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Transport of Diamines by *Enterococcus faecalis* Is Mediated by an Agmatine-Putrescine Antiporter

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Enterococcus faecalis ATCC 11700 is able to use arginine and the diamine agmatine as a sole energy source. Via the highly homologous deiminase pathways, arginine and agmatine are converted into CO₂, NH₃, and the end products ornithine and putrescine, respectively. In the arginine deiminase pathway, uptake of arginine and excretion of ornithine are mediated by an arginine-ornithine antiport system. The translocation of agmatine was studied in whole cells grown in the presence of arginine, agmatine, or glucose. Rapid uncoupler-insensitive uptake of agmatine was observed only in agmatine-grown cells. A high intracellular putrescine pool was maintained by these cells, and this pool was rapidly released by external putrescine or agmatine but not by arginine or ornithine. Kinetic analysis revealed competitive inhibition for uptake between putrescine and agmatine. Agmatine uptake by membrane vesicles was observed only when the membrane vesicles were preloaded with putrescine. Uptake of agmatine was driven by the outwardly directed putrescine concentration gradient, which is continuously sustained by the metabolic process. Uptake of agmatine and extrusion of putrescine by agmatine-grown cells of *E. faecalis* appeared to be catalyzed by an agmatine-putrescine antiporter. This transport system functionally resembled the previously described arginine-ornithine antiport, which was exclusively induced when the cells were grown in the presence of arginine.

In addition to the fermentation of carbohydrates, *Enterococcus faecalis* (previously referred to as *Streptococcus faecalis* [17]) is able to use arginine and the decarboxylated derivative thereof, agmatine, as an energy source for growth (2, 3, 16, 18, 19, 23). Arginine and agmatine are metabolized via the arginine deiminase (ADI) and agmatine deiminase (AgDI) pathway, respectively. Both metabolic routes are very similar and include the sequential action of three enzymes (2). Arginine and agmatine are deiminated by ADI (EC 3.5.3.6) and AgDI (EC 3.5.3.12), respectively. These reactions yield NH₃ and the ureido compound citrulline or carbamoylputrescine. Phosphorolysis of citrulline by a catabolic ornithine carbamoyltransferase (EC 2.1.3.3) results in the formation of carbamoylphosphate and ornithine. In the AgDI pathway, a catabolic putrescine carbamoyltransferase (EC 2.1.3.6) catalyzes the phosphorolysis of carbamoylputrescine into carbamoylphosphate and putrescine (1,4-diaminobutane). The energy-rich phosphate bond of carbamoylphosphate is transferred to ADP, yielding ATP, CO₂, and NH₃, a reaction catalyzed by a carbamate kinase (EC 2.7.2.2). Two carbamate kinases are present in *E. faecalis*, one in the ADI pathway and the other found only in cells grown on agmatine (18). The enzymes of the ADI pathway are coinduced by arginine and repressed by glucose, fumarate, or aerobic growth conditions (19). On the other hand, the enzymes of the AgDI pathway are coinduced by growth on agmatine and repressed by glucose and arginine (18).

Only the guanidine groups of arginine and agmatine are used for carbamoylphosphate synthesis, and ornithine and putrescine are excreted into the growth medium (18, 19). The energy gain of these metabolic routes is low, i.e., 1 mol of ATP produced per mol of arginine or agmatine. Therefore, the energy requirement for uptake of these compounds is also expected to be low. Recently, the mechanism of arginine uptake and ornithine excretion has been elucidated by studies with membrane vesicles derived from the homofer-

mentative lactic acid bacterium *Lactococcus lactis* subsp. *lactis* ML₃ (previously referred to as *Streptococcus lactis* [17]) (4). This organism also metabolizes arginine via the ADI pathway (1, 2). It was found that the uptake of arginine and the excretion of ornithine excretion are catalyzed by the arginine-ornithine antiporter (4, 15, 22). The driving force for arginine uptake in growing cells is supplied by the inwardly directed arginine concentration gradient and the outwardly directed ornithine concentration gradient, which are both maintained during arginine metabolism. Uptake of arginine thus does not require any additional metabolic energy source. This arginine-ornithine antiporter was also found in *E. faecalis* DS5 (15). The similarity between arginine and agmatine metabolism suggests that a similar mechanism would be operational for the uptake and excretion of agmatine and putrescine.

In this paper we demonstrate that in analogy to the arginine-ornithine antiporter, uptake of agmatine and excretion of putrescine in *E. faecalis* ATCC 11700 are coupled via a single transport system which catalyzes heterologous exchange between these diamines. Some characteristics of the agmatine-putrescine antiporter are described and compared with those of the arginine-ornithine antiporter.

MATERIALS AND METHODS

Organisms and growth conditions. *E. faecalis* ATCC 11700 was kindly provided by V. Stalon (Université Libre de Bruxelles, Brussels, Belgium). The old name of this organism is *Streptococcus faecalis* (17). The organism was grown anaerobically at 37°C on an exhausted complex medium as described by Simon et al. (19). Arginine, agmatine, and glucose were added at a final concentration of 10 mM each. Cells were grown overnight, harvested by centrifugation, and washed twice in 50 mM potassium phosphate (pH 7.0). Cells were suspended in 50 mM potassium-phosphate (pH 7.0) to a final protein concentration of 20 to 40 mg/ml and either used immediately or stored in liquid nitrogen in the presence of 30% glycerol as a cryoprotectant. Membrane

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vesicles of *E. faecalis* were prepared by French press treatment as described by Houg et al. (6).

Arginine and agmatine deiminase pathway activity. Cells were washed twice in 0.5 mM potassium phosphate (pH 7.0) supplemented with 50 mM KCl, 50 mM NaCl, and 5 mM MgSO₄ and finally suspended in this buffer to a final protein concentration of 0.05 to 0.08 mg/ml. Arginine or agmatine was added to a concentration of 0.5 mM, and the alkalization of the external medium was followed with a pH electrode. Changes in pH were converted into nanomoles of ammonia by calibration of the cell suspension with 1- μ l portions of 100 mM ammonia. The initial rates of ammonia production were estimated from the pH changes under conditions in which the increase in pH was less than 0.02 pH units and linear over time. Measurements were performed at 20°C.

Determination of intracellular amino acid concentrations. Washed cells were diluted in 2.5 ml of potassium phosphate 50 mM (pH 7.0) supplemented with 50 mM NaCl and 5 mM MgSO₄ to a final protein concentration of 0.80 to 0.85 mg/ml. Samples (1 ml) were taken prior to or after the addition of 1 mM arginine or agmatine and transferred to microfuge tubes containing 0.8 ml of silicon oil (density, 1.03 mg/ml) on top of 0.2 ml of 7% (wt/vol) perchloric acid (PCA) and 4.5 mM EDTA. The mixture was centrifuged for 6 min at 12,000 \times g. After removal of the buffer and 3/4 of the oil layer, 0.15 ml of the PCA fraction was taken and mixed with 0.225 ml of 1 N KOH-KHCO₃ to give a final pH of 9.5. Amino acids were analyzed after separation of the dansylated derivatives on a reverse-phase column (μ Bondapak C18, 3.9 by 30 mm; Waters Associates, Inc., Milford, Mass.) as described previously (14, 15).

Transport assays. Cells were suspended in 50 mM potassium phosphate (pH 7.0) supplemented with 2 mM MgSO₄ to a final protein concentration of 2 to 4 mg/ml. Cells were loaded with putrescine or ornithine by incubation for 1 h at 20°C in this medium containing 500 μ M putrescine or ornithine and subsequently concentrated by centrifugation, yielding a final protein concentration of 40 to 50 mg/ml. For uptake measurements, 2 to 4 μ l of the cell suspension was diluted into 200 μ l of 50 mM potassium phosphate (pH 7.0) supplemented with 2 mM MgSO₄ and 50 μ M [¹⁴C]agmatine, 50 μ M [¹⁴C]arginine, or 25 μ M [¹⁴C]putrescine as indicated. Efflux of putrescine or ornithine was measured as follows. A concentrated cell suspension (40 to 50 mg of protein per ml) was incubated for 20 min with 500 μ M [¹⁴C]putrescine or [³H]ornithine at 20°C, and samples (2 μ l) were subsequently diluted into 200 μ l of 50 mM potassium phosphate (pH 7.0) supplemented with 2 mM MgSO₄ and 500 μ M agmatine, putrescine, arginine, ornithine, or none of these amino acids or diamines. Transport measurements were performed at 25°C as described previously (4, 15).

Other analytical procedures. [U-¹⁴C]Agmatine was enzymatically synthesized from L-[U-¹⁴C]arginine by a procedure originally developed for an arginine decarboxylase activity assay (13). L-[U-¹⁴C]Arginine (180 nmol, 2 MBq) was dissolved in 200 mM sodium acetate, pH 5.2. To this mixture, pyridoxal-5-phosphate was added to a final concentration of 60 μ M, and the reaction was started by the addition of L-arginine decarboxylase (0.25 U) in a final volume of 500 μ l. The suspension was incubated for 30 min at 37°C. Decarboxylation of L-[U-¹⁴C]arginine caused a loss of radioactivity by approximately 30% as a result of liberation of ¹⁴CO₂. The complete interconversion of arginine into agmatine was verified by (i) analysis of the reaction products after dansylation by reverse-phase column chromatography and (ii) the

TABLE 1. Effect of growth medium composition on ADI and AgDI pathway activity in *E. faecalis* ATCC 11700

Energy source (10 mM)	Pathway activity (nmol of NH ₃ /mg of protein per min)			
	Arginine		Agmatine	
	No addition	Ionophores added ^a	No addition	Ionophores added
Arginine	67.9	89.9	<1	<1
Agmatine	<1	<1	74.9	108.8
Glucose	<1	<1	<1	<1

^a Activity was assayed in the presence of 1 μ M valinomycin and 0.1 μ M nigericin.

inability of ornithine-loaded membrane vesicles of *L. lactis* subsp. *lactis* ML₃ to accumulate this compound (4). Protein was measured by the method of Lowry et al. (11) with bovine serum albumin as a standard.

Materials. Agmatine and L-arginine decarboxylase (L-arginine carboxyl-lyase; EC 4.1.1.19) from *Escherichia coli* were purchased from Sigma Chemical Co. (St. Louis, Mo.). [1,4-¹⁴C]Putrescine dihydrochloride (4.37 GBq/mmol) and L-[U-¹⁴C]arginine hydrochloride (11 GBq/mmol) were obtained from Amersham Corp. (Buckinghamshire, England). L-[2,3-³H]Ornithine (1.1 TBq/mmol) was obtained from New England Nuclear Corp. (Boston, Mass.). All other chemicals were reagent grade and acquired from commercial sources.

RESULTS

Arginine and agmatine deiminase pathway activities. The ADI pathway activity can conveniently be determined by following the liberation of ammonia from arginine in a weakly buffered medium with a pH electrode (15). Washed cells of *E. faecalis* ATCC 11700 grown on an exhausted complex broth medium supplemented with arginine as the sole energy source displayed a high ADI pathway activity (Table 1), while cells grown on agmatine as the sole energy source showed virtually no ADI pathway activity. In contrast to the arginine-grown cells, rapid alkalization of the medium was observed when agmatine-grown cells were supplied with agmatine, indicating that the AgDI pathway is inducible (Table 1). Both the ADI and AgDI pathway activities were absent when cells were grown in the presence of glucose. On the other hand, cells grown on equimolar amounts of both arginine and agmatine displayed both activities (data not shown). These results demonstrate that the presence of arginine or agmatine in the growth medium results in selective induction of the ADI or AgDI pathway activity, respectively. Arginine and agmatine probably have to enter the cells to induce them. To investigate whether a proton motive force (PMF) plays a role in these uptake processes and thus is required for induction of the ADI and AgDI pathway, the activities of both pathways were recorded under conditions of high PMF and under conditions in which PMF was absent (Table 1). Dissipation of the PMF by the addition of the ionophores valinomycin and nigericin stimulated ADI and AgDI pathway activities by 33 and 45%, respectively, indicating that a PMF is not required for these activities. Similar phenomena have been observed for arginine metabolism by arginine-grown cells of *L. lactis* ML₃ (15).

Basic amino acids and diamine content of cells. The ornithine, citrulline, putrescine, and carbamoylputrescine contents were determined in washed cells of *E. faecalis* grown with arginine or agmatine as the sole energy source. Small

TABLE 2. Effect of exogenously added arginine or agmatine on the ornithine, citrulline, putrescine, and carbamoylputrescine contents of *E. faecalis* ATCC 11700 grown in the presence of arginine or agmatine^a

Addition	Concn ($\mu\text{mol}/\text{mg}$ of protein) in:			
	Arginine-grown cells		Agmatine-grown cells	
	Ornithine	Citrulline	Putrescine	Carbamoylputrescine ^b
None	0.296	0.019	0.122	0.027
Arginine	0.125	0.274	0.133	— ^c
Agmatine	0.376	0.015	0.068	0.038

^a Arginine- and agmatine-grown cells were suspended in 50 mM potassium phosphate (pH 7.0) supplemented with 50 mM NaCl and 5 mM MgSO_4 to a final protein concentration of 0.11 mg/ml. Arginine or agmatine was added to a final concentration of 1 mM, and the suspension was incubated for 3 min at room temperature. Cells were separated from the medium by silicon oil centrifugation, and amino acids and amines were determined by chromatography after dansylation as described in Materials and Methods.

^b Calculated by using an estimated dansylation efficiency of carbamoylputrescine.

^c —, Not determined.

amounts of citrulline were found in arginine-grown cells and of carbamoylputrescine in agmatine-grown cells (Table 2). On the other hand, the content of ornithine in arginine- and of putrescine in agmatine-grown cells was high. The putrescine content most likely does not represent the free putrescine concentration in the cell, since this basic compound easily forms complexes with intracellular components (20, 21).

Under steady-state conditions of arginine metabolism by arginine-grown cells, the highest citrulline and lowest ornithine content were found. These changes in the intracellular pools were not observed when the cells were supplied with agmatine instead of arginine (Table 2). Similar experiments conducted with agmatine-grown cells indicated that the presence of agmatine resulted in a decrease in the amount of extractable putrescine and an increase in the carbamoylputrescine content (Table 2). When the cells were supplied with arginine, the putrescine pool remained unaltered. These results demonstrate that cells of *E. faecalis* grown on arginine or agmatine maintain high intracellular pools of the end products of the deiminase pathways. The cells, however, seem to be primed for uptake of arginine and agmatine, and uptake is accompanied by a rapid decrease in the intracellular concentration of ornithine and putrescine.

Arginine transport in *E. faecalis* DS5 was found to be catalyzed by an arginine-ornithine antiporter (15). The results presented above suggest that a similar system is present in *E. faecalis* ATCC 11700 and are indicative of an agmatine-putrescine exchange system as the molecular mechanism of agmatine transport.

Uptake of agmatine and arginine. The uptake of arginine and agmatine was studied more directly by the use of radiolabeled compounds. Since [¹⁴C]agmatine is not commercially available, radiolabeled agmatine was enzymatically synthesized from [¹⁴C]arginine by using an arginine decarboxylase from *E. coli* (for experimental details, see Materials and Methods). Arginine-ornithine antiport activity was tested in cells grown in the presence of arginine, agmatine, or glucose as the energy source. Cells were loaded with ornithine and subsequently diluted into a buffer containing [¹⁴C]arginine. Rapid uptake of arginine was only observed with cells grown on arginine (Fig. 1A). The initial phase (up to 2 min) of arginine uptake was not affected by the

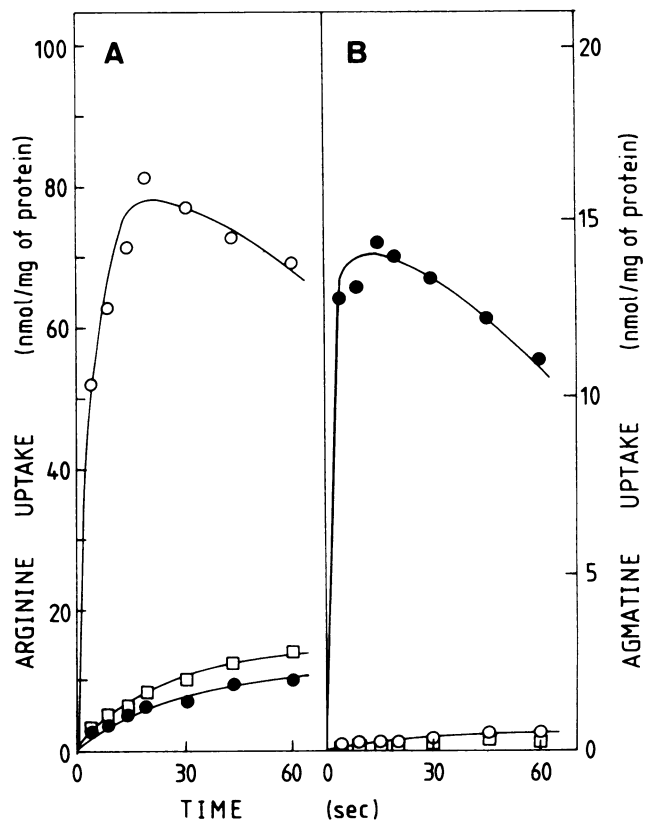


FIG. 1. Uptake of arginine (A) and agmatine (B) by cells of *E. faecalis* ATCC 11700 grown on glucose (\square), arginine (\circ), or agmatine (\bullet) as a sole energy source. Cells were loaded with 500 μM ornithine (A) or putrescine (B) and diluted into a buffer containing [¹⁴C]arginine or [¹⁴C]agmatine, respectively.

ionophores nigericin plus valinomycin, which together dissipate the PMF (data not shown). Cells of *E. faecalis* were also examined for the presence of an agmatine-putrescine antiporter. In this case cells were loaded with putrescine and subsequently diluted into a buffer containing [¹⁴C]agmatine. Agmatine was rapidly accumulated by agmatine-grown cells (Fig. 1B). Virtually no agmatine uptake was detectable in putrescine-loaded cells grown on glucose or arginine. A similar picture emerged when the uptake of [¹⁴C]putrescine was assayed (data not shown). As with arginine uptake, uptake of agmatine (and putrescine) was not affected by the ionophores nigericin plus valinomycin (data not shown). Arginine- and agmatine-grown cells which were not preloaded with ornithine or putrescine also displayed rapid uptake of arginine and agmatine, respectively. This is in accordance with the observation that freshly harvested cells possessed abundant deiminase pathway activities (Table 1) and maintained high pools of ornithine or putrescine (Table 2). Cells frozen in liquid nitrogen lose their ADI and AgDI pathway activities completely, whereas the transport activities are conserved.

The results presented so far support the concept that separate exchange transport systems for arginine and agmatine are induced when cells of *E. faecalis* are grown in the presence of these guanidine compounds.

Efflux of putrescine and ornithine. Arginine- and agmatine-grown cells were loaded with [³H]ornithine and [¹⁴C]putrescine, respectively. Cells were subsequently diluted into a buffer supplemented with unlabeled arginine, ornithine, ag-

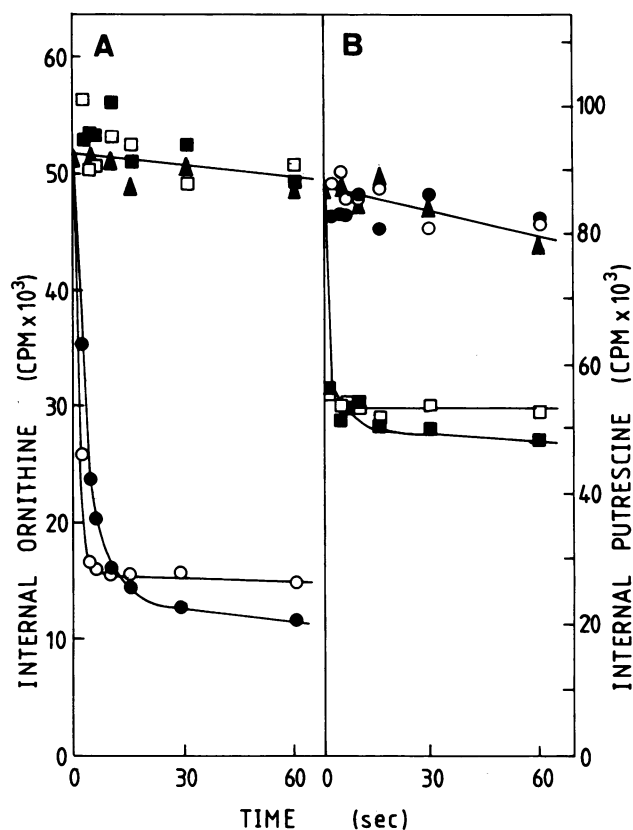


FIG. 2. Ornithine (A) and putrescine (B) efflux from cells of *E. faecalis* ATCC 11700 grown on arginine (A) or agmatine (B) as a sole energy source. Cells were loaded with 500 μM [^3H]ornithine or [^{14}C]putrescine and diluted into a buffer containing 500 μM arginine (●), ornithine (○), agmatine (■), putrescine (□), or none of these amino acids or diamines (Δ).

matine, or putrescine. Rapid release of [^3H]ornithine from arginine-grown cells was observed only when cells were diluted into a buffer containing either unlabeled arginine or ornithine (Fig. 2A). Agmatine and putrescine were without any effect. On the other hand, only putrescine and agmatine were able to elicit [^{14}C]putrescine efflux from agmatine-grown cells. In this case, arginine and ornithine were ineffective. It should be noted that putrescine was only partially released by these cells, suggesting that part of the intracellular putrescine is not present in a free form. Dilution of [^3H]ornithine- and [^{14}C]putrescine-loaded cells into a buffer without any of these amino acids or diamines resulted in slow release of both ornithine and putrescine (Fig. 2). These results directly demonstrate that uptake of arginine and agmatine is accompanied by the excretion of ornithine and putrescine, respectively. The transport system induced in cells grown on agmatine only catalyzes agmatine-putrescine and homologous putrescine exchange. This transport system thus has a substrate specificity which is completely different from that of the arginine-ornithine antiporter.

Kinetic analysis of homologous putrescine exchange. The initial rate of putrescine uptake by agmatine-grown cells loaded with 200 μM putrescine was determined for extracellular putrescine concentrations ranging from 4 to 145 μM . The initial rate was determined from the amount of label accumulated within 3 s. Putrescine uptake displayed saturation kinetics (Fig. 3), and analysis of the kinetic data by an

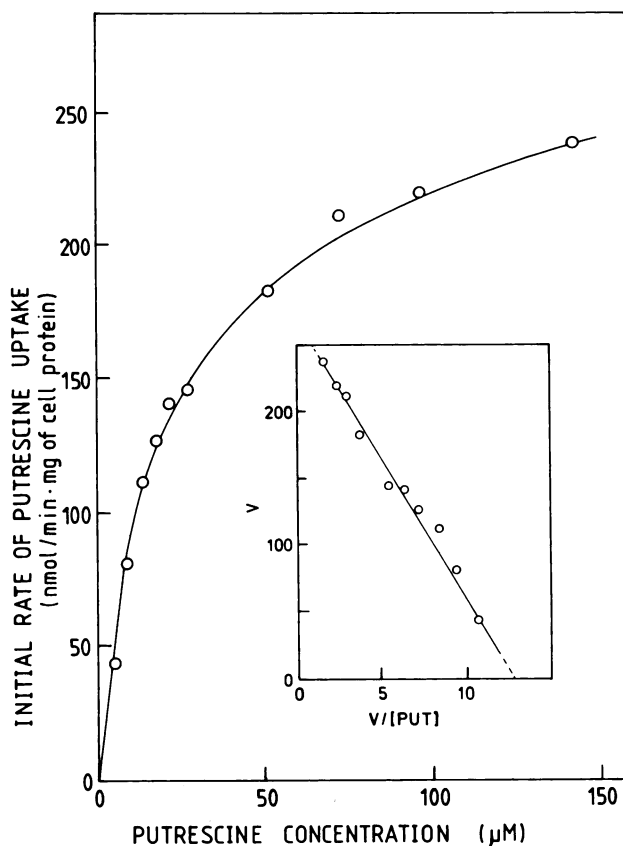


FIG. 3. Kinetic analysis of homologous putrescine exchange by cells of *E. faecalis* ATCC 11700 grown on agmatine. Cells were loaded with 200 μM putrescine, and the initial rate (V) of putrescine uptake was determined from the accumulation of [^{14}C]putrescine within 3 s, with putrescine (PUT) concentrations between 5 and 150 μM . (Inset) Eady-Hofstee plot of the initial rate data.

Eady-Hofstee plot yielded an apparent affinity constant for uptake (K_i) of 20 μM and a maximal velocity (V_{max}) of 262 nmol/min per mg of protein. Agmatine was found to be a competitive inhibitor of putrescine uptake. Initial rates of [^{14}C]putrescine uptake were determined at two concentrations (4 and 23 μM) under conditions in which the agmatine concentration was varied between 0.5 and 500 μM . Dixon plot analysis (Fig. 4) of these data revealed biphasic inhibition kinetics with inhibitor constants (K_i 's) for agmatine of 7 μM (inset, Fig. 4) and 58 μM (Fig. 4).

Agmatine-putrescine exchange in membrane vesicles. In order to obtain conclusive evidence for an agmatine-putrescine antiporter, agmatine transport was studied in membrane vesicles of *E. faecalis*. Inside-out membrane vesicles of agmatine-grown cells were prepared by French press treatment. Membrane vesicles were loaded with 200 μM putrescine and diluted 50-fold into a buffer containing [^{14}C] agmatine. Rapid uptake of agmatine was observed under those conditions (Fig. 5). Low levels of agmatine uptake were found when membrane vesicles that were not loaded with putrescine were used (Fig. 5).

DISCUSSION

In this paper we demonstrate that the uptake of the diamine agmatine by cells of *E. faecalis* proceeds via exchange with intracellular putrescine. Exchange transport

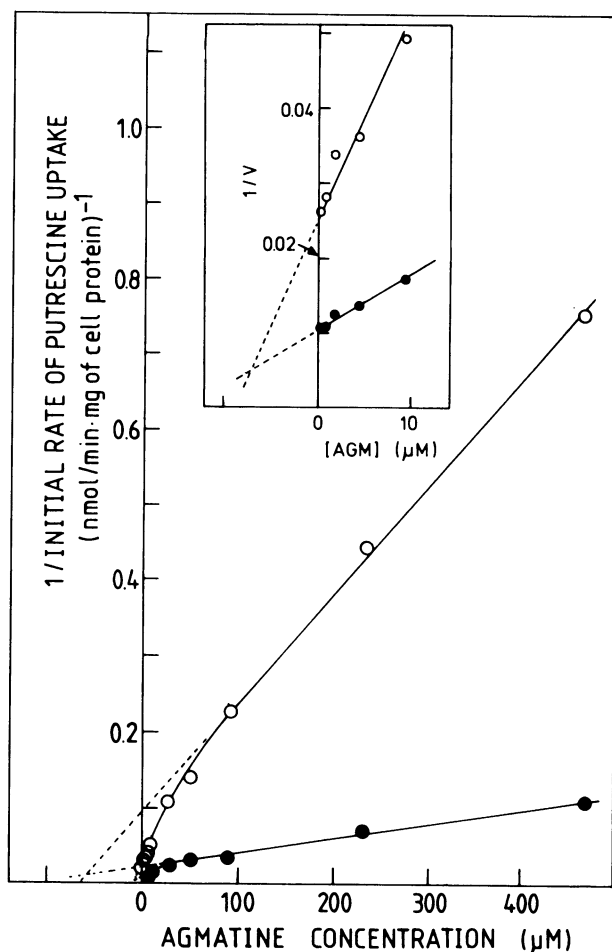


FIG. 4. Competitive inhibition by agmatine (AGM) of [^{14}C]putrescine uptake by putrescine-loaded cells of *E. faecalis* ATCC 11700. The initial rate of [^{14}C]putrescine uptake was determined at a concentration of 4 μM (○) or 23 μM (●) in the presence of agmatine, with agmatine concentrations between 0.5 and 200 μM . (Inset) Inhibition of putrescine uptake by agmatine, with agmatine concentrations between 0.5 and 10 μM . Further experimental detail can be found in the legend to Fig. 3 and Materials and Methods.

systems are commonly found in mitochondria (10) but rarely in bacteria (4, 9, 12). Just like the arginine-ornithine antiporter reported previously (4), the agmatine-putrescine antiporter clearly shows the important role of exchange transport systems in the degradation of guanidine compounds when the metabolic energy yield is low.

Recently, we have shown that an arginine-ornithine antiporter is involved in the uptake and excretion of arginine and ornithine in lactococci, streptococci, and enterococci (4, 15, 22). These bacteria are able to ferment arginine via the ADI pathway and lack an ornithine decarboxylase (2). Ornithine accumulates in the medium. The energy gain of the ADI pathway is low, i.e., 1 mol of ATP produced per mol of arginine consumed. A one-to-one exchange between the metabolite and the end product via an antiport system therefore appeared an attractive possibility. The AgDI pathway is highly analogous to the ADI pathway and also yields 1 mol of ATP per mol of agmatine consumed (2, 18). Both pathways are present in *E. faecalis* and induced when the cells are grown in the presence of these guanidine compounds (3, 16, 18, 19; this paper). When the AgDI pathway

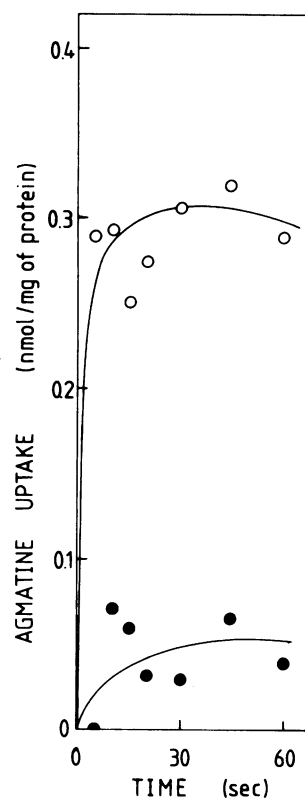


FIG. 5. Agmatine-putrescine transport by inside-out membrane vesicles of *E. faecalis* ATCC 11700. Membrane vesicles incubated in the presence (○) or absence (●) of 200 μM putrescine were suspended in 50 mM potassium phosphate, pH 7.0, supplemented with 2 mM MgSO_4 and 3.6 μM [^{14}C]agmatine. A final protein concentration of 0.08 to 0.09 mg/ml was used.

functions, the end product putrescine accumulates in the medium. Studies on the transport of diamines in bacteria have mainly been carried out with *Escherichia coli* (5, 7, 20). In this bacterium, uptake of di- and polyamines, in particular of putrescine and spermidine, occurs via completely different systems which are driven by the PMF (7). Glycolyzing cells of *E. faecalis* maintain a PMF of about -120 mV, inside negative and alkaline (8). Agmatine and putrescine are both positively charged, and although agmatine uptake may be enhanced by the electrical potential across the membrane ($\Delta\psi$), this $\Delta\psi$ will also oppose the extrusion of putrescine. Coupling of agmatine uptake to putrescine extrusion via the PMF is therefore unlikely, and an antiporter for agmatine uptake and putrescine excretion in *E. faecalis*, as demonstrated in this paper, is a more logical mechanism. Exchange also occurs in the absence of a PMF, which suggests that the antiporter mediates stoichiometric exchange between these diamines. Via this antiport mechanism, uptake of the metabolite agmatine is driven by the outwardly directed concentration gradient of the end product putrescine. Since uptake of agmatine does not occur at the expense of the PMF, cells are able to save the ATP formed during agmatine metabolism for other metabolic-energy-requiring processes.

Attempts have been made to estimate the exchange stoichiometry directly in whole cells (unpublished data). However, problems were encountered in determining the exact intracellular content of free putrescine in whole cells. Exchange experiments revealed that the intracellular putrescine pool is not completely exchangeable with extracellular

agmatine or putrescine (see also Fig. 2B). This phenomenon most likely arises from binding of this basic compound to nucleic acids, ribosomes, or other cellular components (21). Although bound putrescine exchanges slowly with [¹⁴C] putrescine, complete extraction of the intracellular content of putrescine could not be achieved. In this respect, in whole cells of *E. coli* intracellular putrescine is only slowly exchangeable with exogenous putrescine, whereas accumulated putrescine is not released when uncouplers are added. This is a result of binding of putrescine to cytoplasmic components, since these phenomena were not observed with membrane vesicles of *E. coli* (7). In membrane vesicles, accumulated putrescine is freely exchangeable with exogenous putrescine and rapidly released by uncouplers.

Exchange of agmatine and putrescine is an electroneutral process. The steady-state AgDI pathway activity is to some extent stimulated by ionophores which dissipate the PMF (Table 1). This effect has not been studied further. Fine control of the AgDI pathway activity might occur via the intracellular concentrations of (adenine) nucleotides, just as was found in the ADI pathway (15).

The results presented in this paper demonstrate that the arginine-ornithine and agmatine-putrescine antiporters are induced under distinct growth conditions. Both transport systems differ in their substrate specificity. The first system catalyzes the exchange of monovalent positively charged amino acids, which includes homoarginine and lysine (R. van Leeuwen and A. J. M. Driessen, unpublished results), whereas the latter mediates the exchange of divalent positively charged diamines. It is yet not known whether homoagmatine and cadaverine are also transported. Inhibition of homologous putrescine exchange by agmatine displayed biphasic behavior. A possible explanation could be that there are two systems for agmatine uptake, of which one system has a very low affinity for putrescine. Alternatively, binding of putrescine to cellular components might give rise to a second apparent low-affinity binding site.

The results demonstrate that in *E. faecalis* the ADI and AgDI pathways are also similar with respect to the transport mechanism. Uptake of these metabolites results from an energy-independent electroneutral exchange process in which the concentration gradients of the metabolite and the metabolic end product supply the driving force for uptake.

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