CoA to 3-hydroxyisobutyryl CoA at 30° was determined spectrophotometrically at 268 nm according to Stern *et al.*[8] The reaction system contained 70 μmol of Tris-HCl buffer, pH 7.5, 0.06 μmol of methacrylyl CoA, and a cell extract containing 12.5–125 μg dry weight. The reaction was started by adding the cell extract. The total volume of the reaction mixture was 1.00 ml. An enzyme unit is defined as the amount of enzyme which catalyzes the hydration of 1 μmol/min of methacrylyl CoA to 3-hydroxyisobutyryl CoA at 30°.

Results and discussion. The level of enoyl CoA hydratase was low, about 0.02 unit per mg dry weight, when *P. fluorescens* UK-1 was precultured for two generations in glutamate medium. If the cells were then transferred to a medium containing valine or isoleucine, the specific activity of the enzyme began to increase at the same time as the growth of the bacteria. The enzyme was formed in the presence of 2-oxoisovalerate and 2-oxoisocaproate, too. In addition, branched short chain fatty acids isobutyrate and isovalerate caused a marked increase in the enzyme activity. Maximal specific activity was reached in the middle of the exponential phase (Table 1). Glucose, lactate, propionate, and 3-hydroxyisobutyrate have no inducing ability.

The specific activity of enoyl CoA hydratase in the cells of *P. fluorescens* UK-1 grown on several carbon sources is shown in Table 1. The specific activity of the enzyme increased 23-fold on isoleucine and 2-oxoisocaproate, and almost 18-fold on isovalerate. The increase in specific activity on valine, 2-oxoisovalerate, and isobutyrate was 7-, 9-, and 13-fold, respectively.

Fig. 1 shows the kinetics of induction of enoyl CoA hydratase in response to the different growth substrates. Isoleucine and isovalerate were the most powerful inducers among the carbon sources studied. Once induction had begun the differential rate of synthesis of enoyl CoA hydratase

Table 1. Level of enoyl CoA hydratase in *P. fluorescens* UK-1 grown on different carbon sources. Crude extracts were prepared from the cells of the exponential phase and enzyme activities were determined as described under *Preparation and assay of the enzyme.*

Carbon source for growth (10 mM)	Specific activity of enoyl CoA hydratase (mU/mg dry weight)
Isoleucine	450
2-Oxoisocaproate	450
Isovalerate	350
Isobutyrate	250
2-Oxoisovalerate	170
Valine	140
3-Hydroxyisobutyrate	60
Propionate	50
Glucose	40
Lactate	40
Glutamate	30

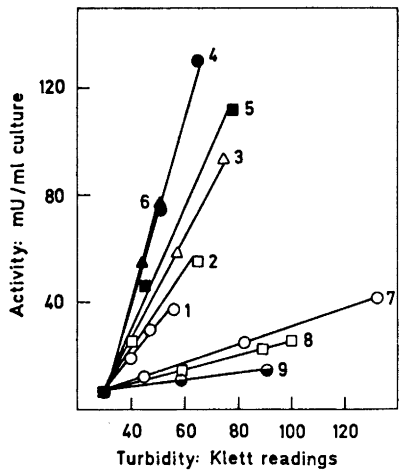

Fig. 1. Differential rate of formation of enoyl CoA hydratase during growth of *P. fluorescens* UK-1 on different carbon sources. The cells had been precultured in a glutamate medium. Samples were withdrawn from the cultures at intervals of 2–6 h, cell extracts were prepared and enzyme activities were determined as described under *Preparation and assay of the enzyme.*

1, Valine. 2, 2-Oxoisovalerate. 3, Isobutyrate. 4, Isoleucine. 5, 2-Oxoisocaproate. 6, Isovalerate. 7, 3-Hydroxyisobutyrate. 8, Propionate. 9, Glucose.

in response to the inducer remained constant.

These data indicate that in *P. fluorescens* UK-1 enoyl CoA hydratase is subject to regulatory control, and the results shown in Table 1 and Fig. 1 suggest that the enoyl CoA hydratase is induced by (growth on) branched chain amino acids, branched chain 2-oxo acids, and branched short chain fatty acids derived from branched chain amino acids, but in *Escherichia coli* enoyl CoA hydratase, which participates in β-oxidation of fatty acids, is induced only by long chain fatty acids.[9,10]

Acknowledgements. I express my sincere gratitude to Professor Veikko Nurmikko, Head of the Department of Biochemistry, for his continued interest in this work and critical reading of the manuscript.

1. Puukka, M., Lönnberg, H. and Nurmikko, V. *Acta Chem. Scand.* **26** (1972) 1271.
2. Puukka, M., Mäntsälä, P., Lönnberg, H., Pajula, R. and Nurmikko, V. *Acta Chem. Scand.* **26** (1972) 1299.
3. Nurmikko, V., Puukka, M. and Puukka, R. *Suomen Kemistilehti* **B 45** (1972) 193.
4. Puukka, M. and Nurmikko, V. *Suomen Kemistilehti* **B 45** (1972) 196.
5. Marshall, V. deP. and Sokatch, J. R. *J. Bacteriol.* **110** (1972) 1073.
6. Stadtman, E. R. In Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Academic, New York and London, 1957, Vol. III, p. 931.
7. Robinson, W. G. and Coon, M. J. In Colowick, S. P. and Kaplan. N. O. *Methods in Enzymology*, Academic, New York and London 1963, Vol. VI, p. 552.
8. Stern, J. R., del Campillo, A. and Raw, I. *J. Biol. Chem.* **218** (1956) 971.
9. Overath, P., Raufuss, E.-M., Stoffel, W. and Ecker, W. *Biochem. Biophys. Res. Commun.* **29** (1967) 28.
10. Weeks, G., Shapiro, M., Burns, R. O. and Wakil, S. J. *J. Bacteriol.* **97** (1969) 827.

Received January 15, 1973.