THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 247, No. 1, Issue of January 10, pp. 298–304, 1972
*Printed in U.S.A.*

# Active Transport in Isolated Bacterial Membrane Vesicles

## V. THE TRANSPORT OF AMINO ACIDS BY MEMBRANE VESICLES PREPARED FROM *STAPHYLOCOCCUS AUREUS**

(Received for publication, August 11, 1971)

STEVEN A. SHORT AND DAVID C. WHITE

*From the Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40506*

H. RONALD KABACK

*From The Roche Institute of Molecular Biology, Nutley, New Jersey 07110*

### SUMMARY

**Concentrative uptake of 16 amino acids by membrane vesicles isolated from *Staphylococcus aureus* is stimulated 3 to 100 times by the conversion of L-α-glycerol phosphate to dihydroxyacetone phosphate. With the exception of ascorbate-phenazine methosulfate, D-lactate, phosphoenolpyruvate, ATP, and a number of other metabolites and cofactors do not replace α-glycerol phosphate. Amino acid transport by these membrane preparations in the presence of α-glycerol phosphate requires oxygen, and is blocked by potassium cyanide, sodium azide, and dinitrophenol; however, uptake is not significantly inhibited by high concentrations of arsenate. Vesicles contain insignificant amounts of ATP; and the level of ATP is not increased by incubation in the presence of α-glycerol phosphate or NADH.**

**The membrane-bound α-glycerol phosphate dehydrogenase is coupled to oxygen via a cytochrome system also present in the vesicle membrane, and spectrophotometric evidence shows that α-glycerol phosphate dehydrogenase, NADH dehydrogenase, L-lactic dehydrogenase, and succinic dehydrogenase all utilize the same cytochrome system. There is no relationship between rates of oxidation of electron donors by the respiratory chain (NADH > α-glycerol phosphate ≫ L-lactate=succinate) and the ability of these compounds to stimulate amino acid transport. *N*-Ethylmaleimide and *p*-hydroxymercuribenzoate inhibit amino acid transport and α-glycerol phosphate oxidation. However, *N*-ethylmaleimide does not significantly inhibit α-glycerol phosphate dehydrogenase with dichlorophenolindophenol as an artificial acceptor, nor does it inhibit oxygen utilization in the presence of NADH. These findings indicate that the site of coupling of α-glycerol phosphate dehydrogenase to amino acid transport lies between the primary dehydrogenase and the cytochrome chain.**

**It is concluded that amino acid transport in *S. aureus* is catalyzed by mechanisms similar to those found in *Escherichia coli* with the exception that α-glycerol phosphate, rather than D-lactate, is the primary electron donor.**

* This study was supported in part under Contract No. 12-14-100-10310(73) with the Agricultural Research Service, United States Department of Agriculture, administered by the Eastern Utilization Research and Development Division, 600 Mermaid Lane, Philadelphia, Pennsylvania, Grant GB-17984 of the Metabolic Biology Section of the National Science Foundation.

The transport of a variety of sugars and amino acids by membrane vesicles isolated from *Escherichia coli* is coupled primarily to a flavin-linked D-lactic dehydrogenase (1–3). Other oxidizable substrates such as succinate, L-lactate, or NADH do not serve as effectively as electron donors for transport, and evidence has been presented which shows that the site of energy coupling of D-lactic dehydrogenase to active transport lies between the primary dehydrogenase and cytochrome $b_1$ (4). In vesicles prepared from *Bacillus subtilis*, the transport of L-serine is stimulated by α-glycerol-P,[1] L-lactate, and, under conditions of vigorous oxygenation, by NADH (5). Most recently, it has been demonstrated that the concentrative uptake of amino acids by membrane vesicles isolated from a variety of organisms can also be coupled to the artificial electron donor system ascorbate-phenazine methosulfate (5, 6).

The mechanism by which amino acids and some sugars are transported in *E. coli* vesicles appears to involve sulfhydryl-containing "carrier" proteins (3). Preliminary evidence has been presented which suggests that the transport-specific "carriers" may be electron transfer intermediates in the respiratory chain; and a conceptual model has been formulated (3). In this model, a critical disulfide group in the carrier molecule is postulated to undergo reduction by D-lactic dehydrogenase or ascorbate-phenazine methosulfate resulting in a conformational change. By this means, substrate is translocated from the outside of the membrane to the inside. Concomitant with the conformational change, the affinity of the carrier for ligand is decreased, and substrate is released on the inside of the membrane. Subsequently, the reduced carrier is reoxidized by cytochrome $b_1$ and ultimately oxygen, and the cycle can then be repeated. An intrinsic feature of this model is that the genera-

[1] W. N. Konings, unpublished information.

tion and utilization of high energy phosphate compounds is not involved in the transport process.

In the present study, data are presented which show that the transport of amino acids by membrane vesicles from *Staphylococcus aureus* is coupled exclusively to L-α-glycerol phosphate dehydrogenase. From the results presented, it appears that the same general transport mechanism proposed for respiration-coupled transport in *E. coli* is operative in the transport of amino acids in *S. aureus*.

## EXPERIMENTAL PROCEDURE

*Growth of S. aureus*—*S. aureus* U-17 was grown with aeration and harvested as described previously (7).

*Preparation of Transport Vesicles*—*S. aureus* membrane vesicles were prepared from lysostaphin protoplasts as described previously (6).

*Measurement of Transport*—Uptake of amino acids was determined by procedures described in previous publications (1, 2, 4–6, 8). The specific activities and final concentrations of the uniformly labeled L-amino acids used in the transport assays were as follows: serine (128 mCi per mmole), $1.56 \times 10^{-5}$ M; leucine (262 mCi per mmole), $7.65 \times 10^{-6}$ M; glutamic acid (206 mCi per mmole), $9.73 \times 10^{-6}$ M; glutamine (219 mCi per mmole), $9.13 \times 10^{-6}$ M; threonine (164 mCi per mmole), $1.21 \times 10^{-5}$ M; glycine (78 mCi per mmole), $2.54 \times 10^{-5}$ M; isoleucine (273 mCi per mmole), $7.31 \times 10^{-6}$ M; aspartic acid (170 mCi per mmole), $1.17 \times 10^{-5}$ M; lysine (255 mCi per mmole), $7.85 \times 10^{-6}$ M; valine (248 mCi per mmole), $8.05 \times 10^{-6}$ M; alanine (137 mCi per mmole), $1.45 \times 10^{-5}$ M; proline (214 mCi per mmole), $9.34 \times 10^{-6}$ M; tyrosine (10 mCi per mmole), $2 \times 10^{-5}$ M; histidine (10 mCi per mmole), $2 \times 10^{-5}$ M; phenylalanine (10 mCi per mmole), $2 \times 10^{-5}$ M; arginine (10 mCi per mmole), $2 \times 10^{-5}$ M.

*ATP Determination*—The ATP content of vesicles was measured with the luciferin-luciferase method described by Ramirez and Smith (9).

*Chromatography*—Amino acids recovered from the vesicles after transport were chromatographed on Silica Gel G thin layer plates as described previously (1). Nonradioactive carrier amino acids were detected by ninhydrin and the unknowns by autoradiography (7).

Dihydroxyacetone phosphate was identified chromatographically with 10 μl of reaction mixture on oxalic acid-washed Whatman No. 1 paper with a solvent of water (15.5%, v/v), 95% ethyl alcohol (32%), and 1-butanol (52.8%) containing 1 mM sodium ethylenediaminetetraacetic acid and 87 mM picric acid (10). Authentic α-glycerol-P ($R_F$ 0.7) and dihydroxyacetone-P ($R_F$ 0.48) were detected with the Haynes-Isherwood spray (11) and unknowns by autoradiography (7). Spots corresponding to the dark areas on the film were cut out and the radioactivity determined in a liquid scintillation spectrometer.

*Oxygen Utilization Measurements*—The rates of oxygen uptake were measured with the Clark electrode (YSI model 53 oxygen monitor) as described (4). The assay mixture (1 ml, total volume) contained 0.50 mg of membrane protein suspended in 50 mM potassium phosphate buffer, pH 7.3, containing 10 mM $MgSO_4$ to which substrates were added at a final concentration of 20 mM (5 mM for NADH). The measurements were performed at 30°.

*Difference Spectra*—Membrane vesicle suspensions containing about 1 mg of membrane protein per ml in a 1-cm cuvette were examined in the Cary 14 CM spectrophotometer at 25°. The vesicles were suspended in 50 mM potassium phosphate buffer, pH 7.3, containing 10 mM $MgSO_4$ and aerated by vigorous agitation with the Vortex mixer to oxidize the respiratory pigments or reduced in the presence of substrate as described previously (4).

*Electron Microscopy*—Sections of glutaraldehyde-fixed vesicle preparations were counter stained with 1% uranyl acetate and examined in the electron microscope as described (12).

*Protein Determination*—Protein was determined by the method of Lowry *et al.* (13).

*Materials*—The radioactive amino acids were obtained from New England Nuclear Co., Boston, Mass. Phenazine methosulfate and L-α-glycerol phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. Other reagents were obtained from sources described previously (7, 14, 15).

## RESULTS

*Characterization of Membrane Vesicles*—*S. aureus* membrane vesicles prepared as described under "Experimental Procedure" contain 7 to 10 times the cytochromes $b_1$ plus $o$ per mg of protein as the intact cells indicating that the vesicles represent at least a 7- to 10-fold purification of the protoplast membrane. Electron microscopy reveals that the preparations as described under "Experimental Procedure" consist of closed membrane vesicles and cell wall fragments (Fig. 1*A*). The vesicles can be resolved of contaminating cell wall fragments by centrifugation at 64,000 × *g* for 2 hours in a discontinuous 20 to 60% sucrose gradient (8). The structures obtained (Fig. 1, *B* and C) consist predominantly of intact "unit membrane"-bound sacs approximately 0.3 μm in diameter. The sacs appear to be empty and without internal structure. It should be emphasized that purified vesicles have approximately twice the specific activity for amino acid transport (*i.e.* initial rate of uptake per mg of protein) as the crude preparations. For the experiments reported here, the vesicle-wall preparation was used (Fig. 1*A*).

*Electron Donor Specificity for Amino Acid Transport*—As shown in Table I, α-glycerol-P is the only substrate of the 41 tested which stimulates threonine uptake by the membrane vesicles. Essentially identical results were obtained with lysine, glutamic acid, and leucine.

The artificial electron donor ascorbate-phenazine methosulfate stimulates the initial rates of serine and lysine uptake 27- and 25-fold, respectively, compared to controls incubated in the absence of ascorbate, phenazine methosulfate, or both. The time course of serine uptake in the presence of ascorbate-phenazine methosulfate is illustrated in Fig. 2. As shown, serine is taken up rapidly for approximately 1 min, reaches a maximum at 2 to 3 min, and is subsequently lost from the vesicles. When ascorbate or phenazine methosulfate is omitted from the reaction mixtures, serine uptake is negligible.

*Effect of α-Glycerol-P on Amino Acid Transport*—Time courses of amino acid transport for 12 amino acids show that α-glycerol-P stimulates both the initial rate of transport and the steady state level of accumulation (Fig. 3). In each experiment shown, amino acid transport (*i.e.* the initial rate, in particular) is almost negligible in the absence of α-glycerol-P. Although detailed time courses are not shown, α-glycerol-P also stimulates the uptake of tyrosine, phenylalanine, histidine, and arginine 9-, 6-, 8-, and 3-fold, respectively, over samples incubated in the absence of α-glycerol-P (3-min incubations). The effect of α-glycerol-P on the transport of other amino acids was not tested.

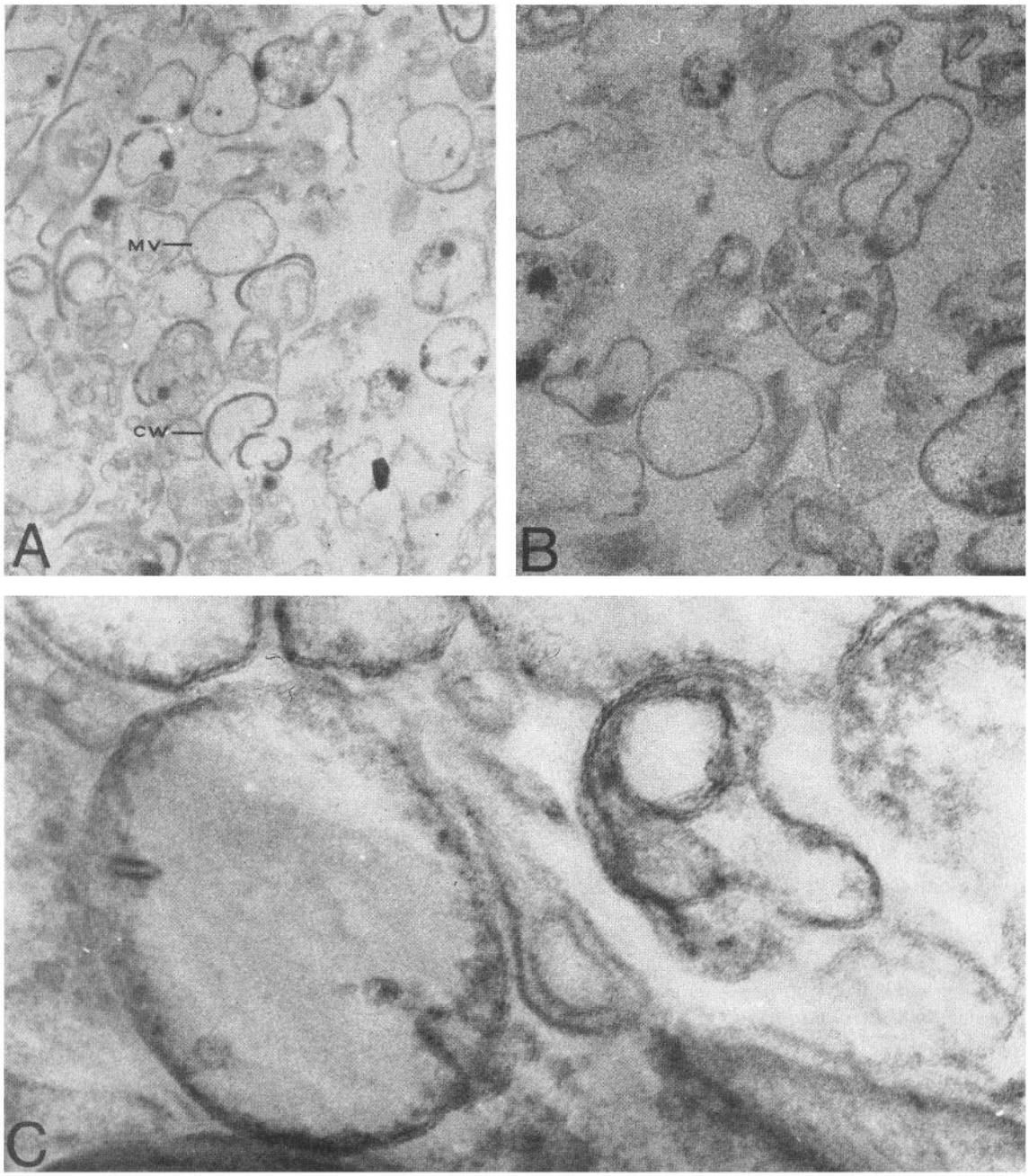


FIG. 1. Electron microscopy of *S. aureus* membrane vesicles. The procedure used is described under "Experimental Procedure." *A*, vesicle-cell wall preparation prior to sucrose density centrifugation. *MV*, membrane vesicle; *CW*, cell wall. Magnification approximately × 12,500. *B*, membrane vesicle preparation purified by sucrose density centrifugation carried out as described previously (8). Magnification approximately × 20,000. *C*, purified membrane vesicles. Magnification approximately × 125,000. The micrographs shown were obtained by Doctors Paul Bartl and Milas Boublik and Mr. Frank Jenkins of The Roche Institute of Molecular Biology.

Each of the 16 amino acids tested was recovered from the vesicles after a 10-min incubation in the presence of α-glycerol-P and subjected to thin layer chromatography as described under "Experimental Procedure." In every case the amino acid studied cochromatographed with authentic standards.

*Product of α-Glycerol-P Oxidation*—When the vesicles were incubated with α-[U-$^{14}$C]glycerol-P under conditions identical with those used to study amino acid transport, the only product obtained from the reaction mixture was dihydroxyacetone-P. Moreover, when studied as a function of time, there was a stoichiometric conversion of α-glycerol-P to dihydroxyacetone-P (Fig. 4). It should be emphasized that dihydroxyacetone-P has no effect on amino acid transport by the vesicles (Table I).

*Effect of Inhibitors on Amino Acid Transport*—Inhibition of cytochrome oxidase activity by azide,[2] cyanide,[2] or anoxia, inhibits amino acid transport by the vesicles. Thus, 70% inhibition of transport was obtained with 10 mM sodium azide, and better than 95% inhibition with 50 mM azide, 10 mM so-

[2] White, D. C., unpublished information.

TABLE I

*Threonine uptake by membrane vesicles from S. aureus U-71*

Threonine uptake was measured in a reaction mixture containing 0.225 mg of membrane protein, 50 mM potassium phosphate buffer, pH 7.3, and 10 mM $MgSO_4$ in a total volume of 100 μl. The assay mixtures were initially incubated for 2 min at 25°, the indicated substrate added,[a] and immediately thereafter [U-$^{14}$C]-threonine (170 mCi per nmole) at a final concentration of $1.17 \times 10^{-5}$ M. The tubes were incubated for 15 sec and the reaction terminated and the samples assayed as described previously (1–4, 6).

| Substrate added (20 mM) | Threonine uptake |
|---|---|
| | *nmoles/mg protein/15 sec* |
| 1. No addition | 0.0 |
| 2. α-Glycerol-P | 0.340 |
| 3. Dihydroxyacetone-P | 0.005 |
| 4. P-enolpyruvate | 0.0 |
| 5. D-Lactate | 0.014 |
| 6. L-Lactate | 0.030 |
| 7. Succinate | 0.0 |
| 8. NADH | 0.008 |
| 9. ATP | 0.0 |

[a] The following compounds produced no significant uptake of threonine, lysine, glutamic acid, or leucine: 6-P-gluconate, glucose, glucose-6-P, glucose-1-P, fructose-6-P, fructose-1,6-$P_2$, glycerol, 3-P-glycerate, 1,2-$P_2$-glycerate, 2-P-glycerate, pyruvate, citrate, *cis*-aconitate, isocitrate, α-ketoglutarate, fumarate, malate, oxalacetate, formate, acetyl-P, acetate, glyoxylate, α-hydroxybutyrate, β-hydroxybutyrate, γ-hydroxybutyrate, α-ketobutyrate, D-glycerate, carbamyl-P, adenosine cyclic 3′,5′-monophosphate, UDP-glucose, FAD, FMN, and acetyl-CoA.

dium cyanide, and anoxia. DNP[3] and the sulfhydryl reagents NEM and PHMB also produce greater than 95% inhibition of transport at 5, 10, and 1 mM, respectively.

Significantly, the steady state levels of amino acids accumulated by *S. aureus* vesicles are not markedly affected by the addition of sodium arsenate to the reaction mixtures (Table II). Even the mild inhibition of serine and proline transport by arsenate is probably due to the increased ionic strength of the assay mixture, as equivalent concentrations of phosphate buffer produce similar degrees of inhibition (data not shown). In the presence of 10 mM arsenate, there is no inhibition of amino acid transport.

*ATP Content of Vesicles*—With the luciferin-luciferase method (9), the vesicles contain an undetectable amount of ATP. The limit of detection under the conditions employed is 0.16 nmole per mg of membrane protein. Incubation of the vesicles with α-glycerol-P or NADH prior to assay makes no observable difference.

*Substrate Oxidation and Amino Acid Transport*—NADH, α-glycerol-P, L-lactate, and succinate stimulate oxygen utilization by *S. aureus* membrane vesicles (Table III). The initial rate of oxygen utilization by vesicles in the presence of 5 mM NADH is 20% greater than in the presence of 20 mM α-glycerol-P. Rates of oxygen utilization in the presence of succinate or L-lactate are 8 to 9 times less than with NADH or α-glycerol-P. NADH, L-lactate, or succinate do not stimulate amino acid

[3] The abbreviations used in this paper are: DNP, 2,4-dinitrophenol; NEM, *N*-ethylmaleimide; and PHMB, *p*-hydroxymercuribenzoate.

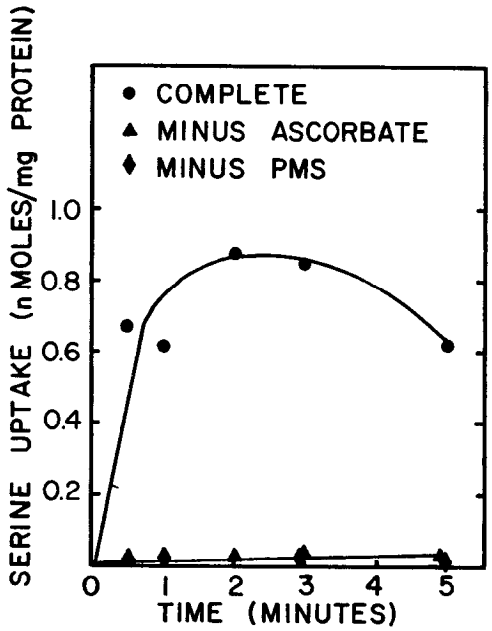


FIG. 2. Time course of serine transport in the presence of ascorbate-phenazine methosulfate. Aliquots (50 μl) of *S. aureus* membrane vesicles containing 0.15 mg of membrane protein were diluted to a final volume of 100 μl containing, in final concentrations, 50 mM potassium phosphate buffer, pH 7.3, 10 mM magnesium sulfate, and 0.1 mM phenazine methosulfate. The reaction mixtures were incubated for 15 min at 25° under oxygen as described previously (6). Ascorbate and [U-$^{14}$C]serine (128 mCi per mmole) were then added at 20 mM and $1.56 \times 10^{-6}$ M, respectively, and the incubations were continued under oxygen for the times given. The reactions were terminated and the samples assayed as described previously (1–3, 4, 6, 8). ●——●, ascorbate plus phenazine methosulfate; ▲——▲, minus ascorbate; ♦——♦, minus phenazine methosulfate (*PMS*).

transport even when the incubations were carried out for 30 min. These findings indicate that the observed specificity of amino acid transport for α-glycerol-P dehydrogenase, as opposed to other dehydrogenases, cannot be accounted for solely on the basis of rates of electron flow to oxygen.

*Utilization of Electron Donors by Cytochrome System*—NADH and α-glycerol-P completely reduce cytochrome $b_1$ plus *o* (maxima near 560 nm (14)) and cytochrome *a* (maximum near 605 nm (14)) (Fig. 5). L-Lactate and succinate also completely reduce these cytochromes (data not shown). In the anaerobic steady state, no further reduction of cytochromes occurs when NADH (Fig. 5, *IV*) or dithionite (data not shown) is added to vesicles reduced in the presence of α-glycerol-P (compare Tracings *II* and *IV*).

These findings indicate that α-glycerol-P, NADH, L-lactic, and succinic dehydrogenases utilize the same cytochrome system for electron flow to oxygen. Thus, the specificity of the coupling between amino acid transport and α-glycerol-P dehydrogenase cannot be related to a unique cytochrome system coupled to α-glycerol-P dehydrogenase. This conclusion is supported by experiments in which amino acid transport was studied in the presence of saturating concentrations of α-glycerol-P plus NADH, L-lactate, or succinate. There was no additional stimulation of the rate of amino acid transport over that obtained with α-glycerol-P alone under any condition studied. Clearly the site of energy coupling for α-glycerol-P dehydrogenase to

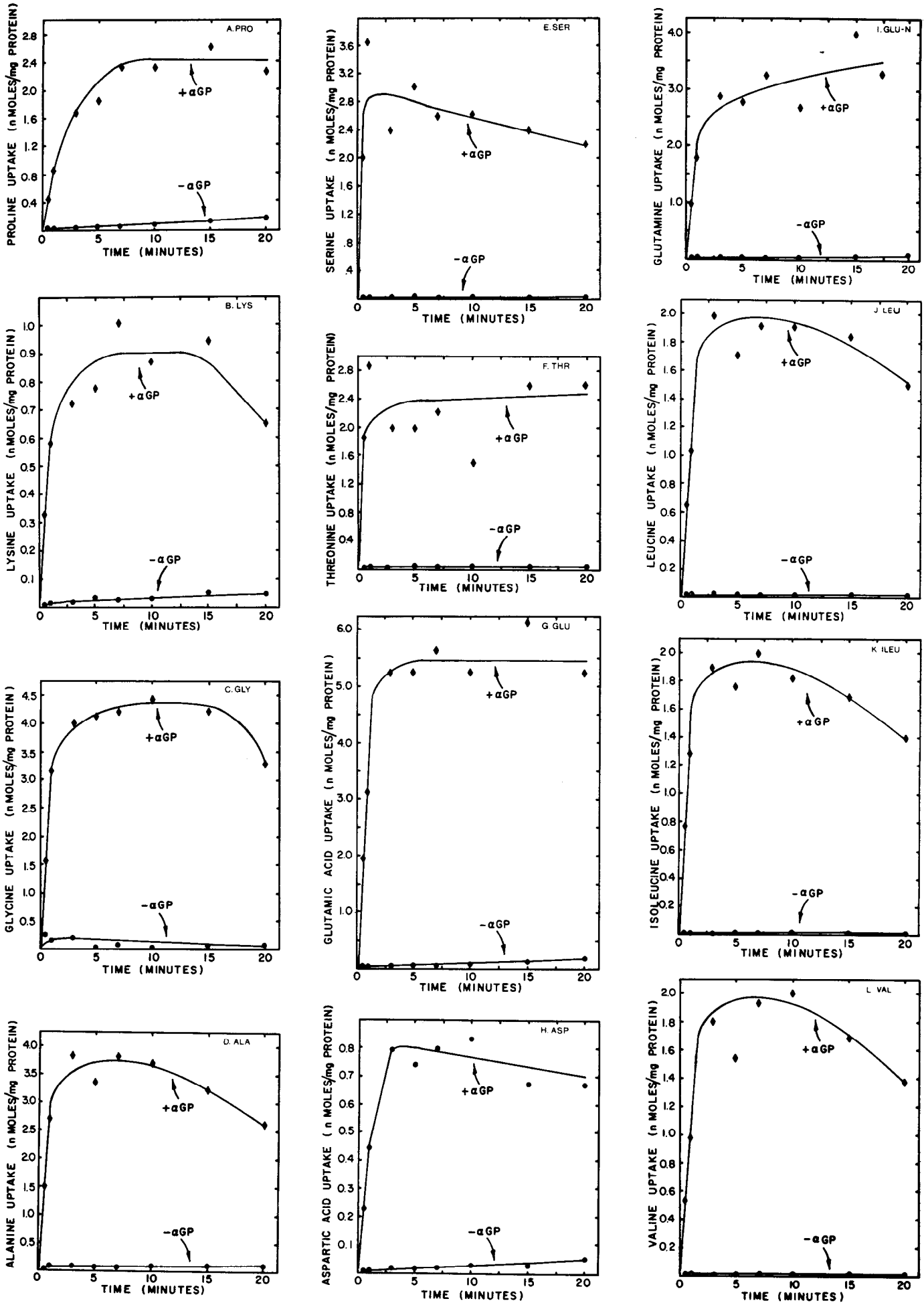


FIGURE 3.

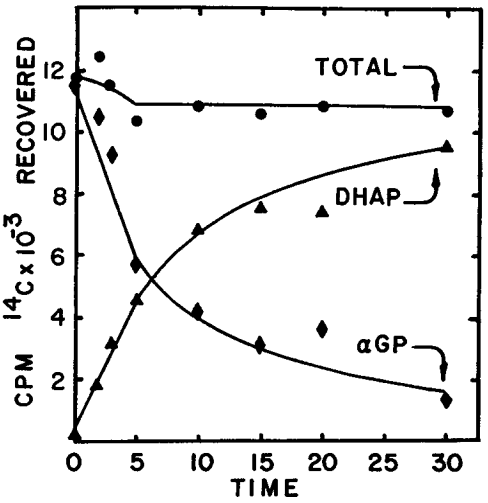


FIG. 4. Time course of L-α-glycerol-P conversion to dihydroxyacetone-P in *S. aureus* membrane vesicles. Membrane suspensions prepared as described in Table I and Fig. 2 were incubated with nonradioactive amino acids, in approximately the same concentrations as those used to study transport, and α-[U-$^{14}$C]glycerol-P. At the times indicated, samples were removed, frozen in Dry Ice-acetone, thawed, and immediately applied to oxalic acid-washed paper. Chromatography was carried out as described under "Experimental Procedure." α-Glycerol-P and dihydroxyacetone-P were located by radioautography, the spots cut out, and the radioactivity determined in a liquid scintillation spectrometer. *α-GP* (◆——◆), α-glycerol-P; *DHAP* (▲——▲), dihydroxyacetone-P; *TOTAL* (●——●), *α-GP* plus *DHAP* recovered.

TABLE II

*Effect of arsenate on transport of amino acids by membrane vesicles of S. aureus*

The transport of amino acids was measured as in Table I in the presence of 50 mM potassium phosphate buffer, or 50 mM potassium phosphate buffer, pH 7.3, plus 50 mM sodium arsenate buffer, pH 7.3, where indicated. The tubes were initially incubated for 15 min before α-glycerol-P (20 mM) and $^{14}$C-amino acids were added. The concentrations and specific activities of the $^{14}$C-amino acids used are given under "Experimental Procedure."

| Amino acid | Transport | | Inhibition |
|---|---|---|---|
| | (−) Arsenate | (+) Arsenate | |
| | *nmoles/mg protein/30 sec* | | % |
| Lysine | 0.44 | 0.37 | 14.7 |
| Threonine | 0.63 | 0.58 | 7.1 |
| Serine | 0.77 | 0.53 | 31.9 |
| Aspartic acid | 0.64 | 0.64 | 0 |
| Glycine | 0.24 | 0.25 | 0 |
| Proline | 0.17 | 0.12 | 26.5 |
| Leucine | 0.17 | 0.18 | 0 |
| Isoleucine | 0.14 | 0.13 | 0 |
| Valine | 0.14 | 0.14 | 0 |
| Alanine | 0.47 | 0.47 | 0 |

FIG. 3. Time courses of proline (*PRO*) (*A*), lysine (*LYS*) (*B*), glycine (*GLY*) (*C*), alanine (*ALA*) (*D*), serine (*SER*) (*E*), threonine (*THR*) (*F*), glutamic acid (*GLU*) (*G*), aspartic acid (*ASP*) (*H*), glutamine (*GLU-N*) (*I*), leucine (*LEU*) (*J*), isoleucine (*ILEU*) (*K*), and valine (*VAL*) (*L*) uptake by *S. aureus* membrane vesicles. Determinations were carried out as described in Table I. +*α-GP* (◆——◆), reactions carried out in the presence of 20 mM α-glycerol-P (*α-GP*); −*α-GP* (●——●), reactions carried out in the absence of α-glycerol-P.

TABLE III

*Oxygen utilization by vesicles prepared from S. aureus*

Oxygen uptake represents the initial rate of oxygen uptake by 0.5 mg of vesicle membrane protein in 1 ml of 50 mM potassium phosphate buffer, pH 7.3, containing 10 mM $MgSO_4$ after the addition of 5 μl of substrate (20 mM final concentration except for NADH which was 5 mM). In the column labeled (+) NEM, the assay mixture was incubated with *N*-ethylmaleimide (10 mM) for 15 min before the addition of substrate.

| Substrate | Oxygen uptake | | Inhibition |
|---|---|---|---|
| | (−) NEM | (+) NEM | |
| | *nmoles $O_2$/min/mg protein* | | % |
| NADH | 82.5 | 75.3 | 8.8 |
| L-α-Glycerol-P | 67.8 | >1.5 | <97.7 |
| L-Lactate | 8.81 | | |
| Succinate | 5.95 | | |
| None | 0.0 | | |

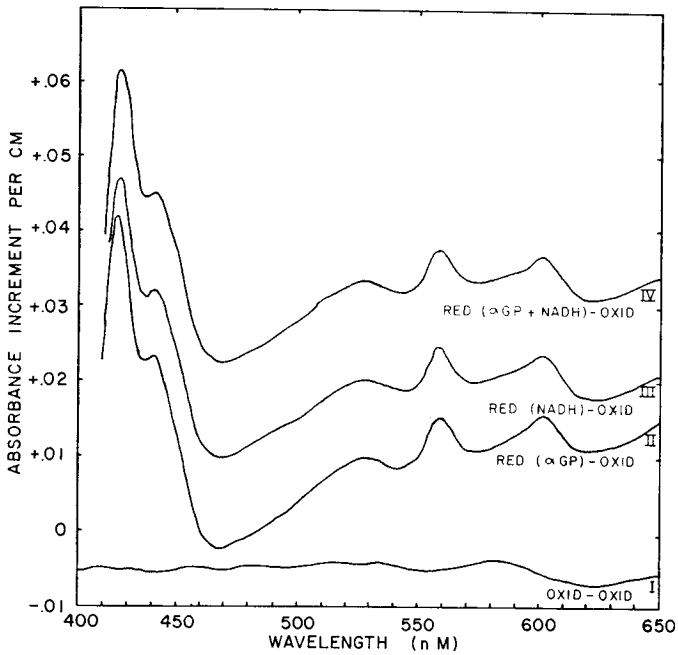


FIG. 5. Difference spectra of vesicles in the presence of various electron donors. Membrane vesicle suspensions were prepared as described under "Experimental Procedure." α-Glycerol-P (*α-GP*) or NADH was added to a portion of the vesicles. After the anaerobic steady state was achieved, difference spectra were recorded between reduced (*RED*) and oxidized (*OXID*) membrane preparations. *I*, difference spectrum of two suspensions in the oxidized state; *II*, difference spectrum of vesicles reduced in the presence of 20 mM α-glycerol-P minus vesicles in the oxidized state; *III*, difference spectrum of vesicles reduced in the presence of 5 mM NADH minus vesicles in the oxidized state; *IV*, difference spectrum of vesicles reduced in the presence of 20 mM α-glycerol-P plus 5 mM NADH minus vesicles in the oxidized state. Addition of sodium dithionite produced the same difference spectra as those observed in *II*, *III*, and *IV*.

transport must occur prior to entry of electrons into the cytochrome system.

*Effect of N-Ethylmaleimide on Oxidation*—As shown in Table III, the addition of NEM to membrane vesicles inhibits oxygen utilization 98% with α-glycerol-P as substrate. At this con-

centration of NEM, amino acid transport is also inhibited by 98%. On the other hand, NADH oxidation is inhibited only 9% in the presence of NEM. Although the data will not be presented in detail, it should be emphasized that inhibition of oxygen uptake by NEM does not appear to be mediated at the level of the primary dehydrogenase for α-glycerol-P. Thus, α-glycerol-P:dichloroindophenol reductase activity in intact vesicles (238 nmoles of dichloroindophenol reduced per min per mg of protein) is insensitive to NEM inhibition. Since neither the primary α-glycerol-P dehydrogenase itself nor NADH oxidation is sensitive to NEM, and since both dehydrogenases are coupled to the same cytochrome chain, the site of inhibition of α-glycerol-P oxidation by NEM must lie between α-glycerol-P dehydrogenase and the cytochromes.

## DISCUSSION

The data presented in this paper show that the transport of a wide variety of amino acids by membrane vesicles isolated from *S. aureus* is coupled exclusively to a membrane-bound α-glycerol-P dehydrogenase. In this respect, the *S. aureus* system differs from the respiration-coupled sugar and amino acid transport systems described in *E. coli* membrane vesicles (1–4, 6) and the amino acid transport systems described in *B. subtilis* vesicles (5). In the *E. coli* system, these transport systems are coupled primarily to D-lactic dehydrogenase; and in the *B. subtilis* system to NADH dehydrogenase, α-glycerol-P dehydrogenase, and to some extent, L-lactic dehydrogenase. It must be emphasized, however, that in every other aspect investigated thus far, amino acid transport in *S. aureus* membrane vesicles appears to be catalyzed by mechanisms which are very similar to those described in the *E. coli* system. Thus, transport is coupled to a specific dehydrogenase, is dependent on electron transfer but independent of oxidative phosphorylation, the site of energy coupling between α-glycerol-P dehydrogenase and transport occurs between the primary dehydrogenase and the cytochrome chain, and there appears to be one or more sulfhydryl components in the respiratory chain between α-glycerol-P dehydrogenase and the cytochrome chain which is (are) essential for transport and α-glycerol-P oxidation.

Recent experiments with an α-glycerol-P dehydrogenase⁻ mutant isolated by Dr. Leonard Mindich of the Public Health Institute of the City of New York which will be published in detail at a later data indicate that the conclusions presented here for isolated membrane vesicles can be extended to whole cells. The optimum generation time of this mutant relative to the parent is dependent on much higher concentrations of amino acids in the growth medium. This observation indicates that the mutant does not transport amino acids as effectively as the parent specifically because of a defect in α-glycerol-P dehydrogenase.

*Acknowledgments*—We would like to express our appreciation to Doctors Paul Bartl and Milas Boublik and Mr. Frank Jenkins for preparing the electron micrographs presented in this paper.

## REFERENCES

1. KABACK, H. R., AND MILNER, L. S., *Proc. Nat. Acad. Sci. U. S. A.*, **66,** 1008 (1970).
2. BARNES, E. M., JR., AND KABACK, H. R., *Proc. Nat. Acad. Sci. U. S. A.*, **66,** 1190 (1970).
3. KABACK, H. R., AND BARNES, E. M., JR., *J. Biol. Chem.*, **246,** 5523 (1971).
4. BARNES, E. M., JR., AND KABACK, H. R., *J. Biol. Chem.*, **246,** 5519 (1971).
5. KONINGS, W. N., AND FREESE, E., *Fed. Eur. Biochem. Soc. Lett.*, **14,** 65 (1971).
6. KONINGS, W. N., BARNES, E. M., JR., AND KABACK, H. R., *J. Biol. Chem.*, **246,** 5857 (1971).
7. SHORT, S. A., AND WHITE, D. C., *J. Bacteriol.*, **104,** 126 (1970).
8. KABACK, H. R., in W. B. JAKOBY (editor), *Methods in enzymology, Vol. XXII*, Academic Press, New York, 1971, p. 99.
9. RAMÍREZ, J., AND SMITH, L., *Biochim. Biophys. Acta*, **153,** 466 (1968).
10. WAWSZKIEWICCZ, E. J., *Anal. Chem.*, **33,** 252 (1961).
11. HANES, C. S., AND ISHERWOOD, F. A., *Nature*, **164,** 1107 (1949).
12. KABACK, H. R., AND DEUEL, T. F., *Arch. Biochem. Biophys.*, **132,** 118 (1969).
13. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J., *J. Biol. Chem.*, **193,** 265 (1951).
14. FRERMAN, F. E., AND WHITE, D. C., *J. Bacteriol.*, **94,** 1868 (1967).
15. SHORT, S. A., WHITE, D. C., AND ALEEM, I. H., *J. Bacteriol.*, **99,** 142 (1969).