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Relationship Between Amino Acid Transport and Electron Transport by Membrane Vesicles of *Micrococcus denitrificans*[1]

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Membrane vesicles were prepared from *Micrococcus denitrificans* by osmotic shock of lysozyme spheroplasts. These vesicles concentrated 4 amino acids via two systems; one for glycine-alanine and the other for asparagine-glutamine. Amino acid transport was coupled to the membrane-bound electron transport system and involved interactions of the primary dehydrogenases, cytochromes, cytochrome oxidase and oxygen. After transport the amino acids were recovered unchanged from the vesicles. The substrates of the membrane-bound electron transport system D-lactate, L-lactate, formate, succinate, NADH, glucose-6-phosphate and α-glycerolphosphate all stimulated transport at least 2-fold. Both oxygen and nitrate could serve as terminal electron acceptors with vesicles prepared from cells grown anaerobically with nitrate. Anaerobic transport in the presence of nitrate was not inhibited by cyanide but was inhibited by nitrite. A system stimulated by substrates of the electron transport system but independent of added terminal electron acceptors was found also in the vesicles prepared from anaerobically grown cells. Addition of one combination of two substrates for electron transport produced an amino acid uptake 12 to 15% greater than the sum of the rates for each substrate added singly. Additions of other combinations gave rates of transport less than the sum of the rates of each added alone. Both the dehydrogenase activities and the coupling of electron transport to amino acid uptake were modified by changing the growth conditions and differences between the effectiveness of each substrate for each of the two transport systems could be detected. The efficiency of the vesicles per protoheme, the prosthetic group of the membrane-bound cytochrome *b*, with D-lactate as substrate was 27% for glutamine and 6% for glycine of the rates of transport of these two amino acids in intact cells when driven by endogenous respiration. Assuming one amino acid transported per electron, the transport of glycine utilized 1% of the respiratory capacity with glucose-6-phosphate as substrate. The coupling to the electron transport with the other substrates was less efficient. It appeared that a small portion of the total capacity of the electron transport system was coupled to amino acid transport and the coupling to respiration, as well as the primary dehydrogenase activities and terminal cytochrome oxidase, were modified in response to the conditions of growth.

Micrococcus denitrificans is a remarkable bacterium which has an electron transport system with a composition of respiratory pigments much like beef heart mitochondria (1) and a lipid composition rich in oleic acid and phosphatidylcholine (2), again more like mitochondria than the enteric bacteria.

Preliminary observations showed that the membrane vesicles prepared from this organism contained an amino acid transport system that was activated by substrates of the electron transport system or by phenazine methosulfate (PMS) with ascorbate, as the electron donors (3). In

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this study we have followed up these initial observations to define more clearly the relationship between the electron transport system and amino acid transport system. In contrast to the other well studied bacterial amino acid transport systems of *Escherichia coli* (4, 5), *Bacillus subtilis* (6), or *Staphylococcus aureus* (7, 8), *M. denitrificans* vesicles showed transport of only 4 amino acids. It appeared that the coupling of the amino acid transport system to the electron transport system was much more like that of *B. subtilis* in which several substrates activated transport than that of *E. coli* or *S. aureus* vesicles in which a single primary dehydrogenase seemed to be most tightly coupled with amino acid transport.

MATERIALS AND METHODS

Materials

Radioisotopes, solvents, reagents were procured as in previous studies (2, 7, 8).

Organism

M. denitrificans ATCC 13543 was obtained from Lucile Smith and was grown at 30°C in a semi-defined medium containing salts, glucose, and casamino acids (Difco Products, Detroit, MI) (2). Anaerobic cultures were grown in the same medium deoxygenated by sparging with argon and with the addition of 20 mM KNO_3.

Preparation of Vesicles for Transport Studies

The vesicle preparation follows that described by Kaback (9). Exponentially growing cells were harvested by centrifugation at a density of 0.41 mg dry wt/ml, washed with an equal volume of cold 10 mM phosphate buffer pH 7.0 and suspended in phosphate buffer containing 0.5 M sucrose at a density of 2.0 mg dry wt/ml. Lysozyme was added (0.15 mg/mg dry wt) and incubated for 30 min at 30°C. At this time a tenfold dilution of the cell mixture into phosphate buffer resulted in a fourfold decrease in turbidity as compared to a similar dilution containing sucrose. The cells were centrifuged and resuspended by gentle homogenization in 30 ml of cold 0.1 M phosphate buffer pH 6.8 containing 0.5 M sucrose and 3 mg each of DNase and RNase. The suspension was added to 6 liters of 50 mM phosphate buffer pH 6.8 at 30°C that was swirling gently. After 15 min potassium ethylenediaminetetraacetate (EDTA) pH 7.0 was added to 10 mM final concentration; after another 15 min $MgSO_4$ was added to 15 mM. Fifteen min later the suspension was centrifuged at 16,000*g* for 30 min at 0°C and resuspended in 0.1 M phosphate buffer containing 10 mM EDTA. The preparation was gently homogenized and centrifuged at 800*g* for 30 min. This low speed centrifugation was repeated twice on the supernatant. The pellets from this series were homogenized in the same buffer and the series of three centrifugations at 800*g* were repeated. The pellets containing whole cells and debris were discarded and the two final supernatants were combined and centrifuged at 16,000*g* for 30 min. The pellet of vesicles was washed with phosphate buffer (0.1 M pH 6.8) at a density of 2.3 mg dry wt/ml. One ml aliquots were placed in plastic test tubes, frozen in liquid nitrogen, and stored at −90°C. Anaerobic vesicles were prepared from *M. denitrificans* grown with nitrate by deoxygenating all solutions by sparging with argon and maintaining a flow of argon over all vessels during their preparation or storage.

Measurement of Transport

Transport activity of the vesicle preparation was determined by incubating about 100 μg of vesicle protein in 50 mM phosphate buffer pH 6.8 containing 10 mM $MgSO_4$ in 0.1 ml for 2 min at 30°C. Transport was initiated by adding substrate (20 mM final concentration) and ^{14}C-labeled amino acid (20 μM final concentration). Transport was terminated by the addition of 2 ml of 0.1 M LiCl and pouring the mixture over a 30-mm Millipore filter. The test tube was washed with an additional 2 ml of LiCl which was also used to wash the vesicles held on the filter. The filters were dried and counted in the scintillation spectrometer. The radioactivity was corrected for nonspecific adsorption of amino acid by subtracting a zero time control. When ascorbate-PMS was used as the substrate the vesicle preparation was saturated with oxygen before adding the substrate. The anaerobic vesicles were assayed using deoxygenated solutions unless otherwise indicated.

RESULTS

Vesicle Preparation from M. denitrificans

Membrane vesicles were prepared from *M. denitrificans* after osmotic shock of lysozyme treated spheroplast preparations using the techniques developed by Kaback (9). These vesicles have much in common with those prepared from *Staphylococcus aureus* (7, 8), *Escherichia coli* (4, 5) and *Bacillus subtilis* (6).

Figure 1 illustrates the kinetics of glycine uptake by the membrane vesicles of *M. denitrificans*. In this preparation the concentrative ability of the vesicles was dependent on the presence of a substrate for the electron transport system. The kinetics

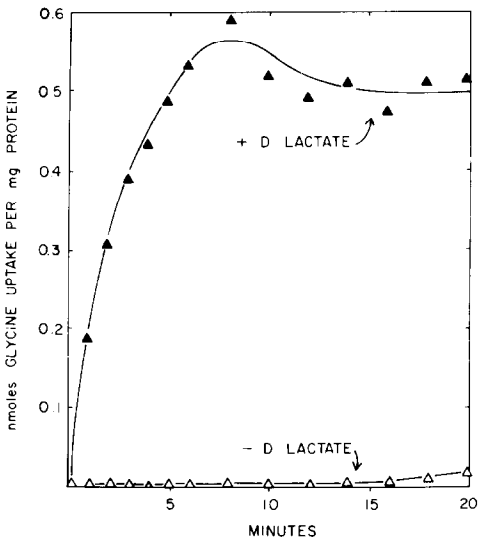

FIG. 1. Kinetics of glycine uptake by vesicles prepared from *Micrococcus denitrificans*. The vesicles (0.112 mg protein) in 0.1 ml 50 mM phosphate buffer pH 6.8 containing 10 mM $MgSO_4$ were preincubated at 30°C for 2 min. D-Lactate (20 mM final concentration) was added to one set of samples (▲). At time zero 1 nmole [1-^{14}C]glycine (165,000 cpm/nmole) was added to the reaction mixtures. The reactions were stopped by the addition of 2 ml of 0.1 M LiCl as described in Methods. All samples were corrected for nonspecific binding.

showed the saturation phenomenon typical of the vesicles prepared from other bacteria. These membrane vesicles contained essentially all the phospholipids and the respiratory pigments found in the intact cells. The vesicle preparation contained 24.1 times the protoheme (the prosthetic group of the cytochromes *b* and *o*) per mg protein as was found in the intact cells. This purification of membrane components during the preparation of the vesicles was similar to vesicles prepared from *S. aureus* in which the elimination of cytoplasmic and cell wall components was established (7).

Dependence Upon the Electron Transport System

Both the presence of a suitable substrate (Fig. 1) and the function of the electron transport system were essential for glycine uptake by the vesicles (Table I). Oxamate which inhibited electron transport at the primary dehydrogenase (10), 2-n-nonyl-4-hydroxyquinoline-*N*-oxide (NOQNO) which blocks between cytochromes *b* and *c* (11, 12) and azide, cyanide and carbon monoxide which inhibited the cytochrome oxidase *a* plus a_3 (11, 12) all decreased the capacity of the membrane vesicles to concentrate glycine in the presence of D-lactate. Oxygen was required as the terminal electron acceptor.

Gassing the preparation with nitrogen inhibited glycine transport (Table I). The inhibitor carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), which blocked oxidative phosphorylation (13), inhibited glycine uptake by these vesicles. Arsenate had little effect on the transport of amino acids. *p*-Hydroxymercuribenzoate (pHMB), which inhibited transport in *E. coli* vesicles by tightly binding to the carrier (10), blocked the concentrative transport of glycine (Table I). Dicyclohexylcarbodiimide (DCCD), an inhibitor of the membrane-bound ATPase in many systems (4), blocked glycine transport. *Micrococcus denitrificans* grown aerobically contained no detectable nitrate reductase activity, nor was nitrate a suitable electron acceptor for glycine concentration under anaerobic conditions. If *M. denitrificans* was grown anaerobically with nitrate, a nitrate reductase was formed and nitrate could then serve as an electron acceptor for anaerobic glycine uptake (Table I). The transport system formed during anaerobic growth used either oxygen or nitrate as a terminal electron acceptor and had the same requirement for substrate as the aerobic cells, but was one-fourth as active with oxygen and one-fifth as active with nitrate. The transport system was inhibited by NOQNO and CCCP with either terminal electron acceptor. The anaerobic system seemed less sensitive to DCCD than the aerobic system. Glycine transport by the vesicles from anaerobically grown cells showed cyanide sensitivity with oxygen as terminal electron acceptor, but not if nitrate was the terminal electron acceptor (Table I).

Number of Amino Acid Transport Systems

In contrast to *E. coli* and *B. subtilis*, which contained nine amino acid transport systems (4-6), or *S. aureus*, which contained twelve amino acid transport systems (8), *M. denitrificans* containd two independent systems which concentrate four amino acids (Table II). Only four amino acids were concentrated if substrates of the electron transport system or the artificial electron donor system ascorbate-PMS were used to stimulate transport. The vesicles did not accumulate mono- or dicarboxylic acids or carbohydrates when stimulated with substrates as ascorbate-PMS. Only glycine-alanine or glutamine-asparagine were concentrated. Vesicles from anaerobically grown cells showed the same specificity.

Recovery of the Transported Amino Acid

It was important to determine whether the mechanism of concentration of the amino acid involved chemical change in the amino acid. Membrane vesicles (1.1 mg protein per ml) were incubated with 0.2 μM [U-^{14}C]glycine or 0.2 μM [U-^{14}C]glutamine in 50 mM phosphate buffer pH 6.8 containing 10 mM $MgSO_4$ and 20 mM D-lactate for 15 min at 30°C. The vesicles were filtered and washed with 0.1 M LiCl. The membrane filters were suspended in 3 ml of distilled water and heated at 40°C for 1 hr. The water was decanted, evaporated, and

TABLE I

EFFECT OF INHIBITORS OF ELECTRON TRANSPORT ON GLYCINE UPTAKE BY MEMBRANE VESICLES OF *M. denitrificans*

Inhibitor	Glycine uptake[a]						
	Aerobic vesicles[b]			Anaerobic vesicles[c]			
	Concentration	nm/min/mg	% inhibited	nm/min/mg	% inhibited	nm/min/mg[d]	% inhibited
Control	—	2.30	—	0.60	—	0.51	—
Oxamate	5 mM	1.32	43	—	—	—	—
NOQNO	0.08 mM	0.82	64	0.31	48	0.22	58
CCCP	0.01 mM	0.30	87	0.16	73	0.25	52
Azide	2 mM	1.4	39	—	—	—	—
DCCD	0.1 mM	1.35	41	0.54	10	0.46	9
Cyanide	2 mM	0.54	76	0.35	41	0.58	0
Carbon monoxide[e]	—	1.13	51	—	—	—	—
pHMB	10 μM	0.06	98	—	—	—	—
Nitrogen[f]	—	0.34	85	—	—	—	—
Na_2HAsO_4	50 mM	2.30	0	—	—	—	—
No D-lactate[g]	—	0.09	97	0.07	89	0.06	89

[a] Glycine transport was measured as described in Methods using 0.11 mg membrane vesicle protein in 50 mM phosphate buffer pH 6.8 containing 10 mM $MgSO_4$ in a volume of 0.1 ml. After 2 min preincubation at 30°C D-lactate (20 mM) and 2 nmoles uniformly labeled [^{14}C]glycine were added and the reaction stopped after 3 min. Transport of glycine in the anaerobic vesicles was measured at 23°C for 5 min. The filters were counted in a scintillation spectrometer with an efficiency of 82%. The abbreviation NOQNO represents 2-*n*-nonyl-4-hydroxy quinoline *N*-oxide; CCCP is *m*-carbonyl cyanide-*m*-chlorophenyl hydrazone; pHMB is *p*-hydroxymercuribenzoate; DCCD is dicyclohexylcarbodiimide.

[b] Aerobic vesicles denotes vesicles prepared from cells grown in the absence of nitrate with shaking.

[c] Anaerobic vesicles denotes vesicles prepared from cells grown without aeration in medium containing 20 mM KNO_3.

[d] Transport of glycine in this series was measured in the presence of 20 mM KNO_3 while gassing the incubation mixture with argon.

[e] Carbon monoxide was blown across the mixture for several minutes before assay.

[f] The incubation was gassed with nitrogen during the entire assay.

[g] Control in the absence of substrate.

TABLE II

AMINO ACID TRANSPORT SYSTEMS OF *Micrococcus denitrificans*

^{14}C Labeled amino acids[a]	% Inhibition by nonradioactive amino acid			
	Glycine	Alanine	Asparagine	Glutamine
Glycine	83.1[b]	63.4	2.7	12.8
Alanine	90.4	77.2	10.9	15.6
Asparagine	2.5	7.6	34.2	53.8
Glutamine	22.0	15.4	62.6	74.6

[a] Amino acid uptake was measured after 5 min as in Table I in the presence of 121 μg vesicle protein for each ^{14}C-labeled amino acid (0.1 μM) in the presence of the non-labeled 0.2 mM amino acids listed.

[b] Data expressed as percent inhibition of the samples in the presence of unlabeled amino acids as indicated when compared with controls in the absence of unlabeled amino acids. Control rates were: glycine, 0.51; alanine, 0.69; asparagine, 0.17; and glutamine, 0.24 nmoles per min per mg protein. Relative stimulation by the addition of 20 mM D-lactate was 136-fold for glycine, 60-fold for alanine, 28-fold for asparagine, and 52-fold for glutamine.

the residues chromatographed on acid washed paper in a solvent of methanol, water, pyridine; 80, 20, 4 (v/v) in the first dimension, and tertiary butanol, methyl ethyl ketone, water, diethylamine; 40, 40, 20, 4 (v/v) in the second dimension. The [U-^{14}C]glycine or [U-^{14}C]glutamine added to the vesicles and that recovered from the vesicles after transport showed identical chromatographic mobilities; glycine was quantitatively recovered and 99.14% of the added glutamine was recovered. The remaining 0.86% of the glutamine was recovered as glutamic acid.

Substrate Dependence of Amino Acid Transport

The vesicle preparations utilized a number of substrates to stimulate the transport of glycine or glutamine (Table III). Glucose-6-phosphate, α-glycerol phosphate (GP), succinate, NADH, D-lactate, L-lactate or formate caused significant uptake of glycine and glutamine.

In cells grown in the presence of nitrate, nitrate was able to serve as a terminal electron acceptor for glycine or glutamine transport (Table III). Using D-lactate as a substrate KNO_3 served as a terminal electron acceptor with a K_m about that of the system formed when the cells were grown in air. Nitrite acted as a competitive inhibitor.

The evidence in Table III suggests that the two different transport systems utilize different portions of the electron transport system. Formate stimulated maximum amino acid transport for both systems with aerobic cells. With anaerobically grown cells D-lactate was the best substrate. The rate of glycine transport was about fourfold greater than for glutamine with aerobic cells. Succinate was a better substrate for glutamine uptake (26% the rate of formate uptake) than for glycine transport (16% of the rate of formate uptake) with vesicles from aerobically grown cells. L-Lactate produced a rate of glycine uptake equal to 34% of the rate of glycine uptake with formate but was only 20% as effective as formate with glutamine transport with vesicles from aerobically grown cells. The rate of ascrobate-PMS induced glycine transport was 2.7 times the rate of glutamine uptake with vesicles from aerobically grown cells (Table III). With vesicles from anaerobically grown cells the transport of glycine and glutamine was about equal with formate as substrate but glycine was transported 20–50% more efficiently than glutamine with the other substrates. The maximal rate of uptake with ascorbate-PMS as electron donor was 77% of the rate of uptake with formate as substrate for glutamine, and 54% of the rate of glycine uptake with formate as substrate in vesicles from aerobic cells.

Effects of Combination of Substrates on Transport

The addition of two substrates for the primary dehydrogenases showed different effects on the rate of amino acid transport than the addition of each substrate alone (Table IV). Additions of some combinations of substrates resulted in a glycine uptake characteristic for one of the substrates rather than the sum of the activities of both when added separately. The addi-

tion of D-lactate and GP on the other hand produced a rate of amino acid transport about 12–15% greater than the sum of the rates for each substrate added singly in both the amino acid transport systems. With vesicles from anaerobically grown cells the combination of GP and D-lactate produced a rate significantly less than the sum of the two rates of glycine transport with GP or D-lactate added singly (Table IV). This was true with the anaerobic system with or without nitrate as added terminal electron acceptor.

Effect of Changing the Growth Medium

As is common with many bacteria, changing the major carbon source in the growth medium changed the activity of the primary dehydrogenases of the electron transport system (14). In semidefined medium containing amino acids, the addition of glucose repressed the formation of formate and lactate dehydrogenases compared to membranes derived from cells grown with succinate (Table V). In these membrane preparations from bacteria grown with either substrate the activity of succinic dehydrogenase was little affected. The addition of 0.4% peptone and 0.2% yeast extract (w/v) to the medium containing succinate increases the formate dehydrogenase activity ninefold and the succinic dehydrogenase activity threefold/mg membrane protein (data not shown).

A comparison of the glycine uptake of vesicles prepared from cells grown with either glucose or succinate in defined media revealed a two- to threefold greater rate of glycine uptake with D-lactate and formate for the vesicles prepared from cells grown with glucose than with those grown with succinate (Table VI). Note that the

TABLE III

AMINO ACID UPTAKE AND OXYGEN UTILIZATION BY MEMBRANE VESICLES OF *Micrococcus denitrificans*

Substrate	Glycine transport[a]	Glutamine transport	Oxygen utilization[b]	Glycine transport[c]			Glutamine transport[d]		Nitrate reductase[e]
				Oxygen	Anaerobic	Anaerobic + nitrate	Anaerobic	Anaerobic + nitrate	
None	0.03	0.006	<1.0	0.01	0.08	0.05	0.007	0.001	20
Glucose	0.03	0.006	<1.0	0.08	0.04	0.05	0.006	0.001	20
Glucose-6-phosphate	0.34	0.06	7.8	—	—	—	—	—	—
α-glycerol-phosphate	0.62	0.21	8.0	0.24	0.24	0.24	0.09	0.09	50
NADH	0.62	0.18	782.0	0.26	0.24	0.24	0.08	0.08	833
Succinate	0.64	0.28	296.0	0.22	0.23	0.21	0.09	0.09	700
D-lactate	1.64	0.41	72.0	0.87	0.27	0.89	0.51	0.65	120
L-lactate	1.35	0.21	42.4	0.57	0.21	0.48	0.35	0.39	70
Formate	4.05	1.05	54.0	0.54	0.15	0.51	0.37	0.52	100
Ascorbate + PMS[f]	2.17	0.81	—	0.74	0.28	0.22	0.09	0.15	144

[a] Transport measured as nmoles amino acid/min/mg vesicle protein with a vesicle preparation from cells grown aerobically without nitrate suspended in 50 mM phosphate buffer pH 6.8 containing 10 mM $MgSO_4$ in 0.1 ml. Transport was measured as in Table I.

[b] Oxygen utilization was measured in nmoles oxygen utilized/min/mg protein with the Clark oxygen electrode in a 1-ml chamber containing 0.60 mg vesicle protein at 30°C. The substrates were present at 20 mM final concentration.

[c] Glycine transport measured with anaerobic vesicle preparation from cells grown anaerobically with nitrate measured with air saturated buffer, anaerobically (argon saturated), and anaerobically with 20 mM nitrate as in Table I.

[d] Glutamine transport in nmoles/min/mg protein measured as in Footnote *c*.

[e] Nitrate reductase measured colorimetrically (19) expressed as nmoles/min/mg vesicle protein.

[f] Oxygen utilization was measured in the presence of 20 mM ascorbate and 0.2 mM phenazine methosulfate (PMS) in buffer saturated with oxygen.

TABLE IV

COMPARISON OF AMINO ACID UPTAKE BY MEMBRANE VESICLES OF *Micrococcus denitrificans* BETWEEN COMBINATION OF SUBSTRATES ADDED TOGETHER OR ADDED SEPARATELY

Substrates	nmoles Transported/min/mg vesicle protein[a]	
	Added simultaneously	Added singly[b]
D-lactate + L-lactate	1.6	2.99
Succinate + GP	0.6	1.24
Succinate + L-lactate	1.4	2.99
D-lactate + NADH	0.7	2.26
D-lactate + GP	2.76	2.26
D-lactate + GP[c]	0.91	0.62
D-lactate + GP[d]	0.23	0.52
D-lactate + GP[e]	0.519	1.13

[a] Glycine transport measured as in Table III with the same vesicle preparation. GP indicates α-glycerol phosphate.

[b] Transport stimulated by the individual substrates taken from Table III.

[c] Glutamine uptake.

[d] Vesicles from cells grown anaerobically tested anaerobically.

[e] Vesicles from cells grown anaerobically tested anaerobically plus nitrate.

dehydrogenase activity and the rate of electron transport with D-lactate and formate was greater with the cells grown with succinate (Table V). The succinic dehydrogenase activity measured as the rate of oxygen utilization was similar in membranes from both types of cells. However, the glycine uptake in the vesicles prepared from glucose grown cells was threefold more tightly coupled to the electron transport system than in vesicles prepared from cells grown with succinate. In these experiments the relative stimulation of transport by the substrate D-lactate was between 18- and 32-fold, formate was between six- and 11-fold, and succinate about three-fold when compared with the endogenous rate of transport (Table V).

Efficiency of the Vesicles for Transport

Transport of glycine and glutamine in membrane vesicles and intact, washed, *M. denitrificans* taken from exponentially growing cells were compared. The cells contained 0.22 nmoles of protoheme/mg protein. The rate of glycine uptake for whole cells was 12.2 μmoles per min per nmole protoheme with endogenous substrate compared to 0.801 μmoles/min/nmole protoheme in the vesicles using D-lactate as substrate. Glutamine uptake in whole cells was 0.7 μmoles/min/nmole protoheme with endogenous substrate compared to 0.20 μmoles/min/nmole protoheme in the vesicles with D-lactate as substrate.

The amino acid transport system of *M. denitrificans* vesicles utilizes a small por-

TABLE V

DEHYDROGENASE ACTIVITIES IN MEMBRANE PREPARATION FROM *Micrococcus denitrificans* GROWN WITH GLUCOSE AND SUCCINATE AS CARBON SOURCES

Substrate	Oxygen uptake nmoles O_2/min/mg protein	
	Medium A[a]	Medium B
D-lactate	8.0	22.4
Formate	<1.0	5.6
Succinate	40.0	42.4

[a] The media was the defined medium described in Materials and Methods containing 20 mM glucose (A) or 20 mM succinate (B). Cells were grown to a density of 1.2 mg dry wt/ml, harvested and treated with lysozyme plus 2 mM EDTA. Then excess $MgSO_4$ and both DNase and RNase were added and the membranes collected and washed with 50 mM phosphate buffer pH 7.6. Oxygen utilization was measured as in Table III.

TABLE VI

GLYCINE UPTAKE BY MEMBRANE VESICLES PREPARED FROM *Micrococcus denitrificans* GROWN IN DEFINED MEDIUM WITH GLUCOSE OR SUCCINATE AS MAJOR COMPONENTS

Substrate	nmoles Glycine/mg protein/3 min[a]	
	Medium A[b]	Medium B
D-lactate	2.11	1.02
L-lactate	1.91	0.62
Formate	0.68	0.33
Succinate	0.29	0.09
α-glycerol phosphate	0.32	0.08
NADH	0.17	0.08
Glucose	0.17	0.04
None	0.12	0.03

[a] Transport was assayed in vesicles prepared as in Table I.

[b] Medium for growth as described in Table V.

tion of the total capacity of the electron transport system. Per electron transferred, about 1% of the capacity of the respiratory system with glucose-6-phosphate as substrate or 0.03% of the capacity with NADH was involved in glycine uptake. The efficiency was much less with anaerobically grown cells. This assumes one glycine transported per electron passed down the respiratory chain.

DISCUSSION

The data in this report suggest very strongly that *M. denitrificans* contained an amino acid transport system that was linked to its membrane-bound electron transport system (Table I) much like that reported in other bacteria (4–8, 15). The amino acid transport system can be concentrated in membrane vesicles in which amino acid transport can be studied in the absence of complications produced by cytoplasmic or wall enzymes. There was no evidence for a phosphotransferase type of system involving covalent changes in the transported amino acids as they were recovered unchanged from vesicles washed after transport. The vesicle system of *M. denitrificans* differs from the other well studied vesicle transport systems in having only two systems, each of which transported only two pairs of amino acids (Table II). No other amino acid carbohydrates, or monocarboxylic acids were transported by the vesicles. The transport system also differed from vesicle systems prepared from other bacteria in having an anaerobic transport system which was functional in vesicles (Tables I and III).

The relative simplicity of the amino acid transport system coupled with the extensive work done on the structure and function of the electron transport system in *M. denitrificans* make it a likely organism in which to study the coupling between the electron transport system and the amino acid transport system.

From a detailed study of the modifications in the functional electron transport system in *Haemophilus parainfluenzae* that result from changes in the growth environment, it seemed reasonable to consider the bacterial membrane as containing a network of cytochrome *c* molecules which connected with the cytochrome oxidases and the multiple primary dehydrogenases (14, 16–19). The primary dehydrogenases were the rate limiting step in the electron transport system (14). Adding substrates for the different dehydrogenases in combination reduced different amounts of cytochrome *c* in the anaerobic steady state. When combinations of substrates were added, the rate of electron transport was less than the sum of each rate when the substrate was added singly (14). Under other conditions the rates of transport of two substrates added simultaneously showed an activation effect of one substrate on another so the rates of electron transport with combinations of substrates exceed the sum of the rates when the substrates were added alone (14, 18).

From the present study it appeared likely that a small portion of the electron transport network surrounding the bacteria had the specialized function of active amino acid concentration. This coupling between the two systems appeared not to involve oxidative ATP coupling or production since 50 mM arsenate had no effect on transport (Table I). This suggestion of a lack of involvement of ATP in amino acid transport was typical of what was found with other bacterial systems. The inhibition by DCCD remains puzzling.

The portion of the electron transport system involved in active transport of the glycine–alanine seemed different from that portion involved with the glutamine-asparagine system as detected by the activity produced by various substrates (Table III). The glutamine-asparagine system always was less tightly coupled than the glycine–alanine system. The proportion of the total capacity of the electron transport network that was involved with active transport of amino acids was between 1% and 0.01% of the total capacity of the system with the various dehydrogenases (Table III). This calculation assumed that one amino acid is transported per electron transferred.

Adding combinations of substrates for the primary dehydrogenases showed that one substrate either competed with or

activated the other. Competition would be expected to result in a significantly slower rate when both were added than would be expected from the sum of both activities when the substrates were added singly (Table IV). This can be interpreted as showing overlapping "domains" of those dehydrogenases which were specially coupled to amino acid transport. The domains of the dehydrogenases for D-lactate and GP, however, showed mutual activation in both transport systems as the rate of the substrate added simultaneously was 12–15% faster than the sum of the individual rates in vesicles prepared from cells grown aerobically (Table IV). Some of the differences could also be explained by problems of substrate access or affinities.

There has been discussion as to whether the vesicle preparations contain sufficient activity to account for the amino acid transport activities of the cells (20). In *S. aureus* washed in 4.1 M NaCl to depress endogenous respiration, the vesicles had the same concentrative power as the intact cells (7). In the *M. denitrificans* system the vesicles with D-lactate as the substrate accounted for 27% for glutamine and 6% for glycine concentrative ability of the intact cells which utilized the presumably more efficient endogenous respiratory activity to drive the uptake.

Changing the growth medium markedly modified the primary dehydrogenase activities in *H. parainfluenzae* (14) and also in *M. denitrificans* (Table V). Not only were the primary levels changed by shifting from glucose to succinate as major carbon source in defined medium, but the activities of the transport systems for glycine seemed to be more tightly coupled to the electron transport system with some growth conditions (Table VI). The ability to shift the coupling between electron transport activity and amino acid transport in response to changing the growth medium was reminiscent of the changes in the coupling between the electron transport system and the oxidative phosphorylation with changing growth conditions (3).

The mechanisms by which the cells control the coupling between electron transport systems and either the oxidative phosphorylation or amino acid transport systems remains as obscure as ever. However, the fact that the coupling between electron transport and amino acid concentration is variable seems to be established.

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