Regulation of Valine Degradation in *Pseudomonas fluorescens* UK-1. Induction of Methylmalonate Semialdehyde Dehydrogenase

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Methylmalonate semialdehyde dehydrogenase catalyzes the oxidation of methylmalonate semialdehyde and propionaldehyde to propionyl CoA in the presence of NAD and CoA. It is active when mercaptoethanol replaces CoA and an active propionyl derivative is then formed as well.[1] Methylmalonate semialdehyde dehydrogenase from *Pseudomonas aeruginosa* has been purified to a form homogeneous by the criterion of disc gel electrophoresis.[1]

The object of the present study was to investigate the induction of methylmalonate semialdehyde dehydrogenase during the growth of *P. fluorescens* UK-1. We have shown earlier that *P. fluorescens* UK-1, when grown with valine as the sole source of carbon and nitrogen, produces inducible branched chain amino acid transaminase,[2] branched chain 2-oxo acid dehydrogenase,[3] enoyl CoA hydratase,[4] 3-hydroxyisobutyryl CoA hydrolase,[5] and 3-hydroxyisobutyrate dehydrogenase.[6] This report shows that methylmalonate semialdehyde dehydrogenase is induced in the presence of valine, 2-oxoisovalerate, isobutyrate, and 3-hydroxyisobutyrate, but not in the presence of glucose or propionate.

Materials. The micro-organism and chemicals used were as described in the preceding paper,[4] in which the culture methods were also described.

Cell extract and enzyme assay. The samples (about 4 mg dry weight) were withdrawn and cell extracts prepared as described earlier.[2] The activity of the enzyme was assayed with freshly prepared cell extracts. The rate of reduction of NAD at 30° was determined spectrophotometrically at 340 nm according to Sokatch *et al.*[7] In 1 ml the reaction system contained 100 μmol of Tris-HCl buffer, pH 9.2, 40 μmol of 2-mercaptoethanol, 160 μmol of propionaldehyde, 1 μmol of NAD and a cell extract with a dry weight of 0.25 mg. The reaction was started by adding the cell extract. A unit of the enzyme is defined as the amount of enzyme which catalyzes the reduction of 1 μmol/min of NAD at 30°.

Results and discussion. The data in Table 1 show the specific activity of methylmalonate semialdehyde dehydrogenase obtained when *P. fluorescens* UK-1 was grown on various single-carbon sources. The enzyme was formed in the presence of valine or its degradation products, 2-oxoisovalerate, isobutyrate or 3-hydroxyisobutyrate, but only slightly in the presence of propionate. In addition, isoleucine, leucine, 2-oxoisocaproate, and isovalerate markedly increased the specific activity of methylmalonate semialdehyde dehydrogenase. The enzyme was produced during all active growth phases, but the maximum level of activity was not reached until the culture approached the stationary phase. This indicates that the formation of methylmalonate semialdehyde dehydrogenase is

Table 1. Effect of growth substrates on the formation of methylmalonate semialdehyde dehydrogenase in *P. fluorescens* UK-1. Bacteria were grown on basal mineral medium supplemented with growth substrates as indicated. Crude extracts were prepared from the cells at the end of the exponential phase and enzyme activities were determined as described in the section *Cell extract and enzyme assay.*

Carbon source for growth	Specific activity of methylmalonate semialdehyde dehydrogenase (mU/mg dry weight)
3-Hydroxyisobutyrate	17.1
Isobutyrate	13.9
2-Oxoisovalerate	12.1
Valine	12.3
Isoleucine	6.9
Leucine	6.8
2-Oxoisocaproate	6.5
Isovalerate	6.2
Propionate	3.4
Acetate	1.1
Glucose	0.5

closely linked with cell multiplication when the inducer is the only source of carbon and energy. It should be noted that very low specific activities of the enzyme were detected when *P. fluorescens* UK-1 was cultured on glucose or acetate (Table 1). Methylmalonate semialdehyde dehydrogenase, like 3-hydroxyisobutyryl CoA hydrolase [5] and 3-hydroxyisobutyrate dehydrogenase,[6] was induced to the maximum level when *P. fluorescens* UK-1 was cultured on 3-hydroxyisobutyrate. This shows that the really effective inducer of methylmalonate semialdehyde dehydrogenase is 3-hydroxyisobutyrate. On the other hand, Marshall and Sokatch [8] have reported that in *P. putida* isobutyrate is about twice as effective as an inducer of methylmalonate semialdehyde dehydrogenase as 3-hydroxyisobutyrate.

Fig. 1 shows the results of experiments in which the deinduction and stability of the induced methylmalonate semialdehyde dehydrogenase were studied. Cells were precultured in a medium containing isobutyrate as inducer and then transferred to a glutamate medium. The cells exhibited a continuous decline in specific activity; the total activity, however, remained practically constant (Fig. 1). This indicates that the induced methylmalonate semialdehyde dehydrogenase is stable *in vivo*, the decreasing specific activity being due solely to reduction of the enzyme concentration during the growth of the bacteria.

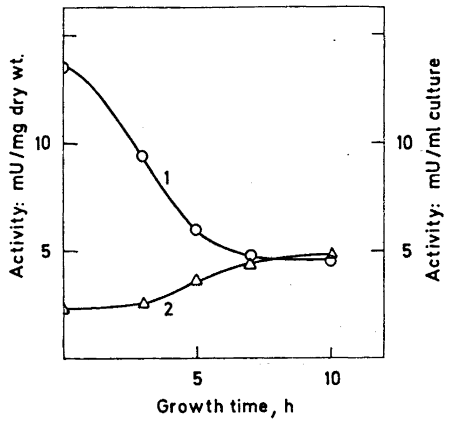

Fig. 1. The stability of induced methylmalonate semialdehyde dehydrogenase during deinduction. Cells were precultured in an isobutyrate medium, and when growth reached the late exponential phase the cells were centrifuged and transferred to a medium containing glutamate as the sole carbon source. Samples were withdrawn from the culture at intervals. Crude extracts were prepared and enzyme activities determined as described under *Cell extract and enzyme assay*.

1. Specific activity of methylmalonate semialdehyde dehydrogenase. 2. Total activity of methylmalonate semialdehyde dehydrogenase.

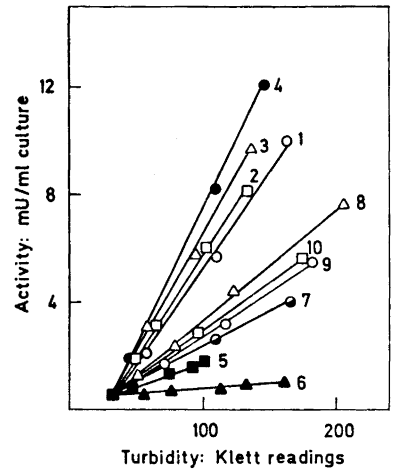

Fig. 2. Differential rate of formation of methylmalonate semialdehyde dehydrogenase during growth of *P. fluorescens* UK-1 on different growth substrates. The cells were precultured in a glutamate medium and transferred to a basal mineral medium supplemented with growth substrates as indicated. The experimental details were the same as described in the legend to Fig. 1.

1, Valine. 2, 2-Oxoisovalerate. 3, Isobutyrate. 4, 3-Hydroxyisobutyrate. 5, Propionate. 6, Glucose. 7, Leucine. 8, Isoleucine. 9, 2-Oxoisocaproate. 10, Isovalerate.

As can be seen from Fig. 2, the differential rate of synthesis of methylmalonate semialdehyde dehydrogenase remained constant to all the inducers studied. The maximal increase in total activity (about 0.02 unit per mg increase in cell mass) was obtained when *P. fluorescens* UK-1 was grown on 3-hydroxyisobutyrate.

These experimental results provide strong evidence that 3-hydroxyisobutyrate

may be the natural inducer for methylmalonate semialdehyde dehydrogenase in *P. fluorescens* UK-1. However, the possibility that methylmalonate semialdehyde, isobutyrate, 2-oxoisovalerate, or valine are also the inducers of the enzyme is not excluded.

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Low-temperature Transitions of Chlorinated Polyethylenes

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Several studies have been made regarding the low-temperature relaxation behavior of solution chlorinated polyethylenes.[1-4] It has been found by dynamic-mechanical studies that the γ-relaxation process occurs below -100°C in amorphous CPE-polymers. Using dielectric relaxation measurements on CPE with low chlorine content the temperature of maximum loss at a frequency of 1 kHz was about -90°C. The γ-transition of commercial polyethylenes has been reported to occur in two temperature regions, -120 to -130°C and -80 to -90°C.[5] In this study, suspension chlorinated low-density polyethylenes were investigated by differential thermal analysis measurements, DTA, in the temperature range -120° to 0°C.

Experimental. The samples were commercial suspension-chlorinated low-density polyethylenes (CPE) of varying chlorine content. The source of all CPE samples, the parent polymer, was a low-density polyethylene which was also used in these studies. The characteristics of the samples are given elsewhere.[6] The Du Pont 900 DTA apparatus was used for these measurements. The instrument was equipped with a low-temperature chamber made of polypropylene which allowed the measurements to be carried out in the temperature range -120° to 0°C. Liquid nitrogen was used as a cooling medium. The procedure employed in the runs was as follows: A macro sample tube was filled with the powdered polymer as received to depth of 5 mm. The sample was melted, pressed with the ceramic sleeve and the thermocouple was inserted in the softened mass. The reference tube consisted of a thermocouple inserted midway into 5 mm of glass beads. The tubes were placed into the low-temperature chamber. The samples were heated above melting points of the polymers, $+120$°C, to remove internal stresses and then cooled to liquid nitrogen temperature. The heating rate was 20°C/min.

Results and discussion. Thermograms of LDPE and CPE are seen in Fig. 1. The composition and transition points of the polymers are given in Table 1.

It can be seen that the transitions of CPE occur in the temperature intervals -38° to -39°C and -104° to -110°C. The latter range is seemingly related to the γ-transition of CPE. It has been found that the mechanical loss maximum at 2.7 Hz of solution chlorinated CPE with a chlorine content 20–45 % lies in the temperature range immediately below -100°C.[1,2] It has been shown that the γ-transition of semi-crystalline PE arises principally from contributions of the non-crystalline regions of the polymer.[8] On the basis of structural studies suspension chlorinated polyethylene, CPE, can be visualized as containing definite amounts of ethylene ($-CH_2-CH_2-$) and vinyl chloride ($-CH_2-CHCl-$) units when the chlorine content of the polymer varies between 0– and 56 %.[9,10] The distribu-