Mechanisms of Active Transport in Isolated Bacterial Membrane Vesicles

FURTHER STUDIES ON AMINO ACID TRANSPORT IN STAPHYLOCOCCUS AUREUS MEMBRANE VESICLES*

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SUMMARY

Active amino acid transport in Staphylococcus aureus U-71 membrane vesicles is coupled to either α-glycerol phosphate dehydrogenase or L-lactate dehydrogenase depending upon the growth conditions of the parent cells. Vesicles prepared from cells grown on gluconate as a primary carbon source exhibit an absolute specificity for α-glycerol phosphate as a physiological electron donor for transport, whereas vesicles prepared from cells grown on glucose as a primary carbon source exhibit an absolute specificity for L-lactate as an electron donor for transport. Both preparations exhibit similar dehydrogenase activities qualitatively, indicating that the coupling between these dehydrogenases and transport is altered. L-Lactate oxidation, L-lactate: dichlorophenol-indophenol reductase activity, and L-lactate-dependent amino acid transport exhibit similar apparent Michaelis constants for L-lactate per se is the rate-limiting step for amino acid transport in the appropriate membrane preparation.

Amino acid transport is dependent on electron transfer, and inhibition of L-lactate oxidation by anaerobiosis, cyanide, 2-heptyl-4-hydroxyquinoline-N-oxide, amytal, and oxalate is directly related to inhibition of amino acid transport. However, only anaerobiosis, cyanide, 2-heptyl-4-hydroxyquinoline-N-oxide, and amytal, each of which inhibits electron transfer after the site of energy coupling, cause efflux. Oxalate, a potent inhibitor of L-lactate dehydrogenase, does not cause efflux despite almost complete inhibition of L-lactate oxidation and amino acid transport. Moreover, oxalate blocks or inhibits efflux caused by each of the other inhibitors and by 2,4-dinitrophenol. These results provide further evidence that active transport is dependent on the oxidation-reduction potential of the respiratory chain at the site of energy coupling.

Cyanide-induced efflux is a saturable process with an apparent affinity constant that is approximately 500 times higher than the affinity constant for active transport. The apparent maximum velocity of efflux, on the other hand, is the same as that of active transport. These findings suggest that one of the primary effects of energy coupling is to change the affinity of the carrier for substrate.

Under anaerobic conditions serine uptake exhibits linear kinetics, indicating that the rate-limiting step for serine uptake under these conditions is a nonsaturable process with an infinite $K_m$. Moreover, approximately 5 min is required for external serine to equilibrate with the intramembranous pool at a variety of concentrations. Thus, it is highly unlikely that facilitated diffusion is the rate-limiting step for active serine uptake.

α-Glycerol-P dehydrogenase-coupled amino acid uptake by Staphylococcus aureus membrane vesicles is catalyzed by 12 distinct and specific transport systems for structurally related amino acids, and the activity of the vesicles is comparable to the transport activity of intact cells (1, 2). Evidence has also been presented which demonstrates that except for differences in physiological electron donors, the characteristics of the transport system in S. aureus vesicles are very similar to those described for the Escherichia coli vesicle system (1-4). In vesicles prepared from both organisms, the site of energy coupling between respiration and active amino acid transport is localized in a segment of the respiratory chain between the primary dehydrogenases (i.e. α-glycerol-P dehydrogenase in S. aureus and α-lactate dehydrogenase in E. coli) and the cytochrome chain. In addition, evidence obtained with both systems indicates that the coupling between respiration and transport does not involve the generation or utilization of high energy phosphate or ATP (1, 3-5).

This paper deals with a further characterization of the amino acid transport systems in S. aureus membrane vesicles.

EXPERIMENTAL PROCEDURE

Growth of S. aureus—S. aureus U-71 (ATCC 27821) was grown aerobically at 37° on either a complex medium containing glucose as a primary carbon source (6) or on a synthetic medium containing glucose as a primary carbon source (7).

Preparation of Membrane Vesicles—Membrane vesicles were prepared from lysostaphin-induced S. aureus U-71 protoplasts as described previously (1, 2). Throughout the paper, vesicles prepared from cells grown on the gluconate-containing medium de-
scribed above are referred to as "gluconate" vesicles, while those prepared from cells grown on the glucose-containing medium are referred to as "glucose" vesicles.

Measurement of Amino Acid Uptake and Efflux—Transport of amino acids was assayed as described previously (1, 2, 8). For efflux experiments, vesicles were incubated in the presence of either α-glycerol-P or L-lactate and an appropriate radioactive amino acid until a steady state level of accumulation was achieved (5 to 10 min). At this time, the indicated electron transfer inhibitor was added or the reaction mixtures were gassed with argon as described previously (8, 9). At an appropriate time, the reactions were terminated and the samples were assayed as described previously (8, 10). Control samples were incubated under atmospheric conditions in the absence of electron transfer inhibitors.

The specific activities and final concentrations of the uniformly 14C-labeled L-amino acids used in these experiments were as follows: serine (140 mCi per mmole), 14.2 μM; leucine (262 mCi per mmole), 6.6 μM; threonine (164 mCi per mmole), 12.1 μM; lysine (312 mCi per mmole), 7.86 μM; proline (260 mCi per mmole), 0.31 μM; alanine (156 mCi per mmole), 14.5 μM; aspartic acid (182 mCi per mmole), 11.7 μM; glutamine (219 mCi per mmole), 9.13 μM.

Oxygen Utilization—Rates of oxygen uptake were determined with a Clark electrode (YSI model 53 oxygen monitor) as described previously (11).

Difference Spectra—Membrane vesicle suspensions containing about 1 mg of membrane protein per ml in a 1-cm cuvette were examined in a Cary 15 spectrophotometer at 25° as described previously (1). The vesicles were suspended in 50 mM potassium phosphate buffer, pH 7.3, containing 10 mM magnesium sulfate, and aerated by vigorous agitation with a Vortex mixer to oxidize the respiratory pigments or reduced in the presence of 20 mM substrate (final concentration).

Protein Determinations—Protein was determined by the method of Lowry et al. (12).

Materials—Radioactive amino acids were obtained from New England Nuclear Corp. Phenazine methosulfate and L-α-glycerol-P were obtained from Sigma Chemical Co. Other reagents were reagent grade and were obtained from commercial sources.

Fig. 1 (left). Amino acid uptake by Staphylococcus aureus membrane vesicles. The time course of serine uptake by glutamate (A) and glucose (B) membrane vesicles was measured in 50-μl reaction mixtures containing 10 mM potassium phosphate buffer (pH 7.3), 10 mM MgSO4, 20 to 50 μg of membrane protein, 20 mM electron donor, and 1.42 × 10-5 M [U-14C]serine (156 mCi per mmole). Reaction mixtures were incubated at 25°, terminated at the times given, and the samples were assayed as described previously (1, 2, 8). Serine transport was measured in the presence of 20 mM α-glycerol-P (O--O), 20 mM L-lactate (Δ--Δ), or no electron donor (■---■). Results obtained with alanine, leucine, threonine, lysine, glutamic acid, and proline were qualitatively identical with those presented for serine.

Fig. 2 (right). A, kinetics of L-lactate dehydrogenase. Initial rates of oxygen consumption by glucose membrane vesicles were examined in the presence of increasing concentrations of L-lactate. Oxygen consumption was measured in 1-ml reaction mixtures containing (in final concentrations) 10 mM potassium phosphate (pH 7.3), 10 mM MgSO4, and 240 μg of membrane protein as described previously (1, 2). B, initial rate of L-lactate:dichloroindophenol reductase activity versus L-lactate concentration. The rate of reduction of 2,4-dichloroindophenol (DCIP) was measured spectrophotometrically at 600 nm as described previously (2). Reaction mixtures (8.5 ml) contained 10 mM potassium phosphate (pH 7.3), 10 mM MgSO4, 0.052% dichloroindophenol, and 23 μg of membrane protein. C, initial rate of amino acid transport as a function of L-lactate concentration. Initial rates of serine uptake were determined in 50-μl reaction mixtures (final volume) containing 10 mM potassium phosphate (pH 7.3), 10 mM MgSO4, 23 μg of membrane protein, and 14.2 μM [U-14C]serine. Reactions were initiated by addition of 5 μl of glucose vesicles to reaction mixtures which had been equilibrated at 25°. Initial rates were determined from samples assayed at 15, 30, and 60 s at each L-lactate concentration. The reactions were linear over this time period. When serine was replaced by lysine, leucine, and proline, the apparent Km of L-lactate dehydrogenase was determined to be 0.210 mM, 0.234 mM, and 0.170 mM, respectively. Insets, data plotted by the method of Hofstee (15).
in both vesicle preparations manifest identical responses to temperature, and exhibit the same respective Michaelis constants and the same structural specificity for amino acids (data not shown). A difference is observed, however, between gluconate and glucose membranes in the stimulation of transport by the artificial electron donor system, ascorbate-phenazine methosulfate (8, 9), relative to the physiological electron donors, \( \alpha \)-glycerol-P and L-lactate. As shown in Table III, amino acid uptake by glucose vesicles incubated in the presence of ascorbate-phenazine methosulfate is much less than that observed in the presence of \( \alpha \)-glycerol-P. On the other hand, amino acid uptake in glucose membranes incubated in the presence of ascorbate-phenazine methosulfate is considerably greater than that observed in the presence of L-lactate.

**Relationship between Electron Transfer and Amino Acid Uptake**—L-Lactate-dependent serine uptake by glucose membranes is markedly inhibited by anaerobiosis and by the electron transfer inhibitors oxalate, amytal, 2-heptyl-4-hydroxyquinoline-N-oxide, and cyanide (Fig. 3). Moreover, as shown in Table IV, inhibition of transport by these inhibitors is directly related to inhibition of L-lactate oxidation. Regarding the sites of action of the inhibitors in the respiratory chain, previous studies (17, 18) have shown that cyanide inhibits cytochrome \( a \), 2-heptyl-4-hydroxyquinoline-N-oxide, and amytal inhibits at the flavin level. Oxalate is a potent competitive inhibitor of L-lactate dehydrogenase with an apparent \( K_i \) of approximately 20 \( \mu M \) (data not shown). As demonstrated by the spectrophotometric measurements presented in Fig. 4, addition of oxalate to anaerobic vesicle suspensions prior to aeration results in re-oxidation of at least 85% of the reduced cytochrome \( b + a \) and reduced cytochrome \( a \) (compare Spectrum III to II). These results demonstrate that the respiratory chain is maintained in an oxidized state in the presence of oxalate.

With the exception of oxalate, the same electron transfer inhibitors and anaerobiosis produce similar degrees of inhibition of \( \alpha \)-glycerol-P-dependent amino acid uptake in glucose vesicles. Oxalate, as expected, has no effect on \( \alpha \)-glycerol-P-dependent amino acid uptake and ascorbate-phenazine methosulfate is much less than that observed in the presence of \( \alpha \)-glycerol-P. On the other hand, amino acid uptake in glucose membranes incubated in the presence of ascorbate-phenazine methosulfate is considerably greater than that observed in the presence of L-lactate.

### Table I

<table>
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<th>Electron donor</th>
<th>Serine uptake</th>
<th>Stimulation Relative to L-lactate</th>
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<tr>
<td>L-Lactate</td>
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<tr>
<td>D-Lactate</td>
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<td>( \alpha )-Glycerol-P</td>
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<td>10</td>
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<tr>
<td>Succinate</td>
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<td>NADH</td>
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### Table II

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<tr>
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<tr>
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<td>nmol/mg protein/min</td>
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<tr>
<td>L-Lactate</td>
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### Table III

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<th>Amino acid</th>
<th>Gluconate membranes</th>
<th>Glucose membranes</th>
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<tr>
<td></td>
<td>( \alpha )-Glycerol-P</td>
<td>Ascorbate-phenazine methosulfate</td>
</tr>
<tr>
<td></td>
<td>Ratio of (I): (II)</td>
<td>Ratio of (III): (IV)</td>
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<tr>
<td>Serine</td>
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<td>2.4</td>
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<tr>
<td>Alanine</td>
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</tr>
<tr>
<td>Leucine</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Lysine</td>
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<td>0.1</td>
</tr>
<tr>
<td>Proline</td>
<td>1.3</td>
<td>0.3</td>
</tr>
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The serine-threonine, alanine-glycine, leucine-isoleucine-valine, lysine, glutamate aspartate, and proline transport systems (2) in both vesicle preparations manifest identical responses to temperature, and exhibit the same respective Michaelis constants and the same structural specificity for amino acids (data not shown). A difference is observed, however, between gluconate and glucose membranes in the stimulation of transport by the artificial electron donor system, ascorbate-phenazine methosulfate (8, 9), relative to the physiological electron donors, \( \alpha \)-glycerol-P and L-lactate. As shown in Table III, amino acid uptake by glucose vesicles incubated in the presence of ascorbate-phenazine methosulfate is much less than that observed in the presence of \( \alpha \)-glycerol-P. On the other hand, amino acid uptake in glucose membranes incubated in the presence of ascorbate-phenazine methosulfate is considerably greater than that observed in the presence of L-lactate.

### Table IV

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Glucose membranes</th>
<th>Glucose membranes</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( \alpha )-Glycerol-P</td>
<td>Ascorbate-phenazine methosulfate</td>
</tr>
<tr>
<td></td>
<td>Ratio of (I): (II)</td>
<td>Ratio of (III): (IV)</td>
</tr>
<tr>
<td>Serine</td>
<td>8.0</td>
<td>2.4</td>
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<tr>
<td>Alanine</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.4</td>
<td>0.1</td>
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<tr>
<td>Glutamic acid</td>
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<td>0.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.2</td>
<td>1.1</td>
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<tr>
<td>Lysine</td>
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<td>0.1</td>
</tr>
<tr>
<td>Proline</td>
<td>1.3</td>
<td>0.3</td>
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</table>

1 The studies referred to were carried out with \( \textit{E. coli} \); however, similar experiments with \( \textit{S. aureus} \) U-71 membrane vesicles indicate that these respiratory inhibitors act at similar sites in this system. Thus, addition of amytal to anaerobic vesicle suspensions prior to aeration results in re-oxidation of approximately 85% of the reduced cytochrome \( b + a \) and cytochrome \( a \); addition of 2-heptyl-4-hydroxyquinoline-N-oxide results in re-oxidation of reduced cytochrome \( a \) only; and addition of cyanide does not result in re-oxidation of any of the cytochrome pigments.
acid uptake and L-lactate oxidation, causes no efflux of serine oxalate, at a concentration which completely inhibits amino preloaded in the presence of L-lactate (Fig. 5). In contrast, substrates in both vesicle preparations.

leucine, proline, lysine, and aspartic acid were used as transport examined in the Cary 15 spectrophotometer at 25°. L-Lactate was added to one cuvette, and after the anaerobic steady state was achieved, difference spectra were recorded. I, difference spectrum of two suspensions in the oxidized state; II, difference spectrum of electron transfer inhibitors on serine uptake by Staphylococcus aureus glucose membrane vesicles. The effect of anaerobiosis, 20 mM potassium cyanide (●—●), 40 μM 2-heptyl-4-hydroxyquinoline-N-oxide (■—■), 10 mM amytal (□—□), and 20 mM potassium oxalate (Δ—Δ) on serine uptake was measured in 50-μl reaction mixtures prepared as described in Fig. 1. The vesicles were preincubated for 1 min at 25°; the inhibitors were added, and the reactions were initiated by addition of L-lactate and [U-14C]serine. ○—○, uptake under aerobic conditions in the absence of the inhibitors. Identical results were obtained with glucose vesicles except that potassium oxalate did not inhibit amino acid uptake by these vesicles.

Fig. 4 (center). Difference spectra of Staphylococcus aureus glucose membrane vesicles. Membrane vesicle suspensions containing about 1 mg of membrane protein per ml in 1-cm cuvettes were examined in the Cary 15 spectrophotometer at 25°. L-Lactate was added to one cuvette, and after the anaerobic steady state was achieved, difference spectra were recorded. I, difference spectrum of two suspensions in the oxidized state; II, difference spectrum of two suspensions in the oxidized state; III, difference spectrum of vesicles reduced in the presence of 20 mM L-lactate minus vesicles in the oxidized state.

TABLE IV

<table>
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<tr>
<th>Inhibitor</th>
<th>L-Lactate oxidation</th>
<th>L-Lactate-stimulated transport</th>
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<tr>
<td></td>
<td>nmole/min/mg protein % inhibition</td>
<td>nmole/min/mg protein % inhibition</td>
</tr>
<tr>
<td>None</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Anaerobiosis</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>KCN</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>Oxalate</td>
<td>26</td>
<td>60</td>
</tr>
</tbody>
</table>

pendent amino acid transport or α-glycerol-P oxidation in gluconate vesicles nor on ascorbate-phenazine methosulfate-dependent transport in either preparation. It is also noteworthy that essentially the same results were obtained when alanine, leucine, proline, lysine, and aspartic acid were used as transport substrates in both vesicle preparations.

Anaerobiosis, amytal, 2-heptyl-4-hydroxyquinoline-N-oxide, and cyanide produce rapid efflux of serine from glucose vesicles preloaded in the presence of L-lactate (Fig. 5). In contrast, oxalate, at a concentration which completely inhibits amino acid uptake and L-lactate oxidation, causes no efflux of serine from the intravesicular pool. Oxalate does not inhibit the exchange of serine across the vesicle membrane (Fig. 6), indicating that it does not have a direct effect on the carriers per se.

Since vesicles incubated in the presence of oxalate do not catalyze net uptake or efflux of serine, it seems likely that the exchange reaction observed in the presence of oxalate is limited to a one-for-one interchange between internal and external serine. Qualitatively similar results were obtained when these experiments were carried out with alanine, leucine, proline, lysine, or aspartic acid, and with the β-galactoside (18), amino acid (19), and valinomycin-induced rubidium uptake (20) systems described previously in E. coli membrane vesicles.

Since the behavior of the carriers has been postulated to reflect the oxidation-reduction state of the respiratory chain at the site of energy coupling (3, 4, 18), oxalate should not only fail to induce efflux, but should also inhibit efflux induced by electron transfer inhibitors which block the respiratory chain after the

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3 Measurements of the stoichiometry of exchange in the presence and absence of oxalate demonstrate that 0.133 nmole of external [3H]serine is exchanged for 0.14 nmole of internal [14C]serine in 1 min; and in 10 min, 0.44 nmole of [3H]serine is exchanged for 0.46 nmole of [14C]serine. During the exchange experiments, the intravesicular serine concentration remained constant at approximately 13.8 mM.

4 The studies with the E. coli membrane vesicle system were carried out with oxamate, another competitive inhibitor of D- and L-lactate dehydrogenases. With S. aureus vesicles, oxalate is a more potent inhibitor of L-lactate dehydrogenase than oxamate.
Fig. 6. Effect of oxalate on amino acid exchange in glucose vesicles. Reaction mixtures (50 μl total volume) were prepared as described in Fig. 1. After 7-min incubation in the presence of 20 mM L-lactate and the 14C-amino acid indicated, the appropriate 14C-amino acid was added to one set of samples to give a final concentration of 100 μM (Δ—Δ), while a second set of samples received 14C-amino acid and potassium oxalate (□—□) in final concentrations of 100 μM and 20 mM, respectively. The time of addition is indicated by the arrows shown in the figure. A third set of samples, the control, received neither 14C-amino acid nor oxalate (○—○). At the times indicated, the reactions were terminated and the samples were assayed as described previously (1, 2, 8, 10).

Fig. 7. Effect of oxalate on serine efflux induced by electron transfer inhibitors and anaerobiosis in glucose vesicles. The effect of 20 mM potassium oxalate on serine efflux induced by anaerobiosis or by the addition of 20 mM potassium cyanide (KCN), 10 mM sodium amytal, 40 μM 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO), and 1 mM 2,4-dinitrophenol (DNP) was assayed in 50-μl reaction mixtures prepared as described in Fig. 1. A, four identical sets of reaction mixtures were prepared. In the first set, serine accumulation by membrane vesicles incubated in the presence of L-lactate and [14C]serine was measured (○—○). In the second set, serine efflux from the vesicles was assayed following the addition of potassium cyanide, amytal, or gassing the reaction mixtures with argon (□—□) (indicated by the arrow at 18 min). The third set of reaction mixtures received potassium oxalate after the steady state level of serine accumulation was obtained (Δ—Δ) (indicated by the arrow at 18 min). In the fourth set of samples, oxalate was added to the reaction mixtures followed 5 min later by the addition of potassium cyanide, amytal, or gassing with argon (■—■). B, efflux induced by 2-heptyl-4-hydroxyquinoline-N-oxide in the presence (■—■) and absence (□—□) of oxalate. C, efflux induced by 2,4-dinitrophenol in the presence (■—■) and absence (□—□) of oxalate. Similar results were obtained when these experiments were repeated with lysine, leucine, and proline.

Kinetics of Amino Acid Uptake and Efflux—In the mechanism proposed for respiration-dependent transport by E. coli membrane vesicles (3, 4, 18), the affinity of the carriers is determined by the oxidation-reduction state of the respiratory chain at the site of energy coupling. As shown in Fig. 7, this prediction is borne out. Addition of oxalate prior to cyanide, amytal, or gassing with argon virtually abolishes serine efflux induced under the latter conditions (Fig. 7A). With 2-heptyl-4-hydroxyquinoline-N-oxide (Fig. 7B), oxalate markedly inhibits the initial rate of efflux, but the effect is not so great as that observed with cyanide, amytal, or anaerobiosis. Finally, as shown in Fig. 7C, oxalate also markedly inhibits efflux in the presence of the uncoupling agent 2,4-dinitrophenol. These experiments provide further support for the hypothesis that carrier activity reflects primarily the oxidation-reduction potential of the energy-coupling site for transport.

Kinetics of Amino Acid Uptake and Efflux—In the mechanism proposed for respiration-dependent transport by E. coli membrane vesicles (3, 4, 18), the affinity of the carriers is determined by the oxidation-reduction state of the respiratory chain at the site of energy coupling. α-Glycerol-P- or L-lactate-dependent amino acid uptake by S. aureus membrane vesicles is a high affinity process, exhibiting apparent Michaelis constants in the micromolar range. With serine, specifically, the apparent Kₘ is 10 to 12 μM and the Vₘₐₓ 5 to 6 nmoles per min per mg of membrane protein (2). Under anaerobic conditions, however, where active serine accumulation and L-lactate (or α-glycerol-P) oxidation are markedly inhibited, the kinetics of serine uptake are strikingly altered (Figs. 8 and 9). As shown in Fig. 8, when the rate and extent of serine uptake are measured under argon, approximately 5 min is required for external serine to equilibrate with the intramembranal pool at serine concentrations ranging from approximately 0.26 to 6.9 mM. A reciprocal plot of the initial rates at each serine concentration is given in Fig. 9. Clearly, the data yield a linear function intersecting the x and y axes at the origin, suggesting that the rate-limiting step for serine uptake under these conditions is a non-saturable process with no Kₘ.
Fig. 8. Kinetics of serine uptake by glucose membrane vesicles under anaerobic conditions. Reaction mixtures (50 μl total volume) contained 10 mM potassium phosphate (pH 7.35), 10 mM MgSO₄, and 200 μg of membrane protein. The vesicle suspensions were gassed with argon for 3 min, L-lactate was added to a final concentration of 20 mM, and the incubation was continued under argon for an additional 10 min at 25°C. [U-¹⁴C]Serine (25.7 mCi per mmole) was then added to the reaction mixtures to give final concentrations ranging from 0.26 to 6.91 mM as shown. At the times indicated, the reactions were terminated as described previously (1, 2) with the exception that 5 ml of 0.1 M LiCl wash and 47-mm cellulose-nitrate filters were used. The filters were dried, dissolved in 10 ml of Instabray scintillator (Yorktown Research, New York, New York), and counted in a Beckman liquid scintillation counter. The broken line in each panel represents the serine concentration of the intravesicular pool at equilibration.

When cyanide is added to vesicles which have been loaded to various intravesicular serine concentrations by incubation with either α-glycerol-P (gluconate membranes) or L-lactate (glucose membranes), the rates of efflux observed exhibit saturation with respect to the internal serine concentration (Fig. 10). The apparent Kₘ values for serine efflux calculated from Hofstee plots (insets in Fig. 10) are 4.66 and 4.58 for gluconate and glucose membranes, respectively, while the Vₘₐₓ is 5 to 6 nmoles per min per mg of membrane protein in both preparations. Thus, compared to the influx process under optimal conditions for transport (i.e. in the presence of α-glycerol-P or L-lactate under aerobic conditions), the apparent Kₘ for serine efflux is approximately 400 to 500 times higher, but the Vₘₐₓ is almost identical. In addition to demonstrating that the S. aureus system exhibits similar properties to those observed previously in E. coli (3, 4, 18-20), these results suggest that the carriers can catalyze facilitated diffusion in the direction of efflux only.

DISCUSSION

Data presented in this paper demonstrate that active transport of amino acids by S. aureus membrane vesicles requires the oxidation of either α-glycerol-P or L-lactate depending on the growth conditions of the parent cells. Thus, vesicles prepared from S. aureus U-71 grown on gluconate as a primary carbon source exhibit an absolute specificity for α-glycerol-P as a physiological electron donor for transport, whereas vesicles prepared from the same cells grown on glucose exhibit an absolute specificity for L-lactate as an electron donor for transport. It is apparently the coupling between these dehydrogenases and transport which is altered rather than the presence or absence of the particular dehydrogenase, as both preparations exhibit
similar dehydrogenase activities qualitatively. Moreover, NADH is oxidized by both vesicle preparations at rates which exceed those of either α-glycerol-P or L-lactate.

The absolute dependence of these respiration-linked transport systems on a specific physiological electron donor is unique to the Staphylococcal membrane vesicle system. In E. coli membrane vesicles, for instance, although N-lactate is by far the most effective physiological electron donor for transport, succinate and NADH, as well as other electron donors, will support transport to some extent when the appropriate dehydrogenases are induced (3, 4, 13, 14, 18–20). A somewhat analogous situation has been reported in N-lactate dehydrogenase mutants of E. coli, however, where it has been shown that the coupling between succinic dehydrogenase and transport is markedly enhanced (22).

Active transport in S. aureus membrane vesicles and in vesicles prepared from a number of other bacterial species is directly dependent on electron transfer (1–4, 9). Inhibition of α-glycerol-P or L-lactate-dependent respiration in S. aureus vesicles by a variety of electron transfer inhibitors results in inhibition of amino acid uptake, and spectrophotometric and other evidence presented in this and other communications (1) demonstrates that the site of energy coupling between active transport and the respiratory chain occurs between the primary dehydrogenase(s) and the cytochrome chain. Although the precise mechanism by which electron transfer is coupled to active transport is unknown, experiments presented here provide a strong indication that carrier activity is related to the oxidation-reduction potential of the respiratory chain at the site of energy coupling. The evidence rests primarily on the observations that inhibition of electron transfer after the energy-coupling site (i.e. with an anacrobosio, cyanide, 2-heptyl-4-hydroxyquinoline-N-oxide, or amytal) induces rapid efflux of solutes accumulated in the intravesicular pool, while inhibition before the site of energy coupling (i.e. with oxalate) does not induce efflux despite almost complete inhibition of oxidation and the initial rate of uptake. Similar arguments have been presented for E. coli membrane vesicles (4, 3, 11, 18). In the Staphylococcal system, moreover, this argument is strengthened by the observation that oxalate not only fails to induce efflux, but also blocks efflux produced by electron transfer inhibitors which inhibit after the energy-coupling site. In addition, the finding that oxalate inhibits the rate of efflux induced by 2,4-dinitrophenol is striking, and suggests that the proton-conducting properties of this uncoupling agent cannot fully explain its inhibitory activity in this system.

In the case of the lactose and proline transport systems in E. coli vesicles, where kinetics of influx and efflux have been studied in detail (18, 19), the concentrating ability of the vesicles is directly related to the ratio of the K_m values for influx and efflux (4). A similar situation exists for the serine transport system in S. aureus vesicles. The K_m for active serine uptake with either α-glycerol-P or L-lactate is 10 to 12 μM, while the K_m for efflux is approximately 5 mM, yielding a ratio of 500. It can be calculated from the data given in Fig. 10 that the vesicles accumulate serine to an intravesicular concentration of approximately 5 mM at an external serine concentration of 10 μM, giving a distribution ratio of 500. These results provide further evidence that one of the primary effects of energy coupling is to change the affinity of the carriers for ligand.

Finally, the possibility that facilitated diffusion is the rate-limiting step for active serine accumulation is inconsistent with at least two observations: (a) the initial rate of serine uptake under anoxic conditions exhibits linear kinetics and it takes approximately 5 min for external serine to equilibrate with the intravesicular pool; (b) oxalate does not induce efflux of serine from the intravesicular pool despite almost complete inhibition of L-lactate oxidation and active serine uptake.

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