pH PROFILES OF PROLINE TRANSPORT WITH MEMBRANE VESICLES FROM MYCOBACTERIUM PHLEI WITH ARTIFICAL AND NATURAL ELECTRON DONORS

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SUMMARY. Proline transport and rates of oxidation with artifical and natural electron donors were examined with membrane vesicles from M. phlei as a function of pH. The levels of transport and rates of oxidation were parallel with generated NADH as substrate. With ascorbate-TPD, both the rate and level of transport increased from pH 7.5 to 9.0. The rate of total oxygen consumption with ascorbate-TPD correlated with the transport studies. However, when oxygen consumption was corrected for the autooxidation of ascorbate-TPD, the corrected oxygen consumption did not correlate with transport. Rates of cytochrome reduction were examined with ascorbate-TPD as electron donors to determine respiratory chain oxidation. For cytochromes c and $a + a_3$, the rates of reduction decreased as a function of pH, while active transport of proline increased.

INTRODUCTION

A number of investigators (1-3) have demonstrated a need for respiration to support active transport processes in bacterial systems. However, the mechanism by which the respiratory chain is coupled to the energy used to support active transport remains unclear. Kaback and collaborators have suggested that transport of amino acids and sugars in membrane vesicles of Escherichia coli is coupled by a respiratory-linked system (4, 5). In addition, transport of metabolites in E. coli and M. phlei may occur independently of oxidative phosphorylation (1-3) or under anaerobic conditions (3, 6). Studies with membrane vesicles (ETP) of M. phlei have shown that when transport of proline is initiated with different substrates, both the rates and levels of accumulation varied (1). Substrates most effective for transport are least effective for oxidative phosphorylation. Ascorbate-tetramethyl-p-phenylenediamine (TPD), which donates electrons to the respiratory chain at the level of cytochrome c (7), supported proline transport in ETP better than generated NADH. In contrast, generated NADH had a greater rate of oxidation by ETP

than that observed with ascorbate-TPD. Thus, one must ask why substrates which use the same portion of the respiratory chain would differ in their ability to support active transport.

METHODS AND MATERIALS

Membrane Preparations - The growth conditions and the preparation of the ETP from M. phlei (ATCC 354) have been described (8). The ETP were suspended in water.

Protein Estimation - Protein was estimated by a modification of the Biuret method (9).

Proline Uptake - The assay system for proline uptake by membrane preparations was similar to that used previously (1). The level of transport refers to the steady state level of transport, i.e., the amount of proline accumulated 15 min following substrate addition, and the rate of transport refers to the initial rate of proline transport, measured within the first minute.

Rate of Oxidation - Oxygen consumption of ETP was measured at 30° with a Yellow Springs Instrument Model 53 Oxygen Monitor with ascorbate-TPD, ascorbate-phenazene methosulfate (PMS), or generated NADH as substrate. Both oxygen consumption and rate and/or level of proline transport were examined as a function of pH from pH 7.5 to pH 9.0, using Tris-HCl buffers. The rates of auto-oxidation (in the absence of ETP) for both the ascorbate-TPD and ascorbate-PMS systems were significant and all rates were corrected for auto-oxidation at each pH value.

Cytochrome Reduction - The rate of cytochrome reduction was followed with an Aminco DW-2 double beam spectrophotometer. The wavelength pairs used were 551-540 nm for cytochrome c and 598-623 nm for cytochromes $a + a_3$ (7).

RESULTS AND DISCUSSION

The rate of oxygen consumption as a function of pH with generated NADH as electron donor was maximal at pH 8.0 (Fig. 1). Under the same conditions, the steady state level of proline transport with generated NADH was found to parallel the rate of oxygen consumption (Fig. 1).

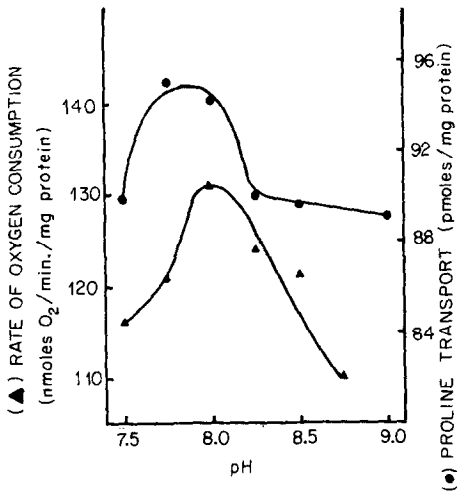


Fig. 1.

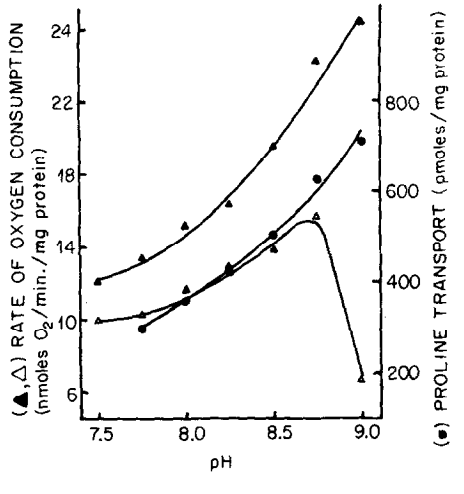


Fig. 2.

Figure 1 (left). pH Profile for Rate of Oxygen Consumption and Proline Transport with Generated NADH.

The system contained 50 mM Tris buffer adjusted to the appropriate pH, 10 mM $MgCl_2$, 25 μM ^{14}C-proline, 0.67 mM NAD^+, 2.2 μM ADH, 6.67 mM semicarbazide (pH 7.2), and 28.6 mM ethanol.

Figure 2 (right). pH Profile for Rate of Oxygen Consumption and Proline Transport with Ascorbate-TPD.

The system contained 50 mM Tris buffer, 10 mM $MgCl_2$, 25 μM ^{14}C-proline, 17 mM Na-ascorbate, and 1.5 mM TPD. The oxygen consumption is expressed as nmoles O_2/min/mg protein for the rate of total oxygen consumption (▲) and the rate of oxygen consumption corrected for the auto-oxidation of ascorbate-TPD (Δ).

With ascorbate-PMS as electron donors, the rate of total oxygen consumption increased from pH 7.5 to pH 9.0 (Table I). When these values were corrected for the auto-oxidation of ascorbate-PMS, the corrected rate of oxygen consumption was highest at pH 7.5, lowest at pH 9.0 and intermediate at all pH levels examined between these extremes (Table I). The rate of proline transport increased from pH 7.5 to 8.5 and then reached a plateau (Table II). The steady state levels of transport also increased with an increase in pH, but began to decrease at pH values of 8.5 and beyond (Table II).

The corrected rate of oxygen consumption with ascorbate-TPD as electron donors increased to pH 8.75 and then dropped at pH 9.0 (Fig. 2). However, when

TABLE I

The Rate of Oxygen Consumption of Ascorbate-PMS as a Function of pH

	nanomoles O_2/min/mg protein		
pH	Total	Auto-oxidation	Corrected
7.50	520	342	178
8.00	591	465	126
8.25	655	532	123
8.50	731	683	93
8.75	807	690	117
9.00	871	825	46

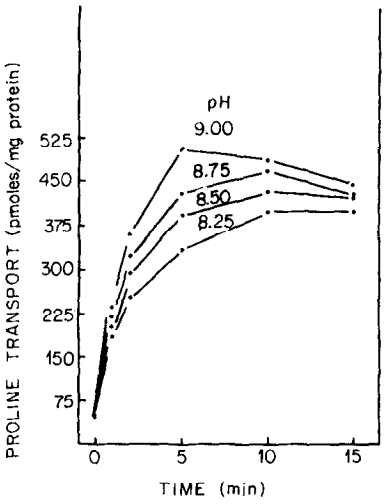
The system for ascorbate-PMS was identical to that for ascorbate-TPD (Figure 2) except that 1.5 mM PMS was substituted for TPD.

TABLE II

pH Profile of Proline Transport with Ascorbate-PMS

Minutes after addition of substrate	picomoles proline/mg protein					
	pH 7.5	pH 8.0	pH 8.25	pH 8.5	pH 8.75	pH 9.0
0	46	45	41	45	40	42
1	391	491	523	554	545	543
2	429	597	664	740	686	723
5	558	821	900	973	883	881
10	695	921	993	1005	902	861
15	791	967	972	955	860	800

the rate of oxygen consumption was not corrected for the auto-oxidation of ascorbate-TPD, the rate of total oxygen consumption increased through the pH range examined and paralleled an increase in the steady state levels of proline transport also examined in the same pH range (Fig. 2). Not only the steady state level, but also the rate of proline transport increased with pH. The data for both rate and level of



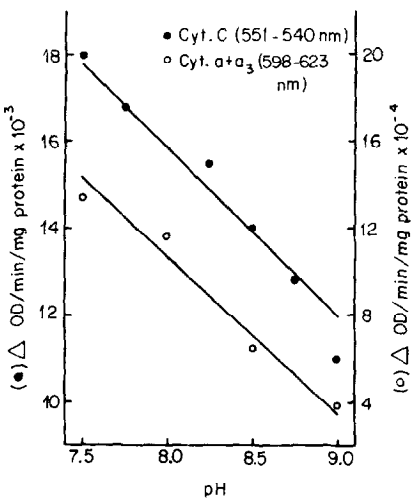


Figure 3 (left). Time Course of Proline Transport as a Function of pH with Ascorbate-TPD.

The system is identical to that described in Figure 2.

Figure 4 (right). Rate of Cytochrome Reduction as a Function of pH with Ascorbate-TPD.

The rates of cytochrome reduction were examined at 30°C. The system was identical to that used with the oxidative studies described in Figure 1 except that the TPD concentration was decreased to 0.15 mM when measuring rates for cytochromes $a + a_3$.

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transport with ascorbate-TPD are shown only for pH 8.25 and above in Figure 3.

It was of interest that under conditions of increased pH there was a drop in the apparent rate of oxidation with a concurrent increase in proline transport when artifical electron donors were used. Since the rate of auto-oxidation increased more readily at the higher pH values than at the lower pH values, it was difficult to determine if the amount of auto-oxidation which occurred in the absence of the membrane vesicles was the same as which occurred in the presence of the membrane vesicles. Hence, the rates of reduction of cytochrome c and $a + a_3$ were examined to determine the rate of oxidation via the respiratory chain.

The initial rate of cytochrome c reduction measured at transition with ascorbate-TPD as electron donors was found to decrease from pH 7.5 to 9.0 (Fig. 4) while both the rate and steady state level of proline transport increased

in the same pH range (Fig. 3). In order to eliminate the possibility that TPD might bypass cytochrome c at high pH values and enter at cytochromes $a + a_3$, rates of reduction of cytochromes $a + a_3$ were examined and found to decrease as the pH was increased (Fig. 4).

Studies were undertaken to determine whether the auto-oxidation of ascorbate-TPD would form a chemical species which would support proline transport. Ascorbate-TPD was added to a system at pH 9.0 in the absence of ETP and allowed to auto-oxidize for either 1, 2, 5, 10 or 15 minutes. The system was then pulsed with ETP for either 30 or 60 seconds and then measured for proline transport. The amount of transport in these pulse experiments was not greater than a normal 30 or 60 second incubation. It was of interest that transport could be initiated by adding TPD alone. It was not possible to detect a difference in transport with TPD alone by varying pH from 7.5 to 9.0.

The dependence of transport upon substrate oxidation would suggest that pH profiles for both transport and oxidation should be parallel. This was observed with generated NADH. When ascorbate-PMS was used as substrate, the rate of increase in oxidation was equal to or greater than transport only when the rate of total oxygen consumption was measured. And as observed with ascorbate-TPD, the level of transport was parallel only to the rate of total oxygen consumption.

In contrast, the corrected rates of oxidation for both artifical electron donors did not correlate with the transport studies that were examined as a function of pH. It should be noted that although the corrected rates of oxygen consumption and the rates of cytochrome reduction decreased with increases in pH, the rates of these parameters could still be sufficient to support proline transport. However, it would not necessarily be expected to continue at an increased level as was observed. In this regard it is of interest that at high pH with generated NADH as electron donor, the rate of oxidation continued to decrease as a function of pH, but that transport was observed to plateau at a low value.

The decrease in rates of cytochrome reduction and the effects on oxygen consumption observed with ascorbate-TPD as a function of pH could be a result of either

conformational effects on the electron transport chain or perturbations to the membrane structure. However, based on these assumptions, it would be difficult to explain both the difference in results between ascorbate-TPD and ascorbate-PMS and the increased levels of proline accumulated as both corrected oxygen consumption and cytochrome reduction decreased. It is also of interest that oxidative phosphorylation in M. phlei has been shown to decrease at high pH values (10).

It has been noted that although generated NADH is oxidized by ETP at a faster rate than ascorbate-TPD, the level of transport with ascorbate-TPD is greater than that supported by generated NADH. This study has shown that proline transport could be correlated to the rate of total oxygen consumption when examined as a function of pH. The oxidation of NADH differs from that of ascorbate-TPD in that artifical electron donors are capable of high levels of auto-oxidation. It would be of interest to further determine the relationship, if any, of auto-oxidation to proline transport. This difference in oxidation of NADH and that described for ascorbate-TPD may explain the high level of transport observed with the artifical electron donors. The decrease in respiratory chain oxidation and the increase in auto-oxidation of ascorbate-TPD and in proline transport may suggest a secondary mechanism which is not a respiratory chain linked process.

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