TRANSIENT pH CHANGES DURING D-LACTATE OXIDATION BY MEMBRANE VESICLES

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SUMMARY

Membrane vesicles from Escherichia coli B/r were incubated anaerobically in the presence of 10 mM D-lactate. Addition of a small quantity of an air-saturated KCl solution resulted in a rapid drop in pH followed by a gradual return to the original value. No pH changes were seen when anaerobic KCl was added, or when air-saturated KCl was added in the absence of D-lactate. Electron transport inhibitors and uncouplers attenuated the pH changes. The pH changes may result from the formation of a transmembrane hydrogen ion gradient during D-lactate oxidation.

INTRODUCTION

Transient pH changes have been observed in anaerobic suspensions of either mitochondria (1), submitochondrial vesicles (2, 3) or whole cells of Micrococcus denitrificans (4) when briefly exposed to oxygen, and in suspensions of chloroplasts (5) or chromatophores (6, 7) in the dark when briefly exposed to light. These results tend to support Mitchell's contention (8, 9) that a transmembrane hydrogen ion gradient is generated during electron transport. According to the chemiosmotic theory of energy conservation, the energy stored in the hydrogen ion gradient can be utilized to effect ATP synthesis (8, 9) or the transport of a variety of substances (10, 11, 12). The present report shows that similar pH changes accompany the oxidation of D-lactate by membrane vesicles obtained from lysed Escherichia coli spheroplasts.

METHODS

E. coli B/r (obtained from Dr. M. Abou-Sabe') was grown in Medium A

(13) with 0.2% glucose. Membrane vesicles were prepared by the lysozyme-lysis method of Kaback (14) and stored in 0.1 M potassium phosphate (pH 6.6) at 0° C. Membranes were suspended in 0.1 M KCl - 5 mM glycylglycine (pH 6.6) at about 1 mg protein/ml. A combination pH electrode (Beckman #39013) was inserted through a hole drilled in a rubber serum stopper that was fitted into the top of the sample cup to provide an air-tight seal. Nitrogen, deoxygenated by bubbling through alkaline dithionite (15), was passed over the suspension (5 ml) throughout the experiment. The pH was monitored with a Beckman Expandomatic pH meter and a recorder. Pulses of oxygen were added as small quantities of 0.1 M KCl saturated with air that had been passed through saturated $Ba(OH)_2$ to remove dissolved CO_2. It was assumed that the oxygen content of the air-saturated KCl was 0.47 µg-atoms per ml (16). The buffering capacity of the suspension was determined by adding small quantities of standard HCl or KOH and noting the pH change. All experiments were carried out at 25 ± 0.02° C and at pH 6.5 - 6.6.

Oxygen uptake studies were done at 25° C using a YSI Model 53 Oxygen Monitor (Yellow Springs Instrument Co.). The membranes utilized oxygen rapidly (> 150 ng-atoms/min/mg protein) in the presence of 10 mM D-lactate or 1 mM NADH. Oxygen uptake was much slower (10 - 20 ng-atoms/min/mg protein) in the presence of either L-lactate, DL-α-hydroxybutyrate or disodium succinate (10 mM each). Endogenous oxygen uptake was negligible (< 2 ng-atoms/min/mg protein). Protein was determined by the method of Lowry et al. (17).

RESULTS

When a small quantity of an air-saturated KCl solution was added to an anaerobic suspension of membrane vesicles in the presence of 10 mM D-lactate, the pH of the suspension fell rapidly and then gradually returned to its original value (Fig. 1-A). No change in pH was observed when anaerobic KCl was added or when air-saturated KCl was added in the

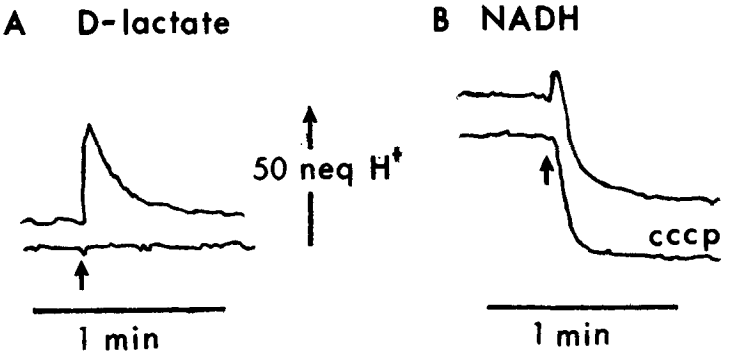

Figure 1. pH changes during D-lactate and NADH oxidation by membrane vesicles. The reaction mixture contained 5 mg membrane protein in 5 ml anaerobic 0.1 M KCl - 5 mM glycylglycine (pH 6.6) plus additions given below. Air-saturated 0.1 M KCl (0.1 ml) added at arrows. A. Upper trace: Reaction mixture contained 10 mM Li D-lactate. Lower trace: D-lactate absent. B. Upper trace: Reaction mixture contained 1 mM NADH. Lower trace: 1 mM NADH plus 10 μM CCCP.

absence of D-lactate (Fig. 1-A). All the oxygen in the added KCl was utilized within a few seconds. The amount of acid produced during the pulse was determined by extrapolating the initial fall in pH and the initial rate of pH decay to their point of intersection. The H^+/O ratio so calculated was 0.94 ± 0.02 equivalents/g-atom (mean of 9 determinations ± standard error of the mean).

No pH changes were generated during a oxygen pulse by membranes in the presence of glucose, glycerol, potassium acetate, sodium pyruvate or DL-β-hydroxybutyrate (10 mM each), substances which are not oxidized by the preparations. If the small number of contaminating whole cells and spheroplasts in the membrane preparations were responsible for the pH changes observed during D-lactate oxidation, at least some of these substrates would be expected to support similar changes. Small but significant pH changes were observed in membrane suspensions containing the poorly oxidized substances succinate, L-lactate and DL-α-hydroxybutyrate.

A trace of the pH changes observed during NADH oxidation is shown in Fig. 1-B. A small drop in the pH immediately preceded a much larger pH rise due to the net consumption of protons according to the reaction:

$$NADH + H^+ + 1/2\ O_2 \longrightarrow NAD^+ + H_2O$$

The small pH drop is reproducible and is not an addition artifact, as seen by its elimination in the presence of 10 μM carbonyl cyanide m-chlorophenylhydrazone (Fig. 1-B). This initial pH drop may represent the counterpart of the pH changes seen during D-lactate oxidation. The net pH change of the reaction precluded calculating an H^+/O ratio.

The effects of electron transport inhibitors and uncouplers on the pH changes observed during D-lactate oxidation are shown in Table I. Potassium

Table I.

Effects of Inhibitors and Uncouplers on pH Changes during D-lactate Oxidation

Addition	Conc. (μM)	O_2 Uptake Inhibition	H^+/O
none	—	0 %	0.94
KCN	1000	71	0.70
	5000	93	0.56
HOQNO	8	16	0.84
	40	42	0.37
CCCP	2	12	0.60
	5	13	0.42
	10	30	0.16
DNP	100	- 7	0.81
	200	0	0.66

Membranes were incubated with KCN for 30 min, with the nitrogen flow discontinued, prior to H^+/O determinations. CCCP and HOQNO were added as ethanolic solutions. Equivalent amounts of ethanol alone were without effect on oxygen uptake or the H^+/O ratio.

cyanide and 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO) reduced the observed H^+/O ratio at concentrations that inhibited D-lactate oxidation.* The uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP) greatly reduced the transient pH changes at concentrations that only slightly inhibited D-lactate oxidation. Substantial reduction in the H^+/O ratio was observed with 200 μM 2,4-dinitrophenol (DNP) although DNP had no effect on the rate of D-lactate oxidation. The inhibitory effects of CCCP and DNP may be related to the ability of these compounds to increase the hydrogen ion permeability of lipid bilayer, mitochondrial and bacterial membranes (8, 11, 18).

The H^+/O ratios given above should not be regarded as accurate measurements of the amount of acid produced during an oxygen pulse. The electrode-pH meter assumbly did not respond rapidly enough to measure accurately pH changes occurring within a few seconds. The addition of valinomycin (5 μg/ml) or KCNS (20 mM) to membrane preparations increased only slightly the observed H^+/O ratios. The addition of $MgSO_4$ (10 mM) had no effect on the magnitude of the pH changes.

DISCUSSION

The data presented above suggest that protons are pumped from the vesicle interior during electron transport and diffuse back once all the oxygen in the pulse is utilized. To maintain electroneutrality, the protons must either be accompanied by anions or exchange for external cations. Alternative explanations cannot be excluded, however. For example, the pH changes could result from the exposure of additional ionizable groups during transient conformational changes in the membrane proteins.

It has been suggested that a transmembrane hydrogen ion gradient provides the immediate driving force for the active transport of a variety of compounds

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* Cyanide (5 mM) did not completely block the observed pH changes although it reduced oxygen uptake by more than 90% when added to membranes oxidizing D-lactate. Cyanide was not nearly as effective, however, when added to anaerobic membranes. When oxygen was added to membranes incubated anaerobically for 30 minutes with 10 mM D-lactate and 5 mM KCN, the initial rate of oxygen uptake was nearly 90% of the rate in the absence of KCN.

in bacterial cells (10, 11, 12). The results reported here, taken together with the known ability of D-lactate oxidation to stimulate transport of amino acids (19) and β-galactosides (20) in membrane vesicles of E. coli, are consistent with this view. It must be emphasized, however, that these studies do not necessarily imply that there is a causal relationship between the apparent pH gradient generated by D-lactate oxidation and the reported stimulation of transport.

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