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Mechanisms of Active Transport in Isolated Bacterial Membrane Vesicles

IX. THE KINETICS AND SPECIFICITY OF AMINO ACID TRANSPORT IN *STAPHYLOCOCCUS AUREUS* MEMBRANE VESICLES*

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SUMMARY

The activity of the α-glycerol-phosphate dehydrogenase-coupled amino acid transport systems in membrane vesicles prepared from *Staphylococcus aureus* is comparable to the transport activity of intact cells. Temperature optima for initial velocities and steady state levels of amino acid accumulation in the vesicles are at approximately 45° and 25°, respectively, and apparent Michaelis constants for the transport of 17 amino acids are in the micromolar range.

There are 12 distinct and specific transport systems for the following families of structurally related amino acids: alanine and glycine; leucine, isoleucine, and valine; serine and threonine; aspartate and glutamate; asparagine and glutamine; lysine; histidine; arginine; phenylalanine, tyrosine and tryptophan; cysteine; methionine; and proline. Structurally dissimilar amino acids do not alter the kinetic constants for the uptake of a given amino acid, and K_i values for amino acids of the same family are essentially identical with the Michaelis constants for the transport of those amino acids. These findings indicate that structurally related amino acids of the same group are transported by the same membrane carrier. Except for the alanine-glycine, leucine-isoleucine-valine, and aspartate-glutamate transport systems in which D-amino acids inhibit the uptake of the appropriate ^{14}C-labeled L-amino acid, each of the transport systems tested is stereospecific for the L-amino acid isomer.

Apparent Michaelis constants for α-glycerol-P:dichloroindophenol reductase and α-glycerol-P oxidation are identical in intact and sonically disrupted membrane vesicles. Moreover, a similar apparent Michaelis constant is obtained for α-glycerol-P with respect to stimulation of amino acid transport. These findings indicate the α-glycerol-P oxidation *per se* is the rate-limiting step for amino acid transport in these preparations.

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In a previous paper (1), it was demonstrated that the transport of amino acids by membrane vesicles prepared from *Staphylococcus aureus* is coupled virtually exclusively to a membrane-bound α-glycerol phosphate dehydrogenase. With this exception, amino acid transport in *S. aureus* vesicles appears to be catalyzed by mechanisms which are very similar to the D-lactic dehydrogenase-coupled sugar and amino acid transport systems described in *Escherichia coli* membrane vesicles (2–8). Specifically, transport is dependent on electron transfer but independent of oxidative phosphorylation, the site of energy coupling 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 (1).

In this paper, data are presented which demonstrate that membrane vesicles prepared from *S. aureus* retain essentially all of the amino acid transport activity of the parent cells. The number and specificity of the amino acid transport systems in the vesicles, as well as their kinetic parameters are described, and evidence is presented which indicates that the transport of α-glycerol-P is not a rate-limiting step for amino acid transport.

MATERIALS AND METHODS

Growth of S. aureus—*S. aureus* U-71 was grown with aeration and harvested as described previously (9).

Preparation of Membrane Vesicles—Membrane vesicles were prepared from lysostaphin-induced protoplasts of *S. aureus* as described previously (1, 10), except that the vesicles were washed in 50 mM potassium phosphate buffer, pH 7.3, instead of 0.1 M

potassium phosphate buffer containing 10 mM EDTA. The membrane vesicles rendered free of contaminating cellular debris were suspended to a protein concentration of approximately 3 mg per ml in 50 mM potassium phosphate buffer, pH 7.3, and frozen in liquid nitrogen.

Measurement of Transport—The uptake of amino acids was assayed in 50-μl reaction mixtures (final volume) by the procedures previously described (1, 10). In the experiments to determine the specificity of amino acid transport, the final concentration of all nonradioactive amino acids was 100 μM unless otherwise indicated. 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), 1.56×10^{-5} M; leucine (262 mCi per mmole), 7.65×10^{-6} M; glutamic acid (206 mCi per mmole), 9.73×10^{-6} M; glutamine (219 mCi per mmole), 9.13×10^{-6} M; threonine (164 mCi per mole), 1.21×10^{-5} M; glycine (78 mCi per mmole), 2.54×10^{-5} M; isoleucine (273 mCi per mmole), 7.31×10^{-6} M; aspartic acid (170 mCi per mmole), 1.17×10^{-5} M; lysine (255 mCi per mmole), 7.85×10^{-6} M; valine (248 mCi per mmole), 8.05×10^{-6} M; alanine (137 mCi per mmole), 1.45×10^{-5} M; proline (214 mCi per mmole), 9.34×10^{-6} M; tyrosine (410 mCi per mmole), 4.44×10^{-6} M; histidine (256 mCi per mmole), 7.85×10^{-6} M; phenylalanine (383 mCi per mmole), 3.58×10^{-6} M; arginine (255 mCi per mmole), 8×10^{-6} M; asparagine (279 mCi per mmole), 7.2×10^{-6} M; tryptophan (340 mCi per mmole), 6.25×10^{-6} M; cysteine (271 mCi per mmole), 7.4×10^{-6} M; methionine (221 mCi per mmole), 8.4×10^{-6} M.

Measurement of α-Glycerol-P Dehydrogenase Activity—The activity of membrane-bound α-glycerol-P dehydrogenase was assayed either spectrophotometrically with 2,6-dichloroindophenol or by oxygen utilization as described previously (1).

Pyridine Hemochromes—Protoheme was measured as the pyridine hemochrome as described by Falk (11).

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

Materials—Radioactive amino acids were purchased from New England Nuclear Co., Boston, Mass. L-α-Glycerol-P, 2,6-dichloroindophenol, nonradioactive amino acids, and lysine analogues were obtained from Sigma Chemical Co., St. Louis, Mo. Other reagents were obtained from sources described previously (1).

RESULTS

Amino Acid Transport in Membrane Vesicles versus Whole Cells—The data presented in Table I demonstrate that the initial rate of α-glycerol-P dehydrogenase-coupled transport of eight representative amino acids by *S. aureus* membrane vesicles is essentially the same as that of whole cells when expressed as nanomoles of amino acid transported per min per nmole of cytochrome $b + o$. As demonstrated previously (1), the vesicles contain 7 to 10 times the cytochromes $b + o$ per mg of protein as the intact cells. Thus, the amino acid transport activity of the whole cells is retained completely by the membrane vesicles.

Effect of Temperature on Amino Acid Transport—The effect of temperature on the initial velocity and steady state level of amino acid accumulation is shown in Fig. 1, *A* and *B*, respectively. Data are presented for the transport of threonine, leucine, and glutamine only, but it should be noted that each of the other amino acid transport systems has been studied in the same manner and displays similar properties. As shown, there is essentially no uptake of threonine, leucine, or glutamine above 60° in initial rate studies (Fig. 1*A*), above 45° in long term incubations (Fig. 1*B*), or at 0° in either case. From 10–42°, the initial rates of leucine, threonine, and glutamine uptake increase 24-, 7-, and 10-fold, respectively (Fig. 1*A*). The steady state level of amino acid accumulation increases 20-fold for leucine, 14-fold for threonine, and 60-fold for glutamine from 10° to 25° (Fig. 1*B*).

Kinetics of Amino Acid Uptake in Membrane Vesicles—Initial

TABLE I

Amino acid transport in membrane vesicles versus whole cells

α-Glycerol-P-dependent transport of the amino acids listed by membrane vesicles and washed intact cells of *Staphylococcus aureus* was assayed as described in Fig. 1 and "Materials and Methods," except that the incubations were terminated at 1 min. Under the conditions employed, the rates of amino acid uptake by the vesicles and the cells were linear for 2 to 3 min. Cytochrome $b + o$ content of vesicles and cells was determined as pyridine hemochrome (11).

Amino acid	Whole cells	Vesicles	(Vesicles/whole cells) × 100
	nmoles transported per min per nmole cytochrome b + o		%
Alanine	3.438	3.303	96
Leucine	1.236	1.734	139
Serine	9.935	9.864	99
Glutamate	1.573	1.325	85
Lysine	1.558	1.908	122
Proline	1.855	1.755	95
Phenylalanine	0.408	0.469	117
Tyrosine	0.434	0.377	87

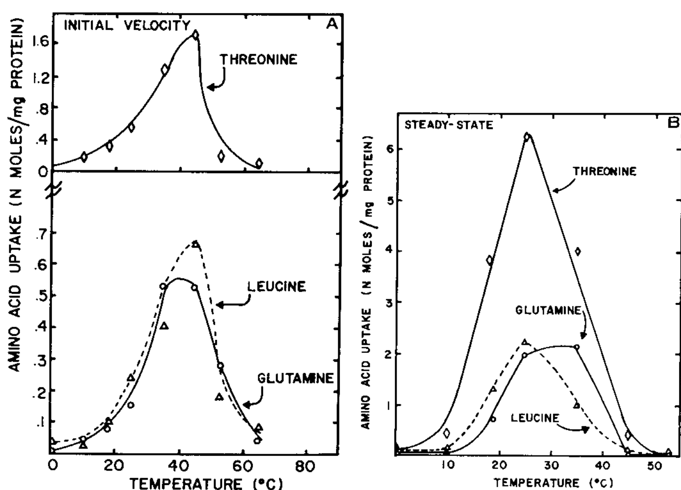

FIG. 1. *A*, effect of temperature on the initial velocity of amino acid transport by *Staphylococcus aureus* membrane vesicles. Initial rates of threonine (◇), leucine (△), and glutamine (○) transport were assayed in 50-μl (final volume) reaction mixtures at the temperatures shown. Reaction mixtures contained (in final concentration) 10 mM potassium phosphate (pH 7.3), 10 mM magnesium sulfate, 20 mM α-glycerol-P, and one of the uniformly ^{14}C-labeled L-amino acids indicated in the final concentration and specific activity given in "Materials and Methods." Reactions were initiated by injection of 10 μl of a membrane suspension containing approximately 30 μg of membrane protein into reaction mixtures which had been previously incubated at the temperatures given for approximately 2 min. Reactions were terminated at 30 s and samples were assayed as described previously (10). *B*, effect of temperature on the steady state level of amino acid accumulation. The effect of temperature on the steady state levels of threonine (◇), leucine (△), and glutamine (○) accumulation was assayed as described in *A* except that the incubations were terminated at 7 min.

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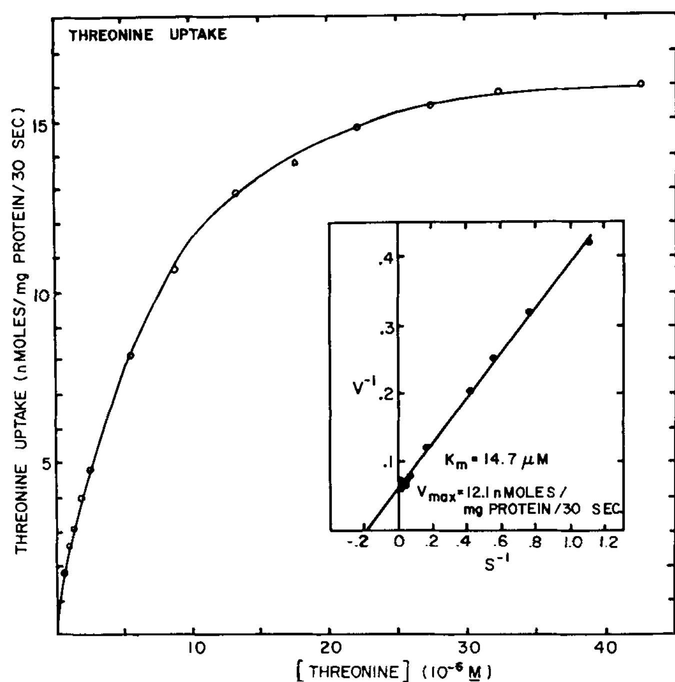

FIG. 2. Kinetics of threonine uptake by *Staphylococcus aureus* membrane vesicles. Initial rates of threonine uptake at the threonine concentrations shown were determined at 30 s as described in Fig. 1*A*. The reactions were carried out at 25°, and the initial rates of uptake were linear at each threonine concentration tested for at least 30 s to 1 min. *Inset*, double reciprocal plot of the data.

velocities of α-glycerol-P-dependent threonine uptake measured as a function of increasing threonine concentration are presented in Fig. 2. The initial rate of transport increases rapidly up to approximately 10^{-5} M and approaches a constant value at concentrations in excess of 3×10^{-5} M. As shown in the *inset* of Fig. 2, these data yield an apparent K_m of 14.7×10^{-6} M and a V_{max} of 24.2 nmoles per mg of membrane protein per min for threonine uptake. Although data are not presented in detail, these results are illustrative of those obtained for each of 16 amino acids studied. Kinetic constants derived from these experiments are summarized in Table II. Proline transport exhibits the lowest apparent K_m (3.51×10^{-6} M), while the aspartate-glutamate transport system exhibits the highest apparent K_m (43.5×10^{-6} M and 38.6×10^{-6} M, respectively), and the serine-threonine system manifests the highest V_{max} (25.0 and 24.2 nmoles per mg of membrane protein per min, respectively). Each of the systems exhibits a monophasic asymptotic function over the concentration range studied. In the absence of α-glycerol-P, the rate of uptake of each amino acid exhibits linear kinetics with no tendency toward saturation at concentrations up to 1 mM (data not shown).

Number and Specificity of Amino Acid Transport Systems—The number and specificity of the amino acid transport systems in the vesicles preparations was determined by measuring the inhibition of transport (*i.e.* initial rates and steady state levels of accumulation) of a given ^{14}C-labeled amino acid by each of 20 nonradioactive amino acids. The results of these experiments (Table III) demonstrate that very few amino acids significantly inhibit the uptake of a given ^{14}C-labeled amino acid. Moreover, in all cases, the inhibition observed is mutual, suggesting that the two amino acids are transported by the same carrier. The following groups of amino acids exhibit mutual inhibition: alanine and glycine; leucine, isoleucine, and valine; serine and threonine; aspartate and glutamate; asparagine and glutamine; lysine; histidine; arginine; phenylalanine, tyrosine and tryptophan; cysteine; methionine; and proline.

TABLE II

Kinetic constants for amino acid transport in Staphylococcus aureus membrane vesicles

Apparent K_m (micromolar) and V_{max} (nanamoles of amino acid transported per min per mg of membrane protein) values were determined from double reciprocal plots obtained from initial velocity experiments carried out as described in Fig. 2.

Amino acid	K_m	V_{max}
	μM	
Glycine	20	6.06
Alanine	16.7	4.00
Leucine	14.3	2.86
Isoleucine	14.3	2.74
Valine	16.7	2.86
Serine	15.2	25.0
Threonine	14.7	24.2
Aspartate	43.5	3.64
Glutamate	38.5	13.32
Asparagine	14.3	1.54
Glutamine	12.5	1.34
Lysine	10.1	6.66
Phenylalanine	25.0	3.64
Tyrosine	28.6	3.34
Tryptophan	26.2	3.34
Proline	3.51	2.36

Similar experiments were carried out with the serine-threonine and lysine transport systems in intact cells (data not shown), and the same pattern of specificity as that shown for the membrane vesicles was observed (*i.e.* only serine and threonine inhibit [^{14}C]serine uptake, and only lysine inhibits the uptake of [^{14}C]-lysine).

Although the data presented above suggest that there is a single carrier for each of 12 groups of structurally related amino acids, the possibility that the transport of amino acids within a specific group may be catalyzed by several carriers with different affinities is not excluded. In order to investigate this possibility, more detailed studies were carried out with some of the transport systems. Initial rates of [^{14}C]serine uptake as a function of serine concentration in the presence of excess glutamate, glycine, threonine, valine, or proline plotted by the method of Lineweaver and Burk (13) are presented in Fig. 3. The addition of glutamate or glycine has no effect at any of the serine concentrations studied nor does valine or proline (data not shown). Threonine, however, behaves as a competitive inhibitor of serine transport (*i.e.* in the presence of this amino acid, the apparent K_m for serine transport is markedly increased while the V_{max} remains unchanged). Kinetic studies of threonine competition for serine transport plotted by the method of Dixon (14) yield an apparent K_i of 13.5 μM (Fig. 4), a value which is similar to the apparent K_m of the serine-threonine transport system for threonine (*cf.* Table II). These results indicate that the same carrier catalyzes the concentrative uptake of serine and threonine. Similar studies were also carried out with the aspartate-glutamate, leucine-isoleucine-valine, and asparagine-glutamine transport systems (data not shown). Apparent K_i values of 40 μM, 12.5 μM, and 15 μM were obtained for glutamate, isoleucine or valine, and asparagine, respectively, with regard to the transport of aspartate, leucine, and glutamine. In each case, the apparent K_i for the competing

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TABLE III

Specificity of amino acid transport in Staphylococcus aureus membrane vesicles

Transport of each uniformly ^{14}C-labeled L-amino acid listed in the vertical column to the left was determined as described in Fig. 1. Specific activities and concentrations of each ^{14}C-amino acid are given in "Materials and Methods." The concentration of each nonradioactive challenging amino acid listed in the upper horizontal line was 100 μM. Reactions were terminated at 30 s (for initial velocity experiments) or 7 min (for steady state experiments). The numbers given represent the percentage of inhibition with respect to amino acid uptake obtained in the absence of unlabeled amino acid. Data are presented for initial velocity experiments (*i.e.* 30-s incubations), although very similar results were obtained under steady state conditions (data not shown).

^{14}C Amino Acid	%Inhibition by Non-Radioactive Amino Acid																			
	Gly	Ala	Leu	Ile	Val	Ser	Thr	Asp	Glu	Asn	Gln	Lys	His	Arg	Phe	Tyr	Trp	Cys	Met	Pro
Gly	96	95	0	12	5	10	10	0	0	4	5	0	1	0	0	0	0	0	4	0
Ala	97	96	0	8	4	20	16	9	11	3	2	3	11	0	0	0	0	0	0	0
Leu	0	0	78	82	76	0	0	0	0	4	6	0	0	0	0	0	0	0	0	0
Ile	0	0	81	78	80	0	0	0	0	0	0	0	5	0	2	5	4	8	9	4
Val	0	0	80	80	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	10	11	0	3	0	80	83	4	3	1	0	0	3	0	0	0	0	0	4	0
Thr	0	3	1	0	3	82	79	0	5	0	0	0	0	0	0	0	0	8	0	0
Asp	20	15	12	10	16	0	8	78	83	16	14	0	2	0	0	0	0	0	2	10
Glu	5	6	0	0	2	3	1	84	81	0	4	0	0	0	0	0	0	0	0	0
Asn	15	13	9	7	11	0	0	4	7	85	80	14	21	15	3	6	8	20	9	0
Gln	15	18	12	14	10	7	5	11	8	90	83	16	18	8	9	13	8	8	12	8
Lys	0	0	4	2	3	6	3	5	2	6	0	86	11	0	8	5	10	15	10	0
His	0	2	0	0	0	4	7	0	0	3	5	6	87	0	3	2	0	0	0	5
Arg	0	0	0	0	0	3	6	2	0	8	2	8	3	84	0	0	0	0	0	0
Phe	0	8	10	3	0	0	0	8	10	3	8	5	15	1	98	100	97	11	15	4
Tyr	0	0	11	0	7	6	10	0	6	0	0	0	0	0	85	89	86	9	13	0
Trp	0	4	0	6	0	0	0	0	0	5	9	0	7	0	88	98	85	1	0	0
Cys	1	0	8	3	0	4	5	0	6	9	5	0	7	9	0	5	9	88	14	4
Met	3	1	4	7	3	0	0	0	0	3	0	0	0	0	3	1	6	9	87	0
Pro	0	0	21	18	25	10	12	11	9	1	0	1	20	0	0	0	0	5	12	97

amino acid is similar to the apparent K_m of the transport system for that amino acid (*cf.* Table II).

The serine-threonine, asparagine-glutamine, and phenylalanine-tyrosine-tryptophan transport systems are specific for L-amino acids as evidenced by the observation that the addition of the appropriate D-amino acids to the reaction mixtures does not inhibit the uptake of these ^{14}C-labeled L-amino acids (Table IV). In contrast, the leucine-isoleucine-valine, alanine-glycine, and aspartate-glutamate systems display decreasing relative affinities for the corresponding D-amino acids. Stereospecificity of the other amino acid transport systems was not studied.

Several analogues of lysine were examined for their ability to compete with L-lysine for transport (Table V). The results suggest that loss of the α-amino group on lysine, as in ϵ-aminocaproic acid, or modification of the α-amino group, as in N-acetyl-L-lysine, abolishes the affinity of these analogues for the lysine carrier as evidenced by their inability to compete with L-lysine for the transport system. Moreover, the lack of inhibition of lysine transport by δ-hydroxy-L-lysine suggests that the δ-amino group on lysine is also an important determinant for substrate binding by the lysine carrier. On the other hand, S-(2-aminoethyl)-cysteine inhibits lysine uptake by 62% and lysine methylester inhibits by 43% compared to L-lysine which inhibits by 86%. These results indicate that modification of the carbon chain between the two amino groups of lysine which does not result in alteration of the molecular geometry of the analogue (*i.e.* S-(2-aminoethyl)-cysteine) or alteration of the carboxyl group of lysine (*i.e.* lysine methylester) does not interfere markedly with analogue-carrier association.

Kinetics of α-Glycerol-P Dehydrogenase with Respect to Substrate Oxidation and Amino Acid Transport—The rate of α-glycerol-P:dichloroindophenol reductase activity in intact and sonically disrupted *S. aureus* membrane vesicles exhibits saturation kinetics when measured as a function of increasing α-glycerol-P concentration, yielding an apparent K_m of 3.85 mM (Fig. 5*A*). A similar value is obtained when oxygen utilization is used to assay α-glycerol-P dehydrogenase activity (data not shown).

The initial rates and steady state levels of proline, glutamic acid, and serine accumulation also exhibit saturation kinetics when measured as functions of increasing α-glycerol-P concentration, and apparent K_m values for α-glycerol-P of 4.35, 4.1, and 5.1, respectively, are observed (Fig. 5*B*). Since the experiments shown in Fig. 1*B* were carried out with amino acid concentrations which exceed the apparent K_m values for these transport systems (*cf.* Table II), those data presented in Fig. 5, *A* and *B*, indicate that the rate of α-glycerol-P oxidation *per se* is the rate-limiting step for amino acid transport in these preparations.

DISCUSSION

Data presented in this paper demonstrate that isolated cytoplasmic membrane vesicles prepared from *S. aureus* transport amino acids as effectively as whole cells. Thus, the transport activity observed in this *in vitro* system is not a trivial fraction of the whole cell transport capacity (15), and conclusions derived from studies utilizing this experimental system are probably highly relevant to the living cell. This observation is also important in at least two other respects. (*a*) It is unlikely that a significant number of vesicles are inverted with regard to the "sidedness" of the membrane (this contention is consistent with a number of morphological observations (16)); and (*b*) assuming

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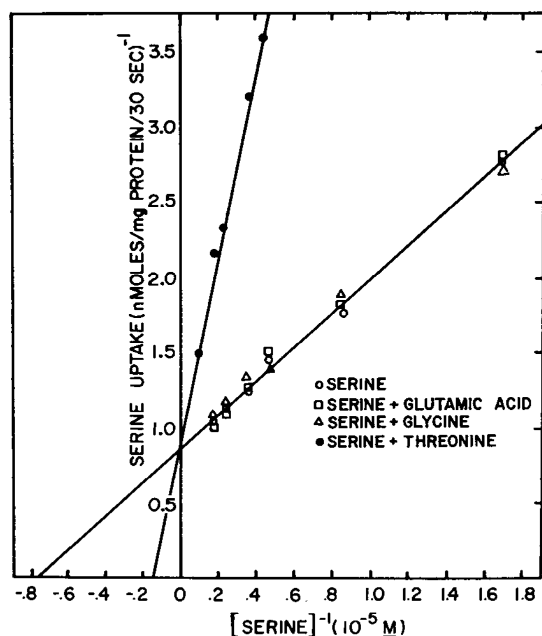

FIG. 3. Specificity of the serine transport mechanism. In this experiment, the effect of several nonradioactive amino acids on the kinetics of L-[U-^{14}C]serine uptake was determined. Reaction mixtures contained (in final concentrations) 10 mM potassium phosphate (pH 7.3), 10 mM magnesium sulfate, 20 mM α-glycerol-P, L-[U-^{14}C]serine (128 mCi per mmole) at the concentrations shown, 30 μg of membrane protein, and one of the nonradioactive amino acids indicated at 100 μM. Reactions were initiated by the injection of 10 μl of the membrane suspension into reaction mixtures which had been previously incubated at 25° for approximately 2 min. At 30 s, the reactions were terminated and the samples were assayed as described previously (10). Results for [^{14}C]serine uptake in the absence of nonradioactive amino acids (○) and for [^{14}C]serine uptake in the presence of threonine (●), glutamic acid (□), or glycine (△) are shown. The results shown were plotted by the method of Lineweaver and Burk (13). Similar experiments in which the kinetics of [^{14}C]serine uptake were measured in the presence of proline or valine yielded results which are identical with those obtained for [^{14}C]serine uptake in the absence of added amino acids (data not shown).

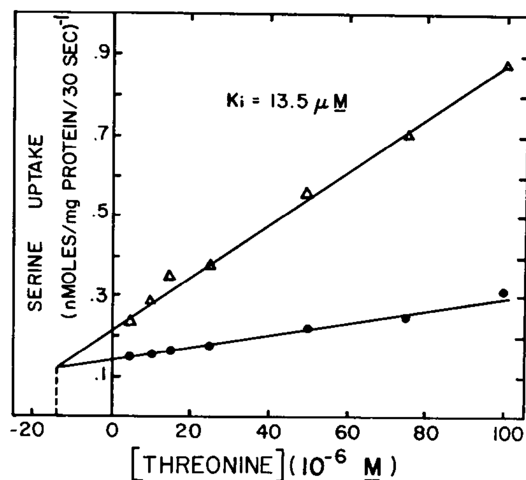

FIG. 4. Initial rates of serine transport in the presence of increasing threonine concentrations. Competition of threonine for the serine-threonine transport system was assayed as described in Fig. 3 at serine concentrations of 1.41×10^{-5} M (△) and 4.11×10^{-5} M (●). The threonine concentration was varied as indicated. The data obtained were plotted by the method of Dixon (14).

TABLE IV

Stereospecificity of amino acid transport in Staphylococcus aureus membrane vesicles

Initial rates of transport of each ^{14}C-labeled amino acid listed were measured as described in Fig. 1. Where indicated, the appropriate D-amino acid was added at a final concentration of 100 μM. Incubations were terminated at 1 min. The specific activities and concentrations of the uniformly ^{14}C-labeled L-amino acids used are given under "Materials and Methods." The numbers given represent the percentage of inhibition with respect to amino acid uptake observed in the absence of the appropriate D-amino acid.

^{14}C-Labeled L-amino acid	Inhibition by D-amino acid isomer
	%
Alanine	55
Leucine	87
Isoleucine	85
Valine	76
Serine	4
Threonine	0
Aspartate	20
Glutamate	18
Asparagine	1
Glutamine	0
Phenylalanine	0
Tyrosine	0
Tryptophan	2

TABLE V

Specificity of lysine transport by Staphylococcus aureus membrane vesicles

The initial rate of L-[U-^{14}C]lysine transport was measured as described in Fig. 1. Where indicated, unlabeled L-lysine or one of the analogues listed was added at a final concentration of 100 μM. Incubations were terminated at 1 min. The specific activity and concentration of the L-[U-^{14}C]lysine used are given under "Materials and Methods." The numbers given represent the percentage of inhibition with respect to lysine uptake observed in the absence of further additions.

Lysine analogue	Inhibition
	%
None	0
L-Lysine	86
ϵ-Aminocaproic acid	12
δ-Hydroxylysine	8
N-Acetyllysine	9
S-(2-Aminoethyl)-cysteine	62
Lysine methyl ester	43

that none of the vesicles is hyperactive, each of the vesicles in the preparation is probably capable of α-glycerol-P dehydrogenase-coupled amino acid transport.

There are 12 distinct transport systems which catalyze the concentrative uptake of specific structurally related groups of amino acids. Each of these transport systems displays an apparent K_m in the micromolar range, and K_i values determined for amino acids transported by the same system are similar to the apparent K_m of the transport system for the inhibiting amino acid. These results indicate that amino acids of the same group are transported by a common carrier. The specificity of amino acid transport systems in *Bacillus subtilis* membrane vesicles exhibits similar properties (19). It is interesting that initial

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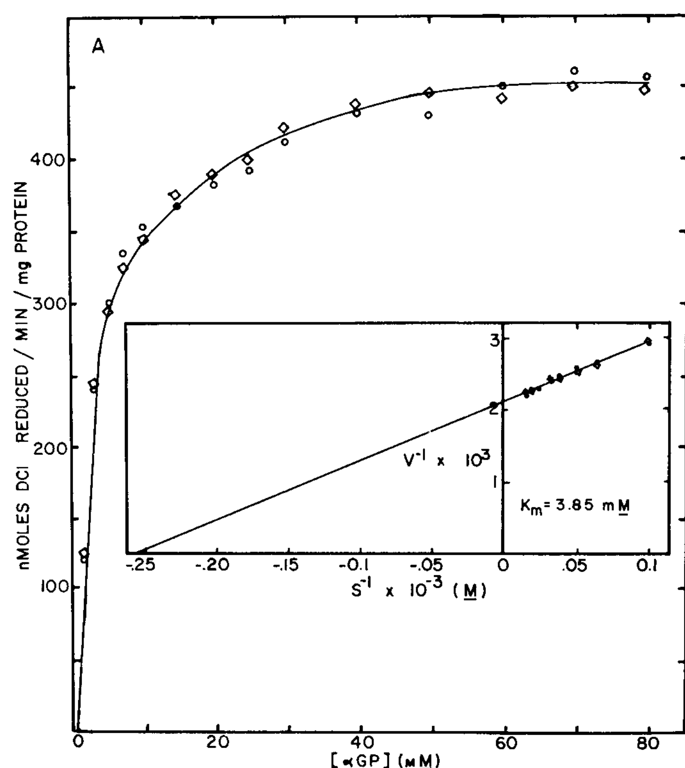
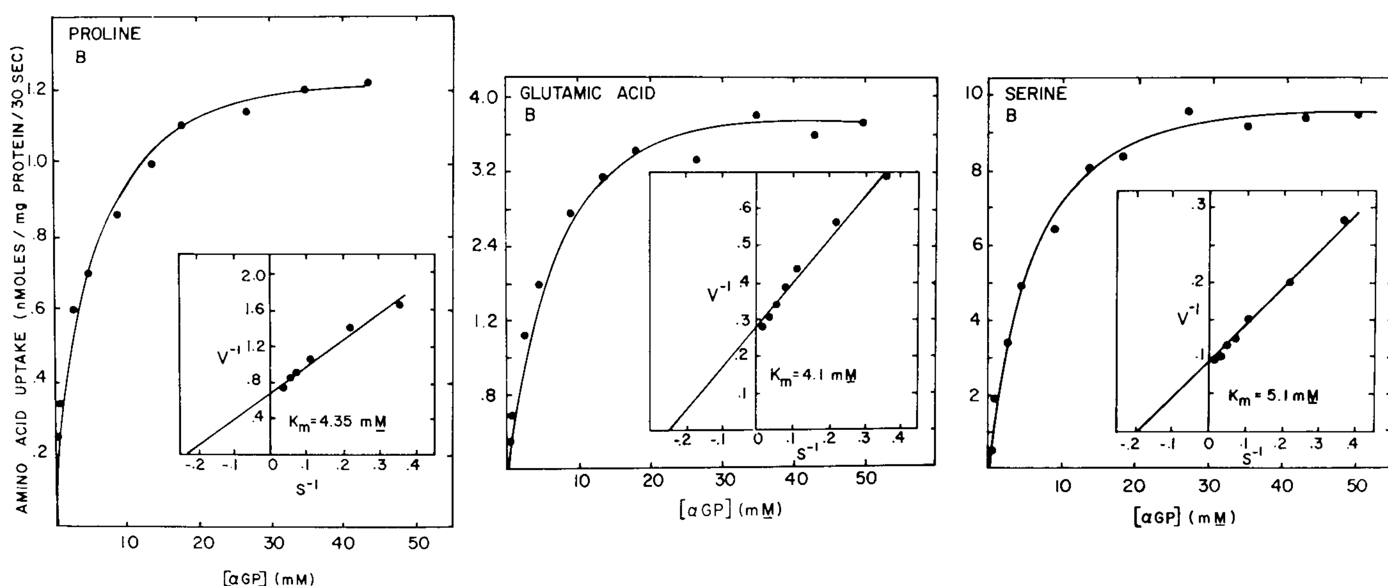


FIG. 5. Kinetics of α-glycerol-P dehydrogenase. *A*, initial rate of α-glycerol-P:dichloroindophenol reductase activity *versus* α-glycerol-P (*α-GP*) concentration in intact (○) and sonically disrupted (◇) membrane vesicles. The rate of reduction of 2,4-dichloroindophenol (*DCI*) was measured spectrophotometrically at 600 nm as described previously (3). Reaction mixtures contained (in final concentrations) 10 mM potassium phosphate (pH 7.3), 10 mM magnesium sulfate, 0.002% dichloroindophenol, and 37.5 μg of membrane protein. Reactions were initiated by the addition of α-glycerol-P in the concentrations shown. For sonic disruption, membrane suspensions containing approximately 3 mg of membrane protein per ml in 50 mM potassium phosphate (pH 7.3) were sonicated in a stainless steel centrifuge tube immersed in an ice bath. Sonication was carried out in two 30-s bursts using a Branson Sonifier set at maximum intensity. Approximately 1 to 2 min was allowed between sonication periods for cooling. *B*, initial rates of amino acid transport as functions of α-glycerol-P (*α-GP*) concentration. Initial rates of serine (*Panel 3*), glutamic acid (*Panel 2*), and proline (*Panel 1*) uptake were determined in reaction mixtures containing (in final concentrations) 10 mM potassium phosphate (pH 7.3), 10 mM magnesium sulfate, 30 μg of membrane protein, 100 μM L-[U-^{14}C]serine, L-[U-^{14}C]glutamic acid, or L-[U-^{14}C]proline, and increasing concentrations of α-glycerol-P as indicated. Reactions were initiated by injection of 10 μl of the membrane suspension into reactions mixtures which had been previously incubated at 25° for 1 to 2 min. Incubations were carried out for 30 s terminated, and assayed as described previously (10). *Insets*, double reciprocal plots.



rates of transport as a function of external amino acid concentration for 16 amino acids exhibit simple monophasic kinetics. In this respect, the leucine-isoleucine-valine and histidine transport systems in *S. aureus* vesicles appear to be less complex than the analogous transport systems in *E. coli* membrane vesicles. In the latter, these transport systems exhibit biphasic kinetics (8).

The access of α-glycerol-P to its dehydrogenase in the vesicles does not appear to be a rate-limiting step for amino acid transport, *i.e.* the transport of α-glycerol-P is not rate limiting. This conclusion is based upon the following observations. (*a*) Kinetic studies of α-glycerol-P:dichloroindophenol reductase activity and α-glycerol-P oxidation exhibit the same apparent K_m in intact and sonically disrupted membrane vesicles. (*b*) Initial rates and steady state levels of proline, glutamic acid, and serine accumulation measured as functions of increasing α-glycerol-P concentration exhibit apparent K_m values for α-glycerol-P which are essentially identical with that obtained for α-glycerol-P:dichloroindophenol reductase and α-glycerol-P oxidation. Although the possibility exists that there is an α-glycerol-P transport system which has identical kinetic constants to those of the dehydrogenase, this possibility seems unlikely, especially since this transport system must be induced in those organisms in which it has been studied (18). It is also noteworthy in this regard that experiments have been carried out in which the vesicles were osmotically shocked in the presence of various concentrations of α-glycerol-P and subsequently assayed for amino acid transport. Previous work (19) has demonstrated that P-enolpyruvate, a relatively impermeable compound, is more accessible to the intravesicular pool during osmotic shock,

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and is thus a more effective P-donor for the P-enolpyruvate-P-transferase-mediated uptake of α-methylglucoside. The effect of α-glycerol-P on amino acid transport is not enhanced by osmotic shock under the same conditions. Similar observations have been made with the D-lactic dehydrogenase-coupled transport systems in *E. coli* membrane vesicles, and in this case as well, transport of electron donors does not appear to be rate-limiting for transport.[1] Moreover, succinate oxidation and amino acid transport as functions of increasing succinate concentration in *Salmonella typhimurium* membrane vesicles prepared from a dicarboxylic acid transport mutant (20, 21)[2] exhibit the same apparent K_m for succinate as that observed in wild type vesicles.[3] Although the active sites of bacterial membrane-bound dehydrogenases may be localized on the inner surface of the vesicle membrane, the access of substrate to these sites is not rate-limiting at the high concentrations used in these studies.

REFERENCES

1. Short, S. A., White, D. C., and Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 298–304
2. Kaback, H. R., and Milner, L. S. (1970) *Proc. Nat. Acad. Sci. U. S. A.* **66,** 1008
3. Barnes, E. M., Jr., and Kaback, H. R. (1970) *Proc. Nat. Acad. Sci. U. S. A.* **66,** 1190; (1971) *J. Biol. Chem.* **246,** 5518–5522
4. Kaback, H. R., and Barnes, E. M., Jr. (1971) *J. Biol. Chem.* **246,** 5523–5531
5. Konings, W. N., Barnes, E. M., Jr., and Kaback, H. R. (1971) *J. Biol. Chem.* **246,** 5857
6. Kerwar, G. K., Gordon, A. S., and Kaback, H. R. (1972) *J. Biol. Chem.* **247,** 291–297
7. Lombardi, F. J., and Kaback, H. R. (1972) *J. Biol. Chem.* **247,** in press
8. Kaback, H. R. (1972) *Biochim. Biophys. Acta,* in press
9. Short, S. A., and White, D. C. (1970) *J. Bacteriol.* **104,** 126
10. Kaback, H. R. (1971) *Methods Enzymol.* **22,** 99
11. Falk, J. E. (1964) in *Porphyrins and Metalloporphyrins,* p. 181, Elsevier Publishing Co., New York
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
13. Lineweaver, H., and Burk, D. (1934) *J. Amer. Chem. Soc.* **56,** 658
14. Dixon, M. (1953) *Biochem. J.* **55,** 170
15. Kepes, A. (1971) *J. Membrane Biol.* **4,** 87
16. Kaback, H. R. (1972) *Biochim. Biophys. Acta,* in press
17. Konings, W. N., and Freese, E., (1972) *J. Biol. Chem.* **247,** 2408–2418
18. Hayashi, S.-, Koch, J. P., and Lin, E. C. C. (1964) *J. Biol. Chem.* **239,** 3098–3105
19. Kaback, H. R. (1970) in *Current Topics in Membranes and Transport* (Kleinzeller, A., and Bronner, F., eds) Vol. 1, p. 35, Academic Press, New York
20. Kay, W. W., and Kornberg, H. L. (1969) *Fed. Eur. Biochem. Soc. Lett.* **3,** 93
21. Kay, W. W. (1971) *J. Biol. Chem.* **246,** 7373–7382

[1] H. R. Kaback, unpublished observations.

[2] The mutant used in these studies was isolated from *S. typhimurium* LT-2 by Dr. J.-S. Hong.

[3] G. Prezioso, J.-S. Hong, and H. R. Kaback, unpublished observations.

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