EVIDENCE FOR AN INDUCIBLE ACTIVE TRANSPORT OF CARNITINE IN PSEUDOMONAS AERUGINOSA

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A permeation of carnitine into intact cells has not been reported. In erythrocytes this quaternary compound is only adsorbed at the cell surface (Thomitzek and Willgerodt, 1965). The present communication deals with the accumulation of carnitine by Ps. aeruginosa. Ps. aeruginosa utilizes L-carnitine and some other quaternary compounds as sole source of nitrogen and carbon (Aurich and Lorenz, 1959; Strack et al., 1962). The ability to oxidize carnitine is acquired by induction (Kleber and Aurich, 1966); only carnitine induces; glucose and glycine betaine repress the formation of the enzymes required for the oxidation of carnitine (Aurich and Kleber, 1966). Carnitine also brings about the sequential induction of the enzymes involved in the degradation of 3-dehydrocarnitine and glycine betaine.

The findings mentioned imply that the bacteria are able to transport carnitine. In this report we present the results of experiments on the transport of carnitine in Ps. aeruginosa.

MATERIALS AND METHODS

Organism and cultural conditions: All experiments described were done

with Ps. aeruginosa A 7244 (NCTC). The bacteria were cultured in a synthetic liquid medium (Buffa and Velluti, 1957) with L-carnitine (0.01 M), glycine betaine, or choline as sole source of carbon and nitrogen, or in a medium containing glucose and ammonium (Aurich and Kleber, 1966).

Preparation of bacteria: Routinely, experiments were performed with cells harvested in mid-exponential phase. Bacterial pellets were washed several times with phosphate buffer (0.07 M, pH 7.5) and the bacteria suspended in the same buffer; the bacterial protein of the suspensions was determined by the method of Lowry et al. (1951).

Determination of labeled and unlabeled carnitine: For the measurement of the transport of carnitine 0.5 ml of the bacterial suspensions (containing between 0.5 and 1.0 mg of protein) were incubated for 2 hours at room temperature with 2.5 µmoles of L-carnitine-$^{14}CH_3$ of specific radioactivity of 0.081 µci/µmole. The uptake of carnitine was stopped by dilution of the suspension into ice-cold KCN (0.01 M). The standard procedures employed for the measurement of accumulated carnitine as well as for the radiochromatographic identification of the carnitine are described in detail elsewhere (Aurich and Kleber, 1966). The results are recorded either as nmoles of carnitine taken up in 2 hours by bacteria equivalent to 1 mg of protein or in relative terms as percentage of uptake by an uninhibited control.

Intracellular free carnitine was estimated in trichloroacetic acid extracts of bacteria by precipitation with ammonium reineckate (Kapfhammer and Bischoff, 1930).

RESULTS AND DISCUSSION

The accumulation of carnitine was found to be a linear function of the cellular concentration (up to 2 mg of bacterial protein) with an average standard error of the rates of uptake of ± 6 %. After an incubation period of 2 hours about 50 % of the radioactivity accumulated by the bacteria are extractable by hot water. Chromatographic analysis (ethanol/NH_3/H_2O = 90/5/5) of the extracted radioactive material revealed that more than 95 % of the extractable radioactivity was recovered as carnitine. The remaining radioactive material chromatographed as a single spot, and was identified as trimethyl-acetonyl-ammonium hydroxide (decarboxylated 3-dehydrocarnitine). We also found this intermediate in the culture medium of bacteria grown on carnitine (Strack et al., 1962) and as product of the activity of carnitine dehydrogenase from Ps. aeruginosa (Aurich and Kleber, 1966).

Transport of carnitine was maximal between 25 - 30°. The temperature coefficient, Q_{10}, as obtained for the range of exponential activation (10 - 23°), was 1.9. The optimal pH (phosphate buffer) was about 7.0.

The accumulation of carnitine by maximally induced bacteria rose linearly during 4 hours. The rate of uptake increased with increasing external concentration (0.01 - 5mM) toward a maximal value of about 70 nmoles of carnitine taken up in 2 hours by bacteria equivalent to 1 mg of protein. The double-reciprocal plot, according to Lineweaver and Burk (1934), of the concentration dependence of the rate of uptake yielded a straight line from which a K_m of 6.3×10^{-4} could be calculated. The intracellular con-

centration of free carnitine determined prior to incubation of the bacteria with labeled carnitine was about 0.2 μmole/mg protein.

The rate of accumulation of carnitine increased by about 55 %, when the bacteria were preloaded with increasing amounts (0.001 - 0.1 M) of unlabeled carnitine by prior growth in the presence of carnitine. The addition of increasing amounts of non-radioactive L-carnitine to bacteria which had been preloaded with labeled L-carnitine displaced labeled carnitine from the bacteria.

The uptake of carnitine was stimulated by Mg^{++}, Na^{+}, and K^{+} in Tris/HCl buffer (pH 7.0). Maximal stimulation was caused by the simultaneous presence of Na^{+} and K^{+} as phosphate buffer (0.07 M).

Metabolic energy apparently was required for the uptake of carnitine by Ps. aeruginosa. Metabolic inhibitors reduced the influx (Table I).

TABLE I

Effect of metabolic inhibitors on L-carnitine uptake in Ps. aeruginosa (percentage of carnitine influx in the presence of various inhibitors in phosphate buffer pH 7.0)

Inhibitor	Concentration in medium (M)	Transport activity (%)
NaN_3	10^{-2}	2
2,4-Dinitrophenol	10^{-2}	5
Monoiodoacetate	$2 \cdot 10^{-3}$	9
KCN	10^{-2}	15
EDTA	10^{-1}	17
G-strophanthin	$2 \cdot 10^{-2}$	63

2,4-Dinitrophenol (0.01 M) and sodium azide (0.01 M) also increased the efflux of carnitine, identified by radiochromatography, from preloaded bac-

teria. G-strophanthin also inhibited the uptake of carnitine; this finding was in agreement with the observation that its transport was stimulated by Na^+ and K^+. Table II shows the inhibition of the uptake of carnitine by analogues. Glycine betaine and choline also displaced previously accumulated carnitine from the bacteria.

TABLE II

Effect of some quaternary compounds on the L-carnitine accumulation, indicated as % inhibition compared to inhibitor-free controls

L-Carnitine:inhibitor (mM)	5:5	5:15	0,5:5	0,5:50
D-Carnitine	0	-	50	79
3-Dehydrocarnitine	-	60	-	-
Glycine betaine	-	34	-	85
Choline·Cl	-	51	-	66

Bacteria grown on carnitine as sole source of carbon and nitrogen showed the highest rate of carnitine transport when harvested in the early log phase. Bacteria grown on sources of carbon and nitrogen other than carnitine accumulated less than 20 % as much carnitine in 2 hours than did the bacteria grown on carnitine. In the case of bacteria grown on sources of carbon and nitrogen other than carnitine the rate of uptake was a non-linear function of time: the rate of uptake increased with the time of incubation with carnitine. The bacteria seemed to acquire the ability to transport carnitine during the 2 hour incubation with carnitine. No increase in the ability to accumulate carnitine was observed when bacteria grown on the glucose-ammonia medium were incubated with carnitine in the presence of chloramphenicol (1 mg/ml). Chloramphenicol didn't affect the accumu-

lation rates of carnitine in completely induced bacteria.

These results, i.e. the requirement for the presence of carnitine as inducer and inhibition of induction of the transport system by chloramphenicol, are taken as evidence that the carnitine transport system includes at least one inducible proteinaceous component. The transport of carnitine shows saturation kinetics, has a high temperature coefficient, and is inhibited by metabolic inhibitors and analogues of carnitine. A Na^+-, K^+-stimulated, G-strophanthin-sensitive ATPase may be involved in the transport of carnitine. In conclusion then carnitine accumulation in Ps. aeruginosa appears to be mediated by an inducible, active carrier transport system.

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