BBA 63256

An inducible carnitine dehydrogenase from *Pseudomonas aeruginosa*

Quaternary compounds such as L-carnitine, 3-dehydrocarnitine or glycine betaine, may serve as sole carbon, nitrogen and energy source for *Pseudomonas aeruginosa*[1,2]. Studies on the assimilation of carnitine derivatives as well as the finding of trimethylacetonylammonium hydroxide (TMAA) in the culture filtrate[3] showed the following carnitine pathway in pseudomonads:

carnitine → 3-dehydrocarnitine → glycine betaine

Serratia marcescens catabolized carnitine in a manner quite different from *Ps. aeruginosa*[4]. We have now studied some properties of the first enzyme of this reaction chain, carnitine dehydrogenase, in cell-free extracts of *Ps. aeruginosa*.

All experiments described were performed with *Ps. aeruginosa* A7244. The bacteria were cultured in a liquid medium with L-carnitine (0.01 M) as sole N and C source[3] and harvested in early log phase by centrifugation. The resulting cell paste was washed several times with 0.05 M Tris–HCl buffer (pH 8.2), suspended and incubated with lysozyme (30 mg/100 ml) and EDTA (200 mg/100 ml) at 30° for 30 min. The resulting viscous solution was centrifuged at 0° and 90 000 × *g* for 60 min. The clear supernatant contained the total enzyme activity. For some experiments the enzyme was brought to a higher specific activity by fractionation with ammonium sulfate between 40 and 65% saturation.

Enzyme assay was based on the detection of NADH by its absorbance at 340 nm. The measurements were conducted at 30° in a Zeiss VSU I spectrophotometer. Each assay cuvette contained 5 μmoles of NAD^+, 200 μmoles of L-carnitine, about 100 μg protein of the enzyme solution, and a sufficient volume of Tris buffer (pH 9.0) to make a total volume of 4.0 ml (10-mm light path).

The activity of carnitine dehydrogenase is, under standard conditions, a linear function of protein content (up to 0.1 mg/ml) and time (up to 4 min). The optimal pH for enzyme reaction is about 9.0 (0.3 M Tris–HCl buffer). We found half-maximal reaction velocity with $8.5 \cdot 10^{-3}$ M carnitine and $4.2 \cdot 10^{-5}$ M NAD^+.

Metabolic inhibitors (NaN_3, NaF, KCN, monoiodoacetate; 10^{-3} M) did not reduce enzyme activity. *p*-Chloromercuribenzoate (10^{-4} M) and hydroxylamine (10^{-3} M) showed a total inhibition, sodium borate ($2 \cdot 10^{-2}$ M) an inhibition of 60% and hydrazine hydrate ($3 \cdot 10^{-2}$ M) of 70%.

To demonstrate the following reaction

L-carnitine + $NAD^+ \rightleftharpoons$ 3-dehydrocarnitine + NADH + H^+

we carried out the experiments summarized in Table I. 3-Dehydrocarnitine was separated by thin-layer chromatography[5] (aluminium oxide D) in several solvents (butanol–acetic acid–H_2O (4:1:1); methanol–dioxan–acetic acid–H_2O (3:4.5:0.5:2); ethyl acetate–acetic acid–H_2O (4:1:1)), stained with iodine and identified by comparison with authentic 3-dehydrocarnitine. After being heated the 3-dehydrocarnitine formed is decarboxylated to TMAA, which was precipitated by sodium triphenylcyanoborate from alkaline solution and then identified by infrared

Abbreviation: TMAA, trimethylacetonylammonium hydroxide.

TABLE I

EVIDENCE OF CARNITINE DEHYDROGENASE IN CELL-FREE EXTRACTS OF *Ps. aeruginosa*

A. Synthesis of NADH and 3-dehydrocarnitine from NAD^+ and carnitine. The complete system contained the enzyme (96 μg protein), 100 μmoles of L-carnitine, 5 μmoles of NAD^+, and 40 μmoles of $MgCl_2$ in 4.0 ml of Tris buffer (pH 9.0) and was incubated for 3 min.

μmoles NADH formed	0.56
μmoles 3-dehydrocarnitine formed (283 nm)	0.55
μmoles 3-dehydrocarnitine formed (530 nm)	0.56

B. Synthesis of NAD^+ and carnitine from NADH and 3-dehydrocarnitine. The complete system contained the enzyme (192 μg protein), 10 μmoles of 3-dehydrocarnitine, 8.25 μmoles of NADH, and 40 μmoles of $MgCl_2$ in 4.0 ml of Tris buffer (pH 9.0) and was incubated for 20 min.

μmoles NADH disappeared	6.9
μmoles 3-dehydrocarnitine disappeared (283 nm)	6.2
μmoles 3-dehydrocarnitine disappeared (530 nm)	6.6
μmoles carnitine formed	6.0

spectroscopy[6]. 3-Dehydrocarnitine (enol form) was determined by the absorbance of its magnesium salt at 283 nm (pH 9.0)[7] and by the absorbance of its iron salt at 530 nm. Carnitine was also identified by thin-layer chromatography, and also, after precipitation by triphenylcyanoborate from weakly acidic solution, by infrared spectroscopy[6]. It was determined by the phenylborate method[8]. Other reaction products than that referred to Table I could not be found.

Systems without enzyme, NAD^+ or carnitine or with heat-denatured enzyme showed no reaction. Structural analogues or derivatives of L-carnitine such as D-carnitine, *O*-acetyl-L-carnitine, L-carnitine ethyl ester, L-carnitine amide, DL-4-dimethylethylammonio-3-hydroxybutyrate, DL-4-dimethylamino-3-hydroxybutyrate, DL-4-amino-3-hydroxybutyrate, DL-3-hydroxybutyrate, choline, DL-β-methylcholine or glycine betaine did not react with the enzyme. Carnitine dehydrogenase is also specific for coenzyme, utilizing only NAD^+ (not $NADP^+$).

The enzyme does not occur if the bacteria are grown with glucose/NH_4^+,

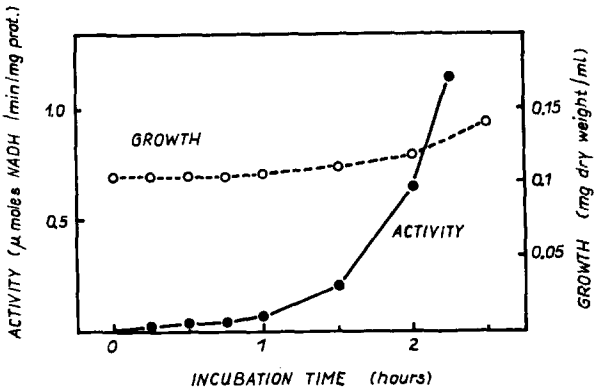

Fig. 1. Induction of carnitine dehydrogenase in *Ps. aeruginosa*. Cells preincubated with glucose/NH_4^+ were washed, centrifuged and then transferred at time 0 into a medium containing carnitine as the sole N and C source and incubated at 30°.

glycine betaine or choline. If cells grown on glucose/NH_4^+ were transferred to a medium containing carnitine, an induction of the enzyme begins (Fig. 1). This induction is completely inhibited by chloramphenicol. In the simultaneous presence of glucose/NH_4^+ and carnitine, the enzyme is only induced if glucose is completely metabolized. Structural analogues of carnitine (D-carnitine, L-carnitine amide, DL-4-dimethylethylammonio-3-hydroxybutyrate, DL-4-dimethylamino-3-hydroxybutyrate, choline, and glycine betaine) are not inducers of carnitine dehydrogenase.

The present data support our concept that the first step of the assimilation of carnitine in *Ps. aeruginosa* is the oxidation of C-3. Carnitine induces the enzyme (NAD$^+$-linked and stereospecific for L-carnitine) called carnitine dehydrogenase (systematic name: L-carnitine:NAD$^+$-oxidoreductase), which is probably a SH-enzyme. The synthesis of carnitine dehydrogenase is repressed by glucose. If it is possible to increase specific activity by purification of the enzyme, carnitine dehydrogenase should be able to serve for the determination of L-carnitine by a simple optical test.

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Received March 31st, 1967

Biochim. Biophys. Acta, 139 (1967) 505–507

BBA 63251

Reactions of the lactate dehydrogenase X-band in human sperm with homologous and heterologous antisera

The electrophoretic separation of homogenates of mature testes from a number of animal species results in the detection of one or more lactate dehydrogenase isozymes (L-lactate:NAD oxidoreductase, EC 1.1.1.27) which are not found in other tissues[1,2]. These isozymes, which have been referred to as the X-bands[1], have been shown to comprise 80 to 100% of the total lactate dehydrogenase activity in sperm, and it appears that sperm may be their source in testes[1,3]. It has been suggested, on the basis of dissociation and hybridization experiments performed with human and rabbit testes, that the X-band consists of subunits which are different from the A or B subunits of isozymes 5 or 1, and that its synthesis is controlled by a third gene[4],