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Published in:
Journal of Bacteriology

Publication date:
1996

JOURNAL OF BACTERIOLOGY, July 1996, p. 3991–3995 Vol. 178, No. 14
0021-9193/96/$04.00+0

Basic Amino Acid Transport in Plasma Membrane Vesicles of *Penicillium chrysogenum*

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Received 18 January 1996/Accepted 7 May 1996

The characteristics of the basic amino acid permease (system VI) of the filamentous fungus *Penicillium chrysogenum* were studied in plasma membranes fused with liposomes containing the beef heart mitochondrial cytochrome *c* oxidase. In the presence of reduced cytochrome *c*, the hybrid membranes accumulated the basic amino acids arginine and lysine. Inhibition studies with analogs revealed a narrow substrate specificity. Within the external pH range of 5.5 to 7.5, the transmembrane electrical potential ($\Delta\psi$) functions as the main driving force for uphill transport of arginine, although a low level of uptake was observed when only a transmembrane pH gradient was present. It is concluded that the basic amino acid permease is a H^+ symporter. Quantitative analysis of the steady-state levels of arginine uptake in relation to the proton motive force suggests a H^+-arginine symport stoichiometry of one to one. Efflux studies demonstrated that the basic amino acid permease functions in a reversible manner.

Amino acids are utilized by fungi as primary or secondary nitrogen sources or as building blocks for the synthesis of proteins and peptides. Systems involved in the translocation of amino acids across the plasma membrane have been studied in only a few filamentous fungi (5, 8, 25). Of these fungi, *Neurospora crassa*, *Aspergillus nidulans*, and, to a lesser extent, *Penicillium chrysogenum* are genetically and biochemically the most extensively characterized species. Two distinct classes of plasma membrane-located amino acid permeases are found in filamentous fungi: systems that catalyze the uptake of structurally related amino acids with a broad substrate specificity, such as the general amino acid permeases of plant and animal cells (2, 15, 18), and systems with a narrow substrate specificity, such as bacterial amino acid transporters (22).

Fungi show a peculiar substrate specificity in their amino acid transport systems, and multiple transport mechanisms seem to exist for several amino acids. In *N. crassa*, five distinct transport systems have been identified, with specificity for aromatic and aliphatic amino acids (system I); aromatic, aliphatic, and basic amino acids (system II); basic amino acids (system III); acidic amino acids (system IV); and L-methionine (system V) (5, 8, 20, 25). Studies with mycelium of *P. chrysogenum* indicate that this fungus possesses at least six distinct amino acid transport systems (1, 5, 8–10, 11, 24). Most of these systems are specific for one amino acid and analogs, except for system III, which is a general amino acid permease, and system IV, which transports acidic amino acids only (8, 10). Amino acid transporters of fungi are assumed to possess some typical properties: (i) they seem to function unidirectionally; i.e., only uphill transport is observed, while efflux or countertransport of the accumulated amino acids is not detected (8, 19); and (ii) their activity is regulated by transinhibition, i.e., a high internal concentration of an amino acid appears to lower the activity of the transport system and thereby inhibits further uptake (8, 10, 21). Transinhibition results in a decrease of the maximal transport rate (V_{max}) without affecting the affinity (K_d) of the system. Transinhibition is thought to occur through binding of the amino acid at a regulation site on the transport system that faces the cytosol. Binding would result in inactivation or a reduction of the transport activity. This phenomenon would allow a regulation of the intracellular amino acid pools and prevent deleterious cytosolic amino acid concentrations in the cell.

Amino acid transport systems from filamentous fungi have been studied mainly in mycelial suspensions. Factors such as growth phase, medium composition, and stage of development strongly influence the expression, regulation, and properties of these systems (1, 3, 8, 23–25). In *P. chrysogenum*, the transport systems for L-arginine and L-lysine (system VI) and for L-cysteine (system IX) are expressed constitutively. Transport systems specific for methionine (system I) and cystine (system II) are expressed under conditions of sulfur starvation, while carbon or nitrogen starvation results in the expression of the transport systems for neutral and basic amino acids (system III) and acidic amino acids (system IV) (1, 8).

Factors such as metabolism and compartmentalization interfere with the analysis of the plasma membrane transport processes in intact mycelium. Internal amino acid pools are sequestered in at least two compartments: the cytosol, with a high turnover rate, and the vacuole, with a low turnover rate (5). To study plasma membrane-located transport systems in *P. chrysogenum*, we have developed a model system devoid of metabolic activities by fusing membrane vesicles with liposomes containing the mitochondrial beef heart cytochrome *c* oxidase (4, 7). We have used this hybrid system to analyze the characteristics of the constitutive basic amino acid transport system in vitro (9, 20). Several properties of this transport system which were difficult to assess in mycelial suspension could be characterized well in this hybrid system. Our data demonstrate that the basic amino acid permease is a reversible proton symporter with a narrow substrate specificity.

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MATERIALS AND METHODS

Organism and culture conditions. The *P. chrysogenum* strains Wisconsin 54-1255 and Panlabs P2 (kindly supplied by Gist-Brocades NV) were grown on production medium (pH 6.3) supplemented with 10 mM glutamate and 10% (wt/vol) glucose as described by Lara et al. (14). Cultures were incubated for approximately 70 h in a rotary shaker at 200 rpm and 25°C. Strain Wisconsin 54-1255 was precultured for 24 h on production medium supplemented with 16% (wt/vol) glucose; phenylacetic acid and lactose were omitted. The P2 strain was precultured on YPG medium (1% [wt/vol] yeast extract, 2% [wt/vol] peptone, and 2% [wt/vol] glucose) for 72 h at pH 7.

Isolation and reconstitution of cytochrome *c* oxidase. Bovine heart mitochondria were obtained by the procedure described by King (13). Cytochrome *c* oxidase was isolated from these mitochondria as described by Yu et al. (26), suspended in 50 mM sodium phosphate (pH 7.5) containing 1.5% (wt/vol) cholic acid, and stored in liquid nitrogen. Cytochrome *c* oxidase was reconstituted in liposomes composed of 75% (by weight) acetone-ether-washed *Escherichia coli* lipid and 25% (by weight) egg yolk L-phosphatidylcholine at a ratio of 0.16 nmol of heme *a* per mg of lipid (4).

Plasma membrane isolation and fusion. *P. chrysogenum* plasma membranes were isolated by the procedure described by Hillenga et al. (7). Cytochrome *c* oxidase-containing liposomes (10 mg of lipid) and plasma membranes (1 mg of protein) were mixed, rapidly frozen in liquid N_2, and thawed slowly at 21°C. The freeze-thaw step was repeated once, and hybrid membranes were sized with a small-volume extrusion apparatus (Avestin Inc., Ottawa, Ontario, Canada) using polycarbonate filters (Avestin) with pore diameters of 400 and 200 nm. Fused membranes had a protein-to-lipid ratio of approximately 0.08 to 0.09 (wt/wt).

Determination of the electrical and pH gradients across the membrane. The transmembrane electrical potential ($\Delta\psi$, interior negative) was calculated from the distribution of the tetraphenylphosphonium ion (TPP^+), assuming concentration-dependent binding to the membranes as described previously (16). The transmembrane distribution of TPP^+ was deduced from measurements with a TPP^+-selective electrode. Hybrid membrane vesicles were added to a buffer at the indicated pH containing 50 mM potassium phosphate, 5 mM $MgSO_4$, and 2 μM TPP^+. A proton motive force (Δp) was generated by the addition of ascorbate (10 mM, adjusted to the desired pH), *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (200 μM), and horse heart cytochrome *c* (20 μM). When added, the ionophores nigericin and valinomycin were used at concentrations of 10 and 100 nM, respectively. The pH gradient across the membrane (ΔpH, interior alkaline) was determined from the fluorescence of pyranine (excitation, 450 nm; emission, 508 nm) on a Perkin Elmer LS50B luminescence spectrometer. Pyranine (100 μM) was entrapped in hybrid membrane vesicles by freeze-thaw extrusion. External pyranine was removed by chromatography of the membrane vesicle suspension over a Sephadex G-25 column (coarse, 1 by 20 cm). Where indicated, valinomycin was added to a final concentration of 50 nM. Energization of hybrid membrane vesicles was carried out as described for $\Delta\psi$ measurements. Where indicated, nigericin was added to a final concentration of 1 μM.

Transport studies. Uptake of arginine and lysine was studied at 25°C and pH 6.5, unless stated otherwise. Mycelium was suspended in 50 mM potassium phosphate at a final density of 10 mg/ml (P2) or 6 mg/ml (Wisconsin 54-1255 [dry wt]) and stored on ice until further use. L-[U-^{14}C]arginine (Amersham; 38 Ci/mol) or L-[U-^{14}C]lysine (Amersham; 43 Ci/mol), previously diluted 10-fold with nonlabeled substrate, was added to the mycelial suspension to 30 μM unless indicated otherwise. At given time intervals, 0.5-ml samples were taken, added to 2 ml of ice-cold 0.1 M LiCl, and filtered immediately on paper filters (296 PE, type 0860; Schleicher & Schuell). Filters were washed once with 2 ml of ice-cold 0.1 M LiCl, and the amount of radioactivity was determined with a liquid scintillation counter (Packard Tri-Carb 460 CD; Packard Instruments). Cells were deenergized by preincubation with the protonophore carbonyl cyanide *m*-chlorophenylhydrazone [$CF_3OPh_2C(CN)_2$; 10 μM] for 5 min at 25°C.

For uptake studies with hybrid membranes, vesicles were suspended to a final concentration of approximately 1.2 mg of protein per ml in 50 mM potassium phosphate (pH 6.5, unless indicated otherwise) containing 5 mM $MgSO_4$. After a 1-min incubation in the presence of the electron donor system ascorbate (30 mM)–*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (150 μM)–horse heart cytochrome *c* (7.5 μM), L-U-^{14}C-labeled amino acids were added to 30 μM unless indicated otherwise. Samples (20 μl each) were taken at given time intervals and processed as described above. Samples were filtered on 0.45-μm-pore-size cellulose-nitrate filters (Schleicher & Schuell).

For efflux studies, hybrid membrane vesicles were washed twice with a 20-fold volume of 50 mM potassium phosphate, pH 6.5. Concentrated suspensions of 25 to 30 mg of protein per ml in 50 mM potassium phosphate (pH 6.5) containing 5 mM $MgSO_4$, 10 μM $CF_3OPh_2C(CN)_2$, and arginine at the indicated concentration were supplemented with a tracer of L-[U-^{3}H]arginine (Amersham; 4 Ci/mmol) and incubated for 3 h at 25°C. Samples (8 μl each) were rapidly diluted into 400 μl of 50 mM potassium phosphate (pH 6.5) containing 5 mM $MgSO_4$ and 10 μM $CF_3OPh_2C(CN)_2$, filtered on 0.45-μm-pore-size cellulose-nitrate filters (Schleicher & Schuell), washed once with 2 ml of ice-cold 0.1 M LiCl, and processed as described above. Kinetic data were analyzed with the GraFit program (Erithacus Software Ltd.).

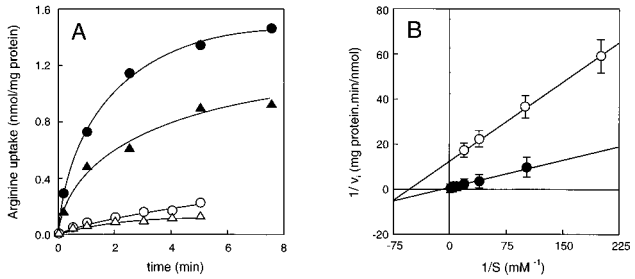

FIG. 1. Kinetics of L-arginine and L-lysine uptake in mycelium and hybrid membranes. (A) Uptake of L-arginine (○ and ●) and L-lysine (▽ and ▼) in mycelium (open symbols) and hybrid membranes (closed symbols) from the Wisconsin 54-1255 strain; (B) kinetics of arginine uptake in mycelium (○) and hybrid membranes (●) from the Wisconsin 54-1255 strain. The error bars indicate the standard error of the mean of three independent experiments.

Other methods. Protein concentrations were determined in the presence of 0.5% (wt/vol) sodium dodecyl sulfate in a modified Lowry et al. assay (17). Bovine serum albumin was used as a standard.

RESULTS

Kinetics of arginine and lysine transport. The constitutive basic amino acid permease was studied in plasma membranes obtained from the *P. chrysogenum* strains Wisconsin 54-1255 and P2. Plasma membranes were fused with cytochrome *c* oxidase containing liposomes by a freeze-thaw extrusion technique. To establish whether membrane isolation or fusion affected the basic amino acid permease, the kinetics of arginine and lysine uptake were determined in both hybrid membranes and mycelial suspensions. Compared with that in mycelium, the specific activity of arginine and lysine uptake was clearly increased in hybrid membranes, i.e., a V_{max} of 0.63 and 4 nmol/min/mg of protein, respectively (Fig. 1). Arginine was accumulated to a greater extent than lysine by the hybrid membranes. An increase in the apparent K_m value of arginine uptake was noted in the hybrid membranes as compared with intact mycelium, i.e., 120 and 25 μM, respectively. Similar results were obtained with mycelia and hybrid membranes of strain P2, yielding V_{max} values of 0.45 and 2.2 nmol/min/mg of protein, respectively, and K_m values of 30 and 170 μM, respectively (data not shown). These studies show that the basic amino acid transport system is active in the hybrid membranes. The proton motive force generated by the beef heart cytochrome *c* oxidase functions as a driving force for the accumulation of arginine and lysine.

Specificity of the arginine/lysine permease. The specificity of the basic amino acid permease was analyzed in competition experiments. Addition of a large excess of lysine completely inhibited arginine uptake and vice versa in hybrid plasma membranes from the Wis 54-1255 strain. On the basis of the observed initial velocity of arginine uptake at three L-[U-^{14}C]arginine concentrations (30, 100, and 300 μM) and various concentrations of unlabeled lysine (0 to 3 mM; Fig. 2), a K_i of 190 μM was calculated for the inhibition of arginine transport by lysine. The observation that the K_i for lysine is in the same range as the K_m for arginine uptake suggests that these amino acids are equivalent substrates for the basic amino acid permease.

The specificity of the basic amino acid permease was further assessed from the extent of inhibition of arginine uptake by addition of a 33-fold excess of analogs and structurally nonrelated amino acids (Table 1). The initial rate of Δp-driven arginine uptake was strongly inhibited by lysine, canavanine,

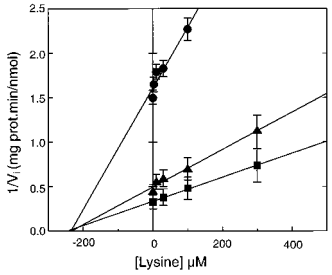

FIG. 2. Competitive inhibition of L-arginine uptake by L-lysine in hybrid membranes. Inhibition of L-arginine uptake by L-lysine was determined in hybrid membranes of Wisconsin 54-1255 at L-[U-^{14}C]arginine concentrations of 30 (●), 100 (▲), and 300 (■) μM. The error bars indicate the standard error of the mean of three independent experiments.

ornithine, and homoarginine. Of the other amino acids only 2,4-diaminobutyric acid and glutamine inhibited the uptake of arginine slightly, while typical substrates for the general amino acid permease, such as leucine and valine, were without effect. The observed inhibition pattern indicates that the specificity of this permease is determined mainly by the presence and structural configuration of the α-amine group. These observations confirm studies observed in mycelial suspensions and are consistent with the narrow substrate specificity described for system VI, the arginine/lysine permease (9, 20).

Effect of ionophores on arginine uptake. The energetic mechanism of arginine uptake in hybrid membrane vesicles from the P2 strain was studied in detail at pH 6.5 by addition of the ionophores nigericin and valinomycin (Fig. 3). The ionophore nigericin collapses the ΔpH by mediating electroneutral K^+-H^+ exchange. Upon a collapse of the ΔpH by nigericin, a compensatory increase in the Δψ occurs. Addition of nigericin enhanced the uptake of arginine (Fig. 3A). The ionophore valinomycin mediates electrogenic diffusion of K^+, thereby collapsing the Δψ. Omitting the Δψ results in a slight increase in the ΔpH. Previous incubation with valinomycin greatly, but not completely, reduced arginine uptake (Fig. 3B). Addition of valinomycin after 5 min of arginine accumulation resulted in a partial efflux of accumulated arginine. No uptake of arginine was observed after collapsing of the Δp with nigericin and valinomycin. Addition of these ionophores after 5 min of arginine accumulation caused a fast and almost complete efflux of the accumulated arginine (Fig. 3C). These results indicate that the Δψ is the major driving force for arginine uptake. The observation that arginine is still accumulated when only a ΔpH is present suggests that the basic amino acid permease is a proton symporter. Similar results were obtained in hybrid membranes of the Wisconsin 54-1255 strain.

TABLE 1. Inhibition of L-arginine transport in hybrid Wisconsin 54-1255 membranes by different L-amino acids

Inhibitor	% Inhibition of L-arginine uptake[a]
Arginine	100
Lysine	72
Canavanine	89
Ornithine	87
Homoarginine	82
Citrulline	17
Histidine	24
2,4-Diaminobutyric acid	55
2-Amino-3-guanidinopropionic acid	29
Glutamate	3
Glutamine	42
Cysteine	8
Serine	12
Valine	6
Leucine	14

[a] Inhibitors were added at a 33-fold-higher concentration than [^{14}C]arginine, which was used at a concentration of 30 μM. Inhibition by arginine was set at 100%.

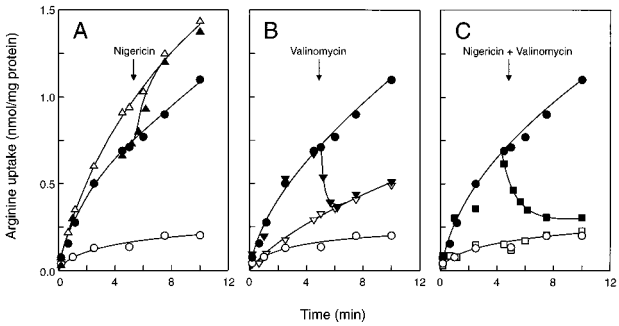

FIG. 3. Effect of ionophores on L-arginine uptake. Uptake of L-arginine in P2 hybrid membranes under energized conditions (●) and after incubation with $CF_3OPh_2C(CN)_2$ (○), nigericin (△) (A), valinomycin (▽) (B), or nigericin plus valinomycin (□) (C). Congruent closed symbols depict the effect of ionophores added after 5 min of arginine accumulation.

pH dependency of the arginine/H^+ symport stoichiometry. The role of the external pH in arginine uptake was studied in hybrid membranes from the Wisconsin 54-1255 strain (Fig. 4). Steady-state levels of arginine uptake were similar at pH 5.5 and pH 6.5, while at pH 7.5 the level of arginine accumulation decreased about twofold. At all pH values studied, addition of nigericin enhanced arginine uptake while valinomycin strongly reduced steady-state levels of arginine uptake.

To determine the H^+/arginine stoichiometry, the magnitude and composition of the generated Δp were determined at different pH values (Fig. 5A). The Δψ and ΔpH were determined in the absence of ionophores. Δψ was highest between pH 6.0 and pH 7.0, with maximum values of about −100 mV. ΔpH was maximal at pH 5.5 and slowly decreased at pH values above 6.0. The Δp, calculated from the Δψ and ΔpH values, was optimum at around pH 6.0 (about −150 mV). Within the pH range studied, the internal pH increased with the external pH (Fig. 5A) but remained within a range of 6.0 to 7.2. Steady-state levels of arginine accumulation were determined under identical conditions as Δψ and ΔpH values (Fig. 5B). From the

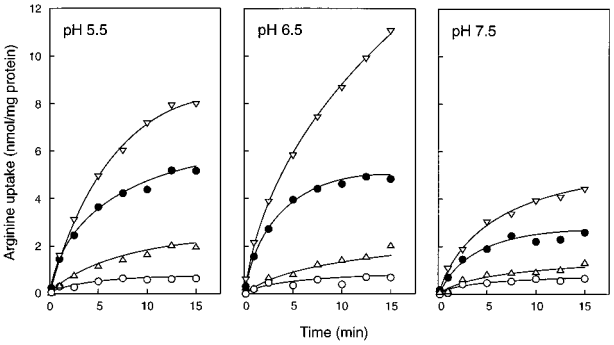

FIG. 4. Effect of the external pH on L-arginine uptake. L-Arginine uptake in hybrid membranes of Wisconsin 54-1255 at pH 5.5 (A), 6.5 (B), and 7.5 (C). Uptake of arginine was determined under energized conditions (●) and after incubation with $CF_3OPh_2C(CN)_2$ (○), valinomycin (△), and nigericin (▽).

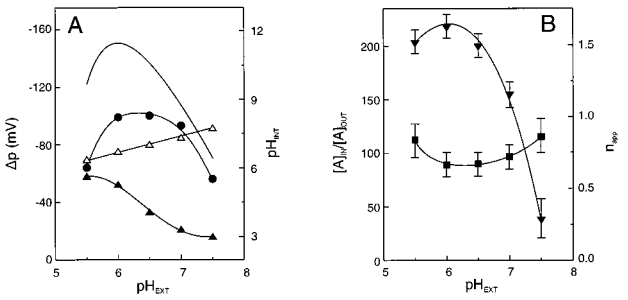

FIG. 5. Relationship between the external pH and the H^+/arginine symport stoichiometry. (A) Effect of the external pH on the internal pH (△) and magnitude and composition of the generated Δp in hybrid membranes of Wisconsin 54-1255. ●, Δψ; ▲, $-Z\Delta pH$; —, calculated total Δp. (B) Effect of the external pH on the steady-state levels of L-arginine accumulation (▼) and the H^+/arginine symport stoichiometry (■). The error bars indicate the standard error of the mean of three independent experiments.

data depicted in Fig. 5A and the steady-state levels of arginine accumulation, the apparent H^+/arginine stoichiometry (n_{app}) was calculated. The n_{app} varied between 0.7 and 0.9 within the examined pH range when it is assumed that arginine is accumulated as a positively charged amino acid (Fig. 5B). This slight variance indicates that there is no strong dependency of the n_{app} on the external or internal pH. Arginine uptake in hybrid membranes relates mainly to the magnitude and composition of the Δp. These data show that the basic amino acid permease takes up amino acids in symport with 1 proton and effectively translocates two positive charges. This is consistent with the observation that the Δψ is the main driving force in amino acid accumulation via the basic amino acid permease.

Arginine efflux and exchange. Counterflow experiments were conducted to assess whether the basic amino acid permease mediates homologous exchange. Hybrid membranes were equilibrated with 0.5 to 10 mM L-arginine and diluted 50-fold into a buffer containing [^{14}C]arginine (33 μM). Under these conditions, no significant uptake of arginine was detected (data not shown). In another experiment, hybrid membrane vesicles were first allowed to accumulate ^{14}C-labeled arginine. Subsequent addition of the protonophore $CF_3OPh_2C(CN)_2$ caused a fast and almost complete efflux of arginine (Fig. 6A), while the addition of an excess of unlabeled arginine resulted in a slow and only partial release of the internal ^{14}C-labeled arginine. Extensive studies showed that the efflux induced by the addition of $CF_3OPh_2C(CN)_2$ was not enhanced when an excess of unlabeled arginine was added simultaneously (data not shown).

Efflux of arginine occurred when the Δp or ΔpH was dissipated by ionophores or protonophores (Fig. 1 and 6A). To further establish that the basic amino acid permease mediates efflux, this process was studied in more detail. In these experiments the protonophore $CF_3OPh_2C(CN)_2$ was added to prevent the buildup of a Δp during efflux. Hybrid membranes were equilibrated with 0.05 to 10 mM arginine supplemented with a fixed amount of tracer [^{3}H]arginine and diluted 50-fold to initiate efflux. The rate at which the radiolabel was released decreased with increasing internal arginine concentrations (Fig. 6B), and when converted to true initial rates the efflux followed Michaelis-Menten kinetics (Fig. 6C). The K_m of the efflux process was shown to be 2 to 6 mM, and the maximum rate of efflux was determined to be 2 to 4 nmol/min/mg of protein. Thus, the K_m values of arginine efflux and Δp-driven arginine uptake differ substantially.

DISCUSSION

The filamentous fungus *P. chrysogenum* is used for the commercial production of penicillins. To study transport processes that play a role in penicillin biosynthesis, a procedure was developed for the isolation of plasma membranes from *P. chrysogenum* (7). Hybrid membrane vesicles were obtained by fusing plasma membranes with cytochrome *c* oxidase vesicles. These vesicles are endowed with a low ion permeability and can be energized easily by the external addition of reduced cytochrome *c*. This system was used to study in detail the kinetic and energetic properties of the constitutive arginine/lysine permease (system VI).

The activity described in this paper cannot be due to the general amino acid permease (GAP), system III. *P. chrysogenum* was grown in the presence of excess lactose and ammonium, the main carbon and nitrogen sources, respectively. These compounds were not exhausted at the time the cells were harvested, while cells were not carbon deprived as substantial amounts of intracellular glycogen and trehalose were present (6). These are conditions under which GAP is not expressed (9). Both cells and hybrid membranes hardly showed any uptake of leucine and methionine, both typical substrates for the GAP (6). Moreover, the narrow substrate specificity of the system described in this paper (Table 1) agrees with the specificity described for the arginine/lysine permease (system VI) (9, 20).

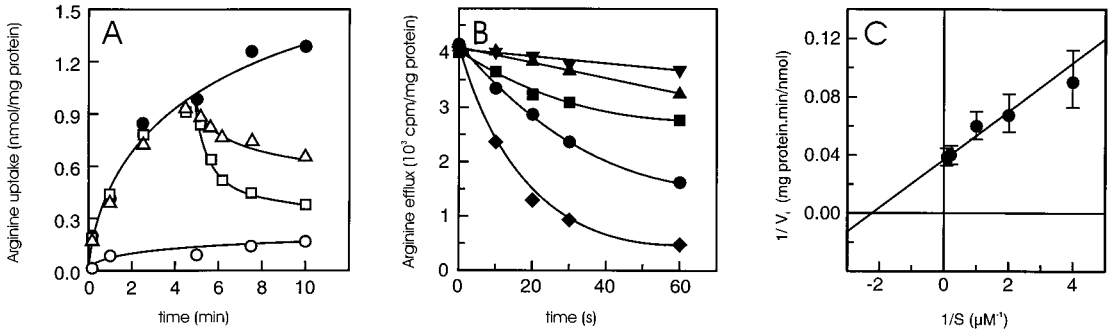

FIG. 6. L-Arginine efflux in hybrid membranes. (A) Uptake of L-arginine in hybrid membranes of Wisconsin 54-1255 under energized conditions (●) or after incubation with $CF_3OPh_2C(CN)_2$ (○). L-[U-^{14}C]arginine efflux or exchange was induced after 5 min of arginine accumulation by the addition of $CF_3OPh_2C(CN)_2$ (□) or a 100-fold excess (3 mM) of nonlabeled L-arginine (△). (B) Efflux of L-arginine in the presence of $CF_3OPh_2C(CN)_2$. Arginine was used at internal concentrations of 0.25 (◆), 0.5 (●), 1 (■), 5 (▲), and 10 (▼) mM mixed with a fixed amount of the tracer [^{14}C]arginine. The data are plotted as the amounts of [^{14}C]arginine (in counts per minute) present in the vesicle lumen as a function of time after 50-fold dilution of the membranes. (C) Kinetics of L-arginine efflux: K_m, 2 to 6 mM; V_{max}, 1 to 4 nmol of arginine per mg of protein per min. The error bars indicate the standard error of the mean of two independent experiments.

In analogy to *Saccharomyces cerevisiae*, a symport stoichiometry of 1 H^+ per amino acid was determined for the basic amino acid permease (5, 19). Uptake was stimulated or inhibited by Na^+ ions (up to 50 mM) (6), excluding the possibility that Na^+ is a cotransported ion. Within the pH range studied, the n_{app} varied only slightly, indicating that the stoichiometry is not dramatically affected by either the internal or external pH.

The K_m for arginine and lysine uptake was significantly higher in hybrid membranes than in mycelium. This alteration might have resulted from the applied isolation or fusion procedures. Plasma membranes form a structurally integrated part of the cell, and isolation inevitably causes the disruption of this organelle and the attached cytoskeleton. It is commonly assumed that these events do not affect properties of transport systems unless important constituents (e.g., binding proteins) or cofactors are lost during the isolation. Some earlier reports implicated amino acid-binding proteins in the uptake of amino acids by filamentous fungi (5). However, no evidence to support these assumptions (e.g., genes) has been presented since, whereas these studies clearly demonstrate that, insofar as such binding proteins are involved, they cannot be soluble constituents. Several studies have shown that alterations in membrane composition strongly affected transport systems. Changes in lipid head group, acyl chain carbon number, and sterol concentration had a pronounced effect on the velocity of L-leucine uptake in *Lactococcus lactis* (12). However, in those studies no alteration of the K_m was noted. Since the kinetic parameters in intact mycelium are less well defined than those in a vesicle system, the discrepancy may arise from the fact that mycelium, in particular that of the P2 strain, is inhomogeneous, while the presence of an anionic cell wall, with a variable thickness, may interfere with the bulk diffusion of cationic solutes such as arginine.

Efflux studies of the basic amino acids in membrane vesicles clearly demonstrated that this symport system functions in a reversible manner. An apparent asymmetry is seen in the half-saturation parameter K_m and not in the V_{max} terms. The K_m value for arginine efflux is almost 50-fold higher than the K_m for Δp-driven arginine uptake. This, however, does not imply that the permease is functionally asymmetric but rather suggests that the K_m is influenced by the Δp. The observed linear correlation between the parameters $1/V$ and $1/[S]$ for arginine efflux indicates that in the concentration range studied "transinhibition" by arginine does not occur. Internal concentrations up to 10 mM did not inhibit the efflux process, but at these high internal concentrations no counterflow was observed in mycelium. Since this "transinhibition" phenomenon appears to be specific for intact mycelium, it could be related to metabolism or compartmentalization in the cell rather than a specific kinetic effect on the permease. Alternatively, the "transinhibition" effect could arise from a regulatory phenomenon which is not operational in isolated plasma membranes.

In conclusion, these studies demonstrated that hybrid membrane vesicles are a powerful tool in investigating characteristics of transport systems from filamentous fungi. Important advantages of hybrid membrane vesicles over mycelial suspension are as follows. (i) Transport systems can be investigated without the interference of compartmentalization or metabolic activities. (ii) The magnitude of driving forces can be determined accurately. (iii) Mechanical properties of transport systems can be assessed more adequately. (iv) The specificity of a system can be resolved with more certainty.

ACKNOWLEDGMENTS

We thank Gist-Brocades NV for helpful recommendations and technical support.

This research was supported by the Technology Foundation (Stichting Technische Wetenschappen) with financial aid from the Netherlands Organization for Pure Scientific Research (N.W.O.) and by a grant from the Royal Gist-Brocades NV, Delft, The Netherlands.

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