It is rather peculiar that the rate constants of the protonated Fe(II)-CyDTA complexes – contrary to the rate constants of the corresponding Fe(II)-EDTA complexes [1] – are smaller than that of the unprotonated complex. At present no explanation can be given for this result.

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Formation of Malonate-semialdehyde: Nicotinamide Adenine Dinucleotide (NAD) Oxidoreductase in *Pseudomonas fluorescens* P-2

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Malonate-semialdehyde:nicotinamide adenine dinucleotide (NAD) oxidoreductase (EC 1.2.1.18) catalyses the formation of acetyl-CoA from malonate semialdehyde. The enzyme from *Pseudomonas fluorescens*, when purified about 30-fold, appeared to be specific for malonate semialdehyde.[1] Both NAD and CoA were required for maximal activity of the purified enzyme.

In the present investigation we have studied the formation of malonate-semialdehyde:NAD oxidoreductase during the growth of *Pseudomonas fluorescens* P-2. The enzyme was formed in the presence of β-alanine, pantothenate, isobutyraldehyde, 3-hydroxybutyrate, 3-hydroxyisobutyrate, acetaldehyde, and acetate and slightly in the presence of malonate semialdehyde, propionaldehyde, and 3-aminobutyrate (Fig. 1). The enzyme activity

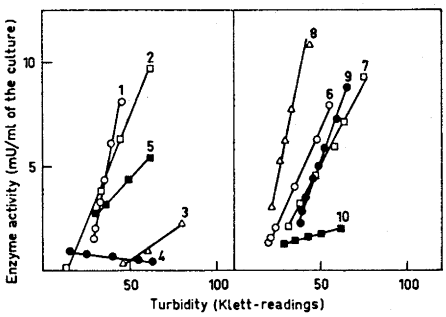

Fig. 1. The formation of malonate-semialdehyde:NAD oxidoreductase on various carbon compounds in *Pseudomonas fluorescens* P-2. Enzyme actvity was determined by the method of Hayaishi *et al.*[1]

1 = β-alanine, 2 = pantothenate, 3 = malonate-semialdehyde, 4 = α-alanine, 5 = acetate, 6 = isobutyraldehyde, 7 = 3-hydroxybutyrate, 8 = 3-hydroxyisobutyrate, 9 = acetaldehyde, 10 = propionaldehyde.

The concentrations of the carbon compounds were 10 mM.

was not found on glyoxylate, succinate, 2-oxoglutarate, 3-oxoglutarate, malonate, pyruvate, propanol, propionate, glycine, *N*-hydroxyethyl-β-alanine, or 4-amino-3-hydroxybutyrate. Although the various compounds mentioned above all caused induction of the enzyme, the real inducer of malonate-semialdehyde:NAD oxidoreductase seems to be β-alanine. 3-Hydroxyisobutyrate and isobutyraldehyde also may function as inducers, because the activity curves obtained on these compounds resembled that obtained on β-alanine, on which activity reached a maximum at the end of the exponential phase of growth. On the other hand, the enzyme activities on acetaldehyde, acetate, and some other compounds reached their maxima during the early logarithmic phase of growth.

Jacoby [2] reported that several aldehydes, including isobutyraldehyde, propionaldehyde, and acetaldehyde, would serve as substrates for aldehyde dehydrogenase, and Burton and Stadtman [3] found that butyraldehyde, propionaldehyde, and acetaldehyde would serve as substrates for CoA-linked aldehyde dehydrogenase, too. Although acetaldehyde and propionaldehyde did not function as substrates for purified malonate-semialdehyde:NAD oxidoreductase, malonate semialdehyde may serve as a substrate for aldehyde dehydrogenase and CoA-linked aldehyde dehydrogenase in crude extracts. This is based on the observations that the activity curves of the enzymes on acetaldehyde, propionaldehyde and malonate semialdehyde differed from those obtained on β-alanine.

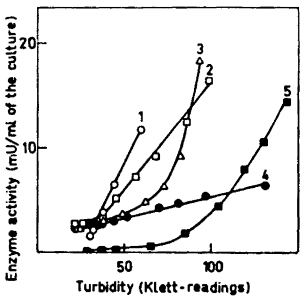

Fig. 2. The effect of α-alanine, pyruvate, succinate and glucose on the formation of malonate-semialdehyde:NAD oxidoreductase when β-alanine was used as inducer of the enzyme.

1 = β-alanine, 2 = β-alanine + succinate, 3 = β-alanine + pyruvate, 4 = β-alanine + α-alanine, 5 = β-alanine + glucose.

The concentrations of the carbon compounds were 10 mM.

Fig 2 shows the effects of α-alanine, pyruvate, succinate, or glucose on the formation of malonate-semialdehyde:NAD oxidoreductase. Among these compounds α-alanine inhibited enzyme synthesis most effectively. Earlier we have found that L-alanine:malonate-semialdehyde amino transferase (EC.2.6.1.18) is induced on β-alanine and repressed on α-alanine in *Pseudomonas fluorescens* P-2.[4] Because malonate-semialdehyde did not serve as a powerful inducer of malonate-semialdehyde:NAD oxidoreductase, we suggest that β-alanine is the real inducer and α-alanine or some derivative of α-alanine is a real corepressor of both L-alanine:malonate-semialdehyde amino transferase and malonate-semialdehyde:NAD oxidoreductase.

Experimental. Malonate-semialdehyde was prepared as described by Deuschel,[5] Deyert *et al.*[6] and Den *et al.*[7] and 3-hydroxyisobutyrate as described by Blaise and Herman.[8] Other compounds were purchased from commercial sources.

Pseudomonas fluorescens P-2 was used as the test organism. The bacteria were cultured in a salt solution described by Goodhue and Snell [9] with 10 mM concentation of the compounds investigated.

Turbidity was measured with a Klett-Summerson colorimeter and filter 62. Samples for assay were taken from the cultures at intervals of 1–3 h and centrifuged at 5000 *g* for 10 min. The enzyme was released from the cells by disrupting them in a sonic oscillator (MSE, 20 kc) for 4 min. Enzyme activity was determined by the method of Hayaishi *et al.*,[1] except that dithiothreitol was used to protect the enzyme during disruption.

Enzyme activity was defined as units of enzyme per ml of the culture.

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