A Study Of Electron Transport In Propionibacterium Shermanii

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ABSTRACT

Propionibacteria possess a membrane-bound electron transport system coupling oxidation of several substrates to reduction of fumarate or oxygen. Published work has indicated that the electron transport system consists of the following: dehydrogenases for NADH, lactate, glycerol phosphate and succinate; menaquinone (II,III-tetrahydro-menaquinone-9); cytochromes b, a, d and possibly o; terminal oxidase(s) and fumarate reductase. Menaquinone has been demonstrated to be an obligate component of electron transport to fumarate but there is disagreement in the literature concerning the role of the b-cytochrome components in the electron transport pathways. In the present study, the effect of a number of inhibitors on electron transport from NADH, D- and L-lactate and succinate to oxygen and to fumarate was investigated with the aim of elucidating the relationship between the aerobic and anaerobic pathways for oxidation of these substrates and the role of the cytochrome components in these systems.

Membranes were prepared by breaking lactate-grown cells of *P. shermanii* ATCC 9614 in a Hughes press and separating membranes from soluble components by differential centrifugation with a final centrifugation over a stepwise sucrose gradient.

Spectrophotometric analysis of pyridine hemochromogen derivatives showed the presence of significant quantities of cytochromes b and d, but did not detect the presence of cytochrome a or c. The cytochromes were also characterized

by redox titrimetry at room temperature and by analysis of low temperature spectra of redox-poised membranes. These experiments indicated the presence of four b-type cytochromes with α-absorption peaks (subscript) and approximate midpoint redox potentials at pH 7 (bracketted figures) as follows: b_{562-3} (+120 mV); b^{HP}_{556-7} (+90 mV); b^{LP}_{556-7} (-20 mV); b_{553-4} (-20 mV). A midpoint potential at pH 7 for cytochrome d of approximately +150 mV was determined from room temperature titrations.

Of several inhibitors surveyed for effects on electron transport activities of membranes, pCMB, dicumarol, UV light and cyanide were selected for detailed study. Dicumarol was particularly effective as an inhibitor of L-lactate dependent activities. L-lactate-dependent reduction of oxygen, DICPIP and $Fe(CN)_6$ was inhibited by a concentration of dicumarol 10^3 times less than that required for comparable inhibition of the corresponding D-lactate and NADH dependent activities. This indicates either that the L-lactate dehydrogenase has distinctive dicumarol-sensitive components or that it is integrated in the membrane in a manner which enhances its accessibility to dicumarol. The fact that the D-lactate-coupled activities are very stable while the L-lactate dependent activities are unstable also indicates that the two lactate dehydrogenase systems have different properties.

Ultraviolet light irradiation caused a rapid inactivation of NADH- and D-lactate-fumarate oxidoreductases but a slower inactivation of NADH and D-lactate oxidases. This was interpreted to indicate that electron transport to

fumarate has an absolute requirement for menaquinone while a portion of the oxidase activity did not require menaquinone.

All electron transport activities were inhibited by pCMB at a concentration around 10^{-4} moles per gram protein but NADH-dependent reduction of oxygen, $Fe(CN)_6$ and DICPIP was inhibited to a maximum of only 50%.

The pCMB titration curves for inhibition of NADH-dependent activities and the significantly higher reduction of cytochrome b by NADH than by D- or L-lactate in the presence of inhibitory concentrations of pCMB suggest the presence of two independent dehydrogenases for NADH. However the complete inhibition of NADH-fumarate oxidoreductase by pCMB suggests that only the pCMB-sensitive dehydrogenase is involved in electron transport to fumarate.

Cyanide inhibited D- and L-lactate- and NADH- dependent oxygen consumption with an I_{50} of about 2 mM. The relatively low sensitivity to cyanide suggests that cytochrome d is the major terminal oxidase. This is consistent with the evidence from difference spectra of cyanide-inhibited aerobic steady state membranes indicating that cyanide binds to cytochrome d and inhibits oxidation of cytochrome b. Although a CO-binding cytochrome b is present, the low cyanide sensitivity and absence of CO inhibition of oxygen uptake suggest that an o-type oxidase does not contribute significantly to oxygen uptake.

The effect of inhibitors on reduction of $Fe(CN)_6$ and DICPIP by all four substrates was also systematically investigated. The results obtained from these studies

suggest multiple sites of interaction of these acceptors with the electron transport pathways, the points of interaction depending on both the nature of the acceptor and of the reducing substrate.

Addition of fumarate caused a decrease of 20-30% in the rate of oxygen consumption and in total oxygen consumption at concentrations between 0 and 1 mM. No further inhibition occurred with increasing fumarate concentrations up to 10 mM, but concentrations above 10 mM inhibited first the rate of oxygen consumption and then the total oxygen consumption until both were completely inhibited at 40 mM fumarate. Inhibition at low concentrations of fumarate is probably due to diversion of a portion of the electron flow from oxygen to reduction of fumarate. This competition between oxygen and fumarate for electrons indicates that the pathways to these two acceptors share common initial steps.

Comparison of cytochrome difference spectra of membranes in the aerobic and anaerobic state in the presence and absence of inhibitors indicated that UV, dicumarol and pCMB inhibited electron transport at sites on the reducing site of the cytochrome b components. Concentrations of pCMB which completely inhibited fumarate reduction appeared to leave cytochrome b_{553-4} largely in the reduced state in the anaerobic steady-state, but not in the aerobic steady state suggesting that this cytochrome is not a component of electron transport to fumarate. The other low potential b type cytochrome b^{LP}_{556-7} was oxidized by fumarate in the presence of pCMB, suggesting that it may be located

specifically on the anaerobic path to fumarate. Since fumarate oxidized at least part of the cytochrome b in UV-irradiated membranes, a previous scheme for electron transport in this organism in which cytochrome b can only be oxidized by fumarate via menaquinone was not supported by the present study.

Spectra of membranes in the aerobic steady state indicated a greater degree of reduction of cytochrome b_{562-3} when succinate was reducing substrate than when D- or L-lactate or NADH was reducing substrate. This suggests that there was a close relationship between succinate dehydrogenase and cytochrome b_{562-3}. Since succinate oxidase activity was low despite the presence of high succinate dehydrogenase activity, it is improbable that b_{562-3} is part of an oxidase.

The present study did not resolve the question as to whether there was a single succinate dehydrogenase which functions *in vivo* as the fumarate reductase. Most evidence is consistent with there being only one enzyme system but the finding of two distinct $K_{m(succinate)}$ values for succinate-DICPIP and succinate-$Fe(CN)_6$ oxidoreductase may indicate the presence of two distinct systems.

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ABBREVIATIONS

a, b, c or d	Cytochromes a, b, c or d
CN	Cyanide
DIC	Dicumarol
DICPIP	2,6-dichlorophenolindophenol
$Fe(CN)_6$	potassium ferricyanide
fp_D	D-lactate dehydrogenase
fp_{FR}	Fumarate reductase
fp_L	L-lactate dehydrogenase
fp_N	NADH dehydrogenase
fp_S	Succinate dehydrogenase
HOQNO	N-heptyl-4-hydroxyquinoline-N-oxide
MQ	Menaquinone
NOQNO	n-Nonyl-4-hydroxyquinoline-N-oxide
pCMB	p-chloromercuribenzoic acid
PMS	Phenazine methosulfate
UV	Ultraviolet light