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Metabolism of D- and L-Lactate by *Pseudomonas putida*

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Abstract

Pseudomonas putida grew at the same rate with the same molar growth yield on D-, L, or DL-lactate as the sole source of carbon for growth. D- and L- lactate were utilized simultaneously and at the same rate when the organism was grown on DL-lactate (ratio of D isomer to L isomer of 1 : 1). Growth on either isomer alone, or in combination, caused the induction of both a D-lactate, and an L-lactate dehydrogenase. Both enzymes were particulate and used dichlorophenolindophenol, or oxygen, but not NAD, as electron acceptor, and were inhibited by cyanide when oxygen was the electron acceptor. The pH optimum for the D-lactate dehydrogenase was about 6·5, and for the L-lactate dehydrogenase was about 8·0. The D-lactate dehydrogenase was more heat-sensitive than the L-lactate dehydrogenase. The stoichiometry of both enzyme reactions was the same with 2 mol of lactate being oxidized by 1 mol of oxygen to form 2 mol of pyruvate. No lactate racemase was detected in the cell extracts.

Introduction

The metabolism of DL-lactate by *Pseudomonas aeruginosa* (Kemp 1972) and *P. citronellolis* (O'Brien 1977) appears to depend on the induction of NAD-independent D- and L-lactate dehydrogenases. Growth of *P. citronellolis* on L-lactate also induced both enzymes, but growth on D-lactate induced only the D-lactate dehydrogenase (O'Brien 1977). Pinchinoty *et al.* (1968) reported briefly that a soil organism, identified as *P. putida*, when grown on DL-lactate, was capable of oxidizing D- or L-lactate using oxygen or dichlorophenolindophenol (DCPIP) as electron acceptor.

This paper presents evidence for the inducible nature of the D- and L-lactate dehydrogenases of *P. putida*, and shows that the induction pattern differs from that of *P. citronellolis*. Some of the properties of the two lactate dehydrogenases are also described.

Materials and Methods

Growth and Harvesting of the Organism

P. putida NCIB 9034 was grown at 30°C on the basal medium previously described (O'Brien *et al.* 1977) using the following carbon sources at a final concentration of 0·5% (w/v): lithium D-lactate, potassium L-lactate, glucose, potassium L-malate, and sodium pyruvate. The lactate salts and sodium pyruvate were sterilized by filtration through a 0·22 μm Millipore filter. The conditions of growth, measurement of growth rate, and harvesting of the cells were carried out as previously described (O'Brien 1977).

Preparation of Cell Extracts and Enzyme Assays

Cell extracts were prepared by sonic disintegration (O'Brien 1977) and were centrifuged at 10 000 *g* for 15 min at 5°C and the supernatant was used for the enzyme assays. Some of the 10 000-*g*

supernatant was again centrifuged at 160000 *g* for 2 h at 5°C. The pellet was resuspended in 0·05 M tris–HCl, pH 7·0, to a protein concentration of about 20 mg/ml.

The NAD-independent D- and L-lactate dehydrogenases were measured by the two procedures previously described (O'Brien and Taylor 1977) using tris–HCl buffer, pH 6·5, for the D-lactate dehydrogenase, and tris–HCl buffer, pH 8·0, for the L-lactate dehydrogenase. The substrates (10 mM) were lithium D-lactate, and potassium L-lactate, respectively. NAD-dependent lactate dehydrogenase (EC 1.1.1.27) was assayed according to Bergmeyer and Bernt (1974).

The stoichiometry of both enzyme reactions was measured by following the oxidation of D- or L-lactate by the 160000-*g* pellet fraction in an oxygen electrode in the presence of 1 mM sodium azide and then stopping the reaction by the addition of 0·2 ml of 30% (w/v) trichloroacetic acid. After removal of the precipitated protein the supernatant was assayed for D- or L-lactate and pyruvate.

Assay Procedures

D-Lactate was assayed according to Gawehn and Bergmeyer (1974); L-lactate according to Gutman and Wahlefeld (1974); and pyruvate according to Bücher *et al.* (1963). Protein was measured by the Biuret method using bovine plasma albumin as the standard (Gornall *et al.* 1949).

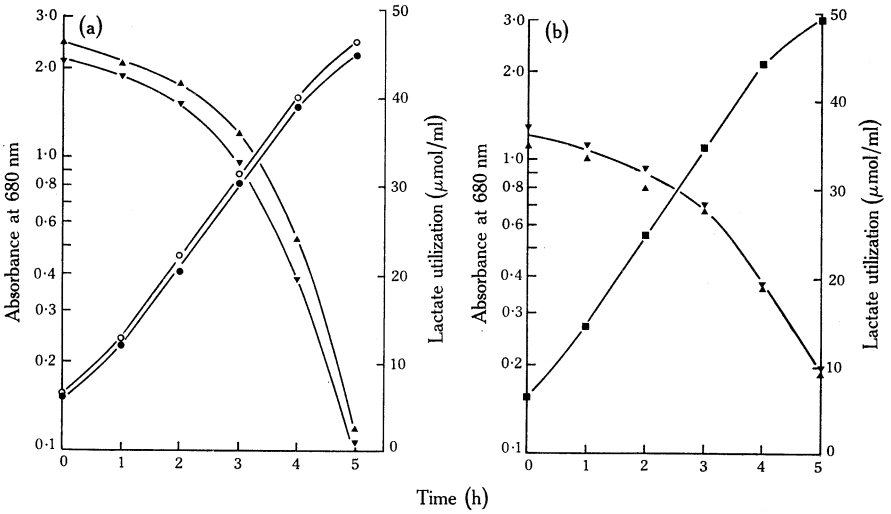

Fig. 1. Growth of *P. putida* on (*a*) D- and L-lactate and (*b*) DL-lactate, and lactate utilization. ○ Growth on D-lactate. ● Growth on L-lactate. ■ Growth on DL-lactate. ▲ D-Lactate utilization. ▼ L-Lactate utilization.

Results

Growth Studies

The growth rate of *P. putida* on D- , L- , or DL-lactate (Figs 1*a* and 1*b*) was the same with a mean generation time of about 60 min. The initial pH of all the cultures was 7·2 and at the time of harvesting it was 8·2. The rates of utilization of D-lactate and L-lactate, when they were used individually as carbon sources, were similar (Fig. 1*a*) and gave molar growth yields of 37 and 35 g dry weight of cells per mole of lactate utilized respectively. When the cells were grown on DL-lactate [both isomers at about 0·4% (w/v) initial concentration] the two isomers were utilized simultaneously, and at the same rate (Fig. 1*b*), giving a molar growth yield (based on total lactate used) of 38 g dry weight of cells per mole of lactate utilized. A similar growth yield was observed when the ratio of D-lactate to L-lactate in the medium was 2:1. The ratio of the two isomers remained constant at 2:1 throughout growth.

Enzyme Studies

The organism was grown on a variety of carbon sources and cell extracts were assayed for NAD-dependent and NAD-independent lactate dehydrogenases and the results are shown in Table 1. No NAD-dependent lactate dehydrogenase activity was detected in any of the extracts.

The activity of both NAD-independent D- and L-lactate dehydrogenases was extremely low in cells grown on glucose, L-malate, or pyruvate but increased dramatically when the cells were grown on D-, L-, or DL-lactate (Table 1). The increase ranged from 20- to 40-fold for the D-lactate dehydrogenase, and from 50- to 100-fold for the L-lactate dehydrogenase. Growth on either isomer of lactic acid resulted in the induction of both enzymes, with L-lactate dehydrogenase being maximally induced by both isomers; D-lactate dehydrogenase was induced some 20-fold by growth on L-lactate and some 40-fold by growth on D-lactate. When DL-lactate was the carbon source the L-lactate dehydrogenase was induced to a lower activity than in cells grown

Table 1. Effect of carbon source during growth on the specific activity of NAD-independent D- and L-lactate dehydrogenases of *P. putida*

Carbon source	Specific activity[A] in cell extracts	
	D-Lactate dehydrogenase	L-Lactate dehydrogenase
DL-Lactate (D:L = 1:2)	0·046	0·26
DL-Lactate (D:L = 1:1)	0·072	0·23
DL-Lactate (D:L = 2:1)	0·119	0·36
D-Lactate	0·114	0·40
L-Lactate	0·068	0·37
Glucose	0·003	0·004
Pyruvate	0·003	0·006
L-Malate	0·003	0·006

[A]Expressed as micromoles of lactate oxidized per milligram protein per minute using DCPIP as electron acceptor.

on either isomer alone; the amount of D-lactate dehydrogenase activity induced depended upon the ratio of D-isomer to L-isomer, increasing as the ratio favoured the D-isomer (Table 1).

The distribution of the two NAD-independent lactate dehydrogenases in supernatant and pellet fractions obtained by centrifuging extracts of cells grown on D-lactate at 160 000 *g* is shown in Table 2. D-Lactate was chosen as carbon source since it induced the highest activity of both enzymes. The results show that both enzymes used DCPIP, or oxygen, as electron acceptors. About 90% of the activity of the 10 000-*g* supernatant fraction was found in the 160 000-*g* pellet fraction. The activity of both enzymes was completely inhibited by 1 mM cyanide when oxygen was the electron acceptor, whereas the presence of cyanide in the DCPIP assay increased the activity by some 30%, by, presumably, preventing the passage of electrons to oxygen. The pH optimum for the D-lactate dehydrogenase was about 6·5 and for the L-lactate dehydrogenase it was about 8·0; at pH 6·5 the activity of the two enzymes was additive. The D-lactate dehydrogenase was not detected when assayed

at pH 8·0. In determining the pH optima of the two enzymes the actual pH of each assay incubation was measured on a pH-meter. Thus even though tris–HCl does not buffer below pH 7·0 the value of 6·5 quoted for the D-lactate dehydrogenase is a true value.

D-Lactate dehydrogenase was more susceptible to heat than the L-lactate dehydrogenase, losing about 75% of its activity after heating for 5 min at 51°C; under these conditions the L-lactate dehydrogenase was unaffected. After 2 min heating at 59°C only some 12% of D-lactate dehydrogenase activity remained, whereas the L-lactate dehydrogenase retained 55% of its activity.

The stoichiometry of the reactions catalysed by both enzymes was measured in the presence and absence of 1 mM sodium azide, which completely inhibited the endogenous catalase activity. In the D-lactate dehydrogenase reaction 0·55 μmol of lactate was oxidized at the expense of 0·21 μmol of oxygen to yield 0·50 μmol of pyruvate. In the L-lactate dehydrogenase reaction the values were: 1·04 μmol lactate oxidized, 0·47 μmol oxygen utilized, and 0·92 μmol pyruvate formed.

Table 2. Distribution of NAD-independent D- and L-lactate dehydrogenases in supernatant and particulate fractions of cell extracts of *P. putida* grown on D-lactate

Enzyme	Electron acceptor	10000-*g* supernatant		160000-*g* supernatant		160000-*g* pellet	
		Sp. act.[A]	Units[B]	Sp. act.[A]	Units[B]	Sp. act.[A]	Units[B]
D-LDH[C]	DCPIP	0·114	20·2	0·026	2·0	0·203	18·5
	Oxygen	0·044	7·8	0·002	0·4	0·078	7·2
L-LDH[C]	DCPIP	0·40	72·8	0·094	7·1	0·711	65·0
	Oxygen	0·204	36·4	0·032	2·4	0·342	31·4

[A]Specific activity expressed as μmoles of lactate oxidized per milligram protein per minute.
[B]Units defined as the specific activity multiplied by the total protein in the extract.
[C]D-Lactate dehydrogenase; L-lactate dehydrogenase, respectively.

When the 160000-*g* pellet was incubated with either D- or L-lactate for 30 min there was no formation of the opposite isomer, indicating that the pellet was free of lactate racemase activity.

Discussion

When *P. putida* was grown on D-, L-, or DL-lactate it was devoid of an NAD-dependent lactate dehydrogenase, but induced two NAD-independent lactate dehydrogenases, one for each isomer of lactic acid. Thus the function of the two enzymes seems to be (1) formation of pyruvate for further oxidation and (2) generation of electrons for the production of energy via oxidative phosphorylation. Inhibition of the oxidation of either D- or L-lactate by cyanide, when oxygen was the electron acceptor, is presumptive evidence for a cytochrome system functioning in the oxidation. Stanier *et al.* (1966) and Pichinoty *et al.* (1968) have reported the presence of cytochromes *b* and *c* in *P. putida*, and Kemp (1972) has shown that cytochromes are involved in the oxidation of DL-lactate by *P. aeruginosa*.

Evidence that two separate enzymes are involved in the oxidation of the two isomers of lactic acid is shown by: (1) the different pH optima for the two reactions; (2) the additive nature of the reactions at pH 6·5; (3) the greater heat sensitivity of

the D-lactate dehydrogenase; (4) the failure to detect any racemase activity; (5) the utilization of both isomers simultaneously, and at the same rate, when the cells were grown on DL-lactate; and (6) the varying amount of activity of each enzyme induced, depending upon which isomer of lactic acid was used as the carbon source for growth.

The rate of utilization of each isomer, when present in the medium at a ratio of 1:1, was the same, even though there was up to a threefold difference in the activities of the two dehydrogenases. These results indicate that the uptake of the two isomers of lactic acid is independent of the activity of the lactate dehydrogenases. A further implication is that the two isomers may be transported into the cells by one transport system. Thus when the cells were grown on DL-lactate (ratio of D isomer to L isomer of 2:1 or 1:1) the ratio of the two isomers in the medium remained constant throughout the growth period at the original starting value and the total lactate (both D- and L-) used was the same for the same amount of growth as that observed in cells grown on either isomer alone.

The pattern of induction of the D- and L-lactate dehydrogenases in *P. putida* differs from that of *P. citronellolis* in that growth of the latter organism on D-lactate caused the induction of only the D-lactate dehydrogenase (O'Brien 1977), whereas in *P. putida* both lactate dehydrogenases were induced. The two organisms were similar in their response when L- or DL-lactate was the carbon source for growth. *P. putida* was able to use both isomers of lactic acid simultaneously when grown on DL-lactate, a property also possessed by *P. citronellolis* (O'Brien 1977).

Both enzymes appear to be similar to the lactate dehydrogenases of *P. aeruginosa* (Kemp 1972) and *P. citronellolis* (O'Brien 1977; O'Brien and Taylor 1977) in that they are inducible, particulate, use oxygen, or DCPIP, but not NAD, as electron acceptors and are inhibited by cyanide. Thus the induction of NAD-independent lactate dehydrogenases by lactate seems to be a general property of *Pseudomonas* species.

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