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Energetics of Arginine and Lysine Transport by Whole Cells and Membrane Vesicles of Strain SR, a Monensin-Sensitive Ruminal Bacterium

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Strain SR, a monensin-sensitive, ammonia-producing ruminal bacterium, grew rapidly on arginine and lysine, but only if sodium was present. Arginine transport could be driven by either an electrical potential or a chemical gradient of sodium. Arginine was converted to ornithine, and it appeared that ornithine efflux created a sodium gradient which in turn drove arginine transport. There was a linear decline in arginine transport as pH was decreased from 7.5 to 5.5, and the cells did not grow at a pH less than 6.0. The Eadie-Hofstee plot was biphasic, and arginine could also be taken up by a high-capacity diffusion mechanism. Because arginine was a strong inhibitor of lysine transport and lysine was a weak inhibitor of arginine transport, it appeared that both lysine and arginine were taken up by an arginine-lysine carrier which had a preference for arginine. The rate of lysine fermentation was always proportional to the extracellular lysine concentration, and facilitated diffusion was the dominant mechanism of lysine transport. When SR was grown in continuous culture on arginine or lysine, the theoretical maximum growth yield was similar (13 g of cells per mol of ATP), but the apparent maintenance energy requirement for arginine was greater than lysine (9.4 versus 4.4 mmol of ATP per g of cells per h). On the basis of differences in yield and maintenance energy, it appeared that active arginine transport accounted for approximately 40% of the total ATP.

In the early 1960s, Bladen et al. (1) showed that a variety of ruminal bacteria could deaminate amino acids, but the specific activities could not explain ammonia production in vivo (16). Strain SR, a recently isolated, monensin-sensitive, ruminal bacterium, grew rapidly on short peptides or amino acids (4). Although SR was present at low concentrations in ruminal fluid (10^7/ml), it had a very high specific activity of ammonia production (>310 nmol of ammonia per mg of protein per min) (5). On the basis of its capacity to produce ammonia, it appeared that SR could be a significant contributor to ruminal amino acid degradation, and its sensitivity to monensin provided an explanation for the "protein-sparing" effects of ionophores (6, 15). Strain SR deaminated arginine, lysine, serine, and glutamine, but it grew most rapidly on arginine and lysine (5).

Streptococci, halobacteria, clostridia, eubacteria, and mycoplasmas ferment arginine by the arginine deiminase pathway (12). Because this pathway of arginine metabolism yields only one ATP by substrate-level phosphorylation, the energetics of arginine fermentation were curious. Recent work by Driessen et al. (8), however, showed that *Lactococcus* (*Streptococcus*) *lactis* had an arginine-ornithine exchanger. Since neither a proton-motive force nor ATP was required for transport, it appeared that all of the ATP from substrate-level phosphorylation could be used for growth. In *L. lactis*, lysine is taken up by the arginine-ornithine exchanger and by a separate system which is proton-motive force driven (9).

Experiments with strain SR indicated that an outwardly directed ornithine gradient could drive arginine transport, but the mechanism did not involve a direct exchange of arginine and ornithine. High-affinity arginine transport had an absolute requirement for sodium, and ornithine efflux created a sodium gradient which then drove arginine transport. At high arginine concentrations, the rate of transport was proportional to the external concentration, and it appeared that arginine was also taken up by a facilitated diffusion mechanism. The arginine carrier was also able to transport lysine, but in this case facilitated diffusion was the predominant mechanism.

MATERIALS AND METHODS

Cell growth. Strain SR (5) was grown anaerobically in a medium containing (per liter) 292 mg of $K_2HPO_4 \cdot 3H_2O$, 240 mg of KH_2PO_4, 480 mg of Na_2SO_4, 480 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 0.6 g of cysteine hydrochloride, 4 g of Na_2HCO_2, vitamins, and minerals (5). Trypticase (BBL Laboratories, Cockeysville, Md.), arginine, or lysine was provided as an energy source (15 g/liter). The medium pH was 6.9, and cultures were incubated at 39°C. The sodium-deficient medium had potassium salts instead of sodium salts and a purified amino acid mixture resembling Trypticase (0.5 g/liter) as the carbon source.

Exponentially growing cultures were harvested by centrifugation (12,000 × *g*, 12 min, 5°C) at an optical density at 600 nm of approximately 0.8 (1-cm light path). Membrane vesicles were prepared as previously described (17) by using lysozyme and mutanolysin. Vesicles were resuspended in either 100 mM sodium phosphate buffer (pH 7.0, with or without 100 mM ornithine) or 100 mM potassium phosphate buffer (pH 7.0, with or without 100 mM ornithine) and stored in liquid nitrogen until use.

Continuous cultures (39°C, 360-ml vessel, 57 mM arginine or lysine, and 1 g of Trypticase per liter) were grown at

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dilution rates ranging from 0.047 to 0.256 h^{-1}, and the culture vessel was purged with O_2-free carbon dioxide. At least a 98% turnover of medium passed through the culture vessel before samples were obtained. Supernatants and cell pellets were stored (−4°C) for further analysis.

Transport assays. Cells were harvested by centrifugation (11,000 × *g*, 10 min, 5°C), and cells or vesicles were washed twice and resuspended in potassium phosphate (100 mM K, pH 7.0). Washed cells were incubated on ice for 30 min and diluted 50-fold into sodium phosphate buffer (100 mM Na, pH 7.0) which contained ^{14}C-labeled arginine. An artificial membrane potential ($\Delta\psi$) was created by treating washed cells or vesicles with valinomycin (5 μM) and loading them with K (100 mM potassium phosphate, pH 7.0, 30 min on ice). Valinomycin-treated cells or vesicles were diluted 50-fold into 100 mM sodium phosphate (pH 7.0) to create an artificial $\Delta\psi$ and a chemical gradient of sodium ($\Delta\mu$Na). Potassium-loaded cells were diluted into 100 mM sodium phosphate and 100 mM potassium phosphate to create a $\Delta\mu$Na in the absence of a $\Delta\psi$. An artificial $\Delta\psi$ in the absence of a $\Delta\mu$Na was produced by diluting K- and Na-loaded cells (5 μM monensin and 5 μM valinomycin) into 100 mM sodium phosphate. Controls (no driving force) were loaded with potassium or potassium and sodium and diluted into potassium or potassium plus sodium, respectively. Sodium-plus-ornithine-loaded vesicles (100 mM sodium phosphate plus 100 mM ornithine, 0°C, 1 h) were diluted 50-fold into potassium phosphate (pH 7.0) to create a chemical gradient of ornithine. Cells (3.8 mg of protein) were loaded with ^{22}Na (2.1 μCi) and 100 mM ornithine (0°C, 24 h) and diluted into potassium phosphate (pH 7.0) to monitor sodium efflux.

Concentrated cells or vesicles (4 μl, 3 to 14 μg of protein) were diluted 50-fold into buffer (see above) which contained 100 nCi of ^{14}C-labeled arginine, lysine, or glutamine. After 0, 5, 10, 30, 60, or 120 s, transport was terminated by adding 2 ml of ice-cold LiCl (100 mM) and filtering the mixture through cellulose nitrate membrane filters (0.45-μm pore size). The filters were rinsed with 2 ml of LiCl, dried (105°C) for 20 min, and counted by liquid scintillation.

Proton-motive force. Strain SR was grown in batch culture with 15 g of arginine per liter, and pH was decreased by the addition of concentrated HCl. Samples (2 ml) were incubated anaerobically with [^{14}C]tetraphenylphosphonium bromide (1.0 μCi, 30 μCi/μmol), [7-^{14}C]benzoate (1.0 μCi, 21.8 μCi/μmol), [1,2-^{14}C]polyethylene glycol (1.0 μCi/mg), or ^3H_2O (4.00 μCi, 3.6 μCi/μmol) for 5 min (14). Cultures were centrifuged through silicon oil (equal-part mixture of Dexter Hysol 550 and 560; Hysol Co., Olean, N.Y.) in microcentrifuge tubes (13,000 × *g*, 5 min, 22°C), and 20-μl samples of supernatant were removed. The tubes and contents were frozen (−15°C), and the bottoms (cell pellets) were removed with dog nail clippers. Supernatant and cell pellets were dissolved in scintillation fluid and counted. Intracellular volume (2.42 μl/mg of protein) was calculated from the difference between ^3H_2O and [^{14}C]polyethylene glycol. The electrical potential across the cell membrane ($\Delta\psi$) was calculated from the uptake of [^{14}C]tetraphenylphosphonium bromide according to the Nernst relationship. Nonspecific tetraphenylphosphonium bromide binding was estimated from cells which had been treated with valinomycin and nigericin (5 μM). The pH gradient across the membrane (ΔpH) was determined from the distribution of [^{14}C]benzoate by using the Henderson-Hasselbalch equation. $\Delta\psi$ and the chemical gradient of protons (ZΔpH) were corrected for extracellular contamination.

Analyses. Volatile fatty acids from metaphosphoric acid-treated samples (6% [wt/vol], final concentration) were measured with a Gow Mac model 580 flame ionization gas chromatograph (Supelco 1220 column, 1% H_3PO_4, 100/120 mesh). Amino acid samples (15 μl) were treated with 985 μl of 40 mM Na_2CO_3 (pH 9.5) and 500 μl of dansyl chloride (1.5 mg/ml in acetonitrile) and incubated for 1 h in the dark. An excess of methylamine (20 μl, 2% [wt/vol]) was added to terminate the reaction. Dansyl derivatives were detected by high-pressure liquid chromatography (254 nm, model 160 UV detector [Beckman]) with an acetonitrile gradient. Samples (20 μl) were injected into 30 mM sodium phosphate buffer (pH 6.5) which passed through a C-18 reversed-phase column at 1.0 ml/min (28°C). The acetonitrile gradient was increased linearly to 55% over the next 30 min.

Ammonia was assayed by the colorimetric method of Chaney and Marbach (3). Cell protein was measured by the method of Lowry et al. (13) after the cells had been heated to 100°C in 0.2 N NaOH for 15 min. To determine the dry matter, cells were washed in 0.9% NaCl and dried at 105°C to a constant weight in aluminum pans. The weight of the NaCl was then subtracted. Cell pellets (silicon oil as described above) were dissolved in 3 N HNO_3 and allowed to digest for at least 24 h. Sodium and potassium were analyzed with a Cole Parmer 2655-00 flame analyzer (Cole Parmer Instrument Co., Chicago, Ill.).

Materials. ^{14}C and ^{22}Na compounds were obtained from Amersham Corp., Arlington Heights, Ill. All chemicals and reagents were reagent grade.

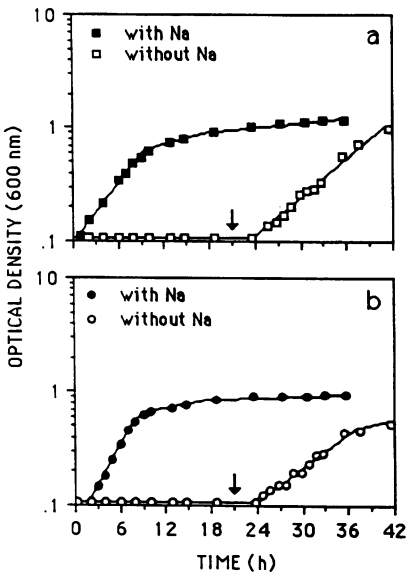

FIG. 1. Growth of strain SR on 15 g of arginine (a) or lysine (b) per liter in sodium-deficient medium with and without 100 mM sodium chloride. Arrows indicate the addition of 100 mM sodium chloride to the sodium-deficient cultures. The media contained a low concentration (0.5 g/liter) of an amino acid mixture with a composition similar to that of Trypticase (based on manufacturer's specifications).

RESULTS

Cell growth. Strain SR grew on arginine or lysine (15 g/liter) as long as a low concentration (0.5 g/liter) of other amino acids was provided as a carbon source (Fig. 1). Little growth (optical density, <0.3) was observed if arginine and lysine were deleted. When the sodium salts were replaced by

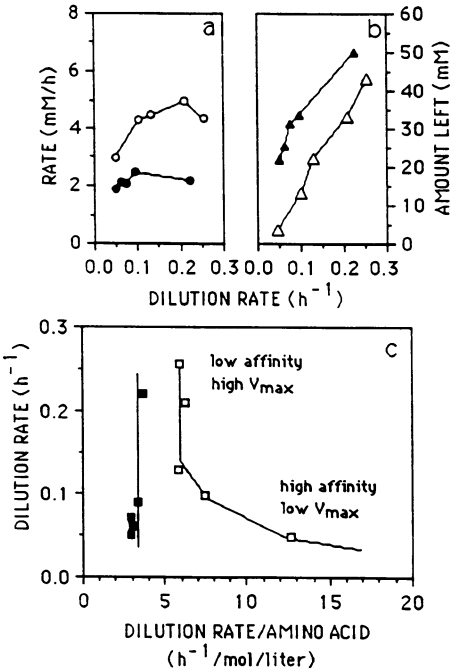

FIG. 2. Rate of amino acid utilization (a), amount of substrate remaining in the culture vessel (b), and an Eadie-Hofstee plot of amino acid utilization (c) by strain SR cells when arginine (open symbols) or lysine (closed symbols) was provided as the energy source in continuous culture.

potassium salts, the washed cells were no longer able to utilize arginine or lysine as an energy source. If sodium chloride (100 mM) was added to the sodium-deficient medium, growth was observed. When SR deaminated arginine, the increase in optical density closely paralleled the accumulation of ammonia and more than 80% of the arginine was converted to ornithine. The remaining arginine was converted to citrulline and acetate. Acetate and butyrate (equimolar concentrations) were the end products of lysine fermentation by strain SR.

When strain SR was grown in continuous culture (pH 6.9), acetate was not a significant end product of arginine metabolism and the ratio of ornithine to citrulline (5 to 1) was not significantly influenced by the dilution rate. Preliminary experiments indicated that the cultures were energy limited (optical density increased when more arginine was added), but there was a linear increase in the amount of arginine left when the dilution rate was increased (Fig. 2). Although the amount of arginine left increased, there was, at least initially, an increase in the overall rate of arginine fermentation (amount of arginine used times dilution rate). An Eadie-Hofstee plot indicated that arginine utilization did not follow typical saturation kinetics (Fig. 2c). The high-affinity system could not account for arginine utilization at rapid dilution rates, but the low-affinity system had a very high maximum velocity (intercept). Similar results were observed when lysine was the substrate, but in this case the rate of lysine fermentation was always proportional to the amount of lysine left (dilution rate per lysine millimolar was constant).

At high dilution rates the yield from lysine was similar to the arginine yield (Fig. 3). However, a Pirt plot (1/ATP yield versus 1/dilution rate) indicated that lysine was utilized more efficiently for growth than arginine at low dilution rates and

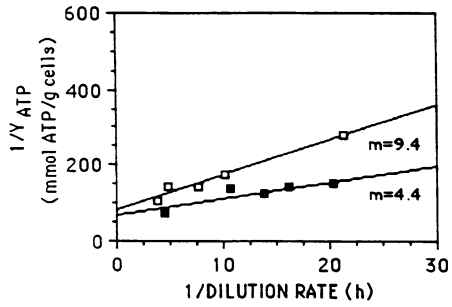

FIG. 3. A Pirt plot of ATP production by strain SR when grown in continuous culture with 57 mM arginine (open symbols) or lysine (closed symbols). Trypticase (1 g/liter) was provided as a carbon source. Y_{ATP}, ATP yield.

that this difference was due to a higher maintenance energy (slope). Although the maintenance term for arginine was higher, the theoretical maximum growth yields for arginine and lysine (intercepts) were similar.

Proton- and sodium-motive force. Exponentially growing cells (15 g of arginine per liter, pH 7.0) had little if any pH gradient across the cell membrane, but the electrical potential ($\Delta\psi$) was −117 mV (Table 1). During this time, the cells were able to maintain a 40-fold concentration gradient (inside higher) of potassium. The intracellular concentration of sodium was threefold lower than the outside concentration, but it is likely that much of this sodium was cell bound (20). Monensin, a sodium-proton antiporter which inhibited growth, had little effect on $\Delta\psi$, and there was an increase in cellular sodium and a decrease in potassium (Table 1). Valinomycin, a potassium uniporter, had little effect on growth, proton-motive force, or sodium, but there was a 40% decrease in cellular potassium. Dicyclohexylcarbodiimide, an inhibitor of proton ATPases, had little effect on growth, potassium and sodium concentrations, or $\Delta\psi$, but there was an increase in $Z\Delta pH$. A combination of valinomycin and nigericin was used as a correction for nonspecific tetraphenylphosphonium bromide binding, and this treatment caused a large decrease in the level of potassium and an accumulation of sodium. The protonophore 3,3',4',5-tetrachlorosalicylanide completely inhibited growth and lowered the cellular potassium concentration.

When concentrated HCl was added to batch cultures (15 g of arginine per liter) and pH was lowered in stepwise incre-

TABLE 1. Effects of various inhibitors on $\Delta\psi$, $Z\Delta pH$, intracellular Na, and intracellular K of strain SR

Treatment[a]	mV		Intracellular concn (nmol/mg of protein)[b]	
	$\Delta\psi$	$Z\Delta pH$	Na	K
Control	−117	0	92 (38)	705 (291)
Monensin	−124	0	275 (114)	181 (75)
Valinomycin	−86	11	52 (22)	410 (170)
DCCD	−106	−49	110 (46)	680 (281)
Nigericin-valinomycin	0	0	316 (131)	246 (102)
TCS	ND[c]	0	133 (55)	406 (168)

[a] DCCD, dicyclohexylcarbodiimide; TCS, 3,3',4',5-tetrachlorosalicylanide.

[b] The intracellular concentrations of Na and K (in parentheses) were based on an intracellular volume of 2.42 μl/mg of protein. The extracellular concentrations of K and Na were 8 and 92 mM, respectively.

[c] ND, not determined.

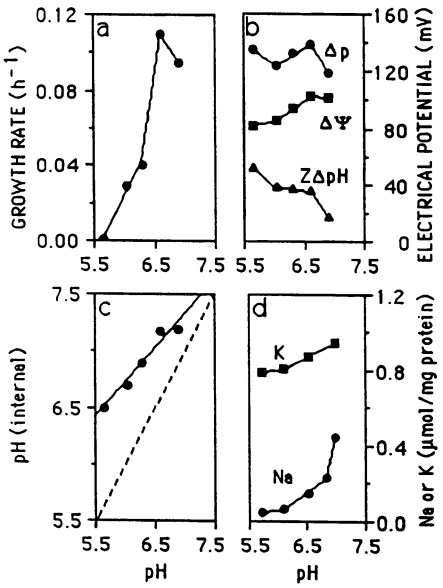

FIG. 4. Effects of pH on growth rate (a), proton-motive force (b), internal pH (c), and cellular sodium and potassium levels (d) on SR batch cultures (15 g of arginine per liter). pH was decreased with concentrated HCl over a period of 4 h.

ments from 6.9 to 5.65, there was a dramatic decrease in growth rate (Fig. 4a). No growth was observed at pH values less than 6.0. As extracellular pH declined, the electrical potential ($\Delta\psi$) across the cell membrane decreased (Fig. 4b) and this decline was only partially compensated for by an increase in ΔpH (Fig. 4c). During this time, there was also a decrease in cellular potassium and sodium concentrations (Fig. 4d).

Transport. Preliminary experiments indicated that arginine transport was not significantly affected by the presence of oxygen and that the rate was directly proportional to cell protein and time as long as the transport time was not longer than 30 s. When washed cells were incubated with 3 to 477 μM arginine, the Eadie-Hofstee plot was nonlinear (Fig. 5). The affinity constant (K_t) of the high-affinity system was less than 1.0 μM, but its V_{max} was 20-fold lower than the ammonia production of batch cultures (16 versus 310 nmol/mg of protein per min). At higher arginine concentrations, the transport rate was nearly proportional to the substrate concentration.

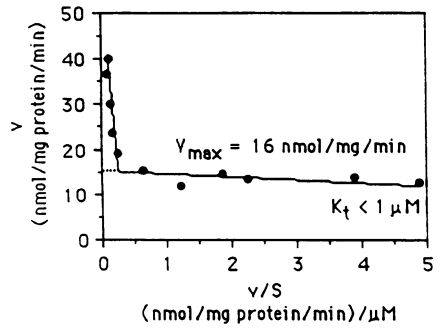

FIG. 5. An Eadie-Hofstee plot of arginine transport showing initial rates (30 s) of uptake (v) versus initial rates of uptake/substrate (v/S). Whole cells were washed with potassium phosphate and diluted into sodium phosphate (pH 7.0).

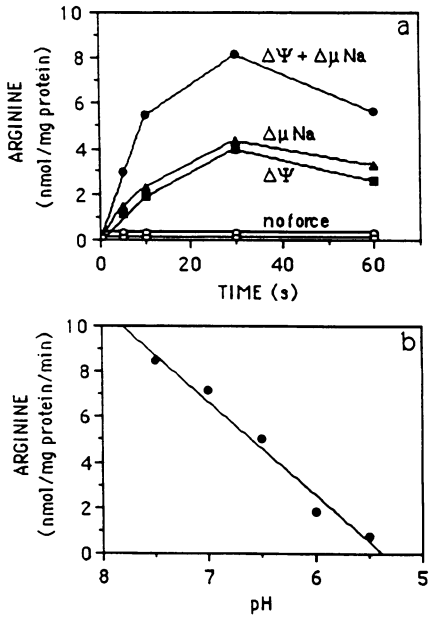

FIG. 6. (a) Transport of arginine (pH 7.0) by valinomycin- and monensin-treated (5 μM) cells which were loaded with potassium and diluted into sodium (●) or sodium plus potassium (▲) or potassium (□). Sodium- and potassium-loaded cells were diluted into sodium (■) or potassium plus sodium (○). (b) Effects of pH on the transport (10 s) of arginine by strain SR ($\Delta\psi$+$\Delta\mu$Na).

When valinomycin- and monensin-treated cells were diluted into sodium to create an artificial $\Delta\psi$ and a $\Delta\mu$Na, the rate of arginine uptake was twice as high as that observed with only $\Delta\psi$ or $\Delta\mu$Na (Fig. 6a). No transport was observed if sodium was deleted or if cells were loaded with K or K plus Na and diluted into K or K plus Na, respectively (no driving force). Similar results were observed with lysine. When the pH of the transport buffer was decreased, there was a linear decrease in the rate of arginine transport even though the driving force ($\Delta\psi$ plus $\Delta\mu$Na) remained constant (Fig. 6b). An artificial $\Delta\psi$ was unable to drive arginine uptake in the absence of sodium, and the arginine carrier had a low affinity for sodium (Fig. 7a). Since the Hill plot slope was 0.94, it appeared that the arginine carrier had a single binding site for Na (Fig. 7b). The lysine carrier had a slightly lower affinity (K_t = 17 versus 10 mM) and one site for sodium (data not shown).

Valinomycin-treated membrane vesicles which were loaded with potassium (pH 7.0) and diluted into sodium phosphate (pH 7.0) to create an artificial $\Delta\psi$ and a $\Delta\mu$Na also transported arginine at a high rate (Fig. 8a). No transport was observed when the same vesicles were diluted into potassium phosphate buffer, and the rate of transport was decreased if the dilution buffer contained sodium and potassium ($\Delta\mu$Na but no $\Delta\psi$). Untreated membrane vesicles took up arginine nearly as fast as valinomycin-treated vesicles, but untreated vesicles which were loaded with sodium and diluted into potassium did not take up arginine (Fig. 8b).

Ornithine-sodium efflux. The idea that ornithine translocation was indeed creating a sodium gradient was further supported by the effect of ornithine on glutamine uptake. Cells which were loaded with sodium and diluted into sodium or potassium were unable to take up glutamine. However, if the cells were loaded with ornithine and sodium and diluted into buffer lacking ornithine, rapid uptake was observed (Fig. 9). Little glutamine transport was seen if ornithine was added

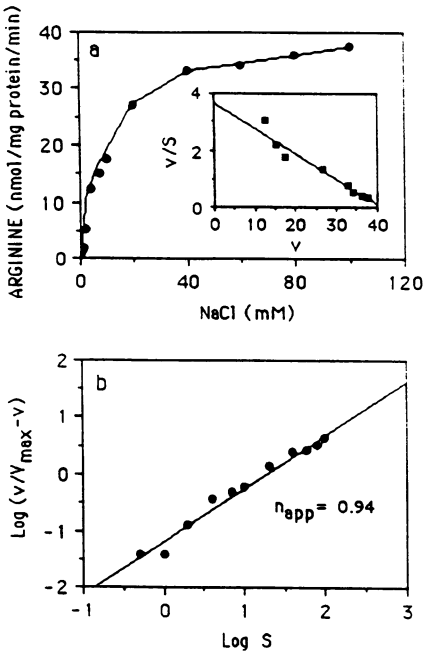

FIG. 7. (a) Effect of added NaCl on the initial rate (10 s) of arginine transport by strain SR cells loaded with K in the presence of valinomycin (5 μM) and diluted 50-fold into K-phosphate (pH 7.0). An Eadie-Scatchard plot is shown in the inset. (b) A Hill plot of the data in panel a; n_{app}, slope of the plot.

to the dilution buffer. Similar results for arginine were observed in membrane vesicles (Fig. 8b). Cells which were loaded with sodium plus ornithine and diluted into potassium lost more ^{22}Na than cells which were diluted into potassium plus ornithine (Fig. 10). Flame photometry likewise indicated

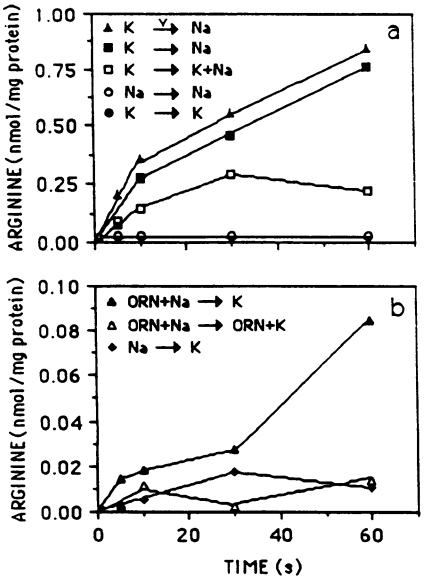

FIG. 8. Uptake of arginine by strain SR vesicles (pH 7.0) which were loaded with potassium, sodium, or ornithine and diluted to create chemical gradients of sodium or ornithine. In panel a some cells (▲) were treated with valinomycin (5 μM).

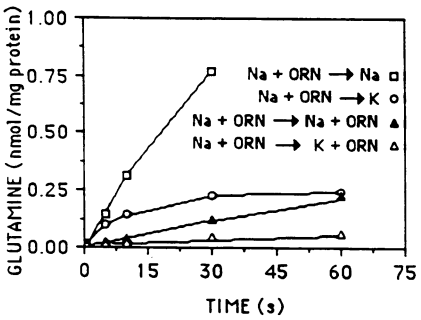

FIG. 9. Uptake of glutamine by SR cells which were loaded with sodium (100 mM) plus ornithine (100 mM) and diluted 50-fold into buffers containing 100 mM sodium or potassium, with or without ornithine (100 mM).

that intracellular sodium concentrations were lower when ornithine rushed out (no extracellular ornithine).

DISCUSSION

In *L. lactis* arginine uptake is mediated by an electroneutral arginine-ornithine exchange mechanism which does not respond to an electrical membrane potential and is not inhibited by protonophores or ionophores (7). Strain SR also produces ornithine, but it did not appear that arginine transport involved an arginine-ornithine exchanger. With strain SR, high-affinity arginine transport (i) was observed only when sodium was present, (ii) was inhibited by monensin, and (iii) used either $\Delta\psi$ or $\Delta\mu Na$ as a driving force. To our knowledge, this is the first report of sodium-dependent arginine transport in bacteria.

The arginine-ornithine exchanger of *L. lactis* provides a non-energy-requiring mechanism to transport arginine (7). SR also conserves the energy of ornithine efflux, but the mechanism was distinctly different. Preliminary experiments showed that ornithine-loaded cells could take up arginine at a higher rate than cells which were not loaded with ornithine, but these experiments did not exclude the possibility that ornithine fermentation was providing ATP that subsequently drove sodium efflux and arginine uptake. Because cells which were loaded with ^{22}Na and diluted into ^{23}Na lost the ^{22}Na in less than 5 s, with or without arginine or ornithine, it appeared that there was a rapid exchange of ^{23}Na for ^{22}Na. Nonetheless, it appeared that ornithine efflux was indeed generating a chemical gradient of sodium (Fig. 11), and this conclusion was supported by the following observations: (i)

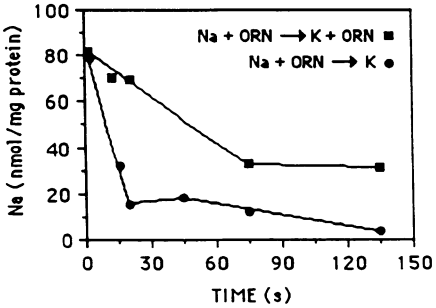

FIG. 10. Loss of cellular sodium by SR cells when loaded with sodium (100 mM) plus ornithine (100 mM) and diluted 50-fold into 100 mM potassium (●) or potassium plus 100 mM ornithine (■).

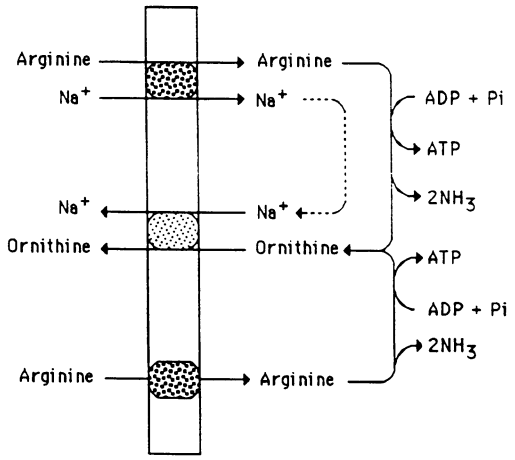

FIG. 11. Hypothetical scheme of arginine transport by strain SR.

whole cells which were loaded with sodium and diluted into potassium lost ^{22}Na at a higher rate if ornithine was allowed to rush out, and (ii) arginine transport in membrane vesicles could be driven by an outwardly directed ornithine gradient.

Because the Eadie-Hofstee plot of arginine transport was biphasic and v/S was nearly constant at high arginine concentrations, it appeared that arginine was also taken up by a facilitated-diffusion mechanism. The V_{max} of the high-affinity system could not account for arginine utilization at rapid growth rates, but the V_{max} of the low-affinity system was very high. Since monensin-treated cultures continued to ferment arginine and some ammonia was produced when sodium salts were replaced by potassium salts, it appeared that only high-affinity transport was sodium dependent. *Escherichia coli* (2) and *Pseudomonas putida* (11) also have both high- and low-affinity transport systems for arginine.

When lysine was present at low concentrations (e.g., 1.5 μM) a $\Delta\psi$ or $\Delta\mu Na$ was required for transport, but the V_{max} of this system was very low. Because the rate of lysine transport in continuous culture was always proportional to the external lysine concentration (v versus v/S constant), it appeared that facilitated diffusion was the dominant mechanism of lysine uptake. Unlabeled arginine strongly inhibited ^{14}C-lysine transport, but unlabeled lysine was a weak inhibitor of ^{14}C-arginine transport. On the basis of these results, it appears that arginine and lysine are transported by the same carrier and that the carrier has a preference for arginine. In *L. lactis*, lysine can be translocated across the cell membrane by the arginine-ornithine exchanger or a separate carrier (7).

In clostridia, lysine is converted to acetate, butyrate, and ammonia by a pathway which gives 1 mol of ATP per mol of lysine via substrate-level phosphorylation, and the ATP yield of the arginine deiminase pathway is also 1 mol of ATP per mol (12). The theoretical maximum growth yield of SR was approximately 13 g of cells per mol of ATP when either arginine or lysine was the energy source, but there was a marked difference in the apparent maintenance energy requirement (Fig. 3). This difference was a curiosity. Why would cells grown on arginine require more energy than cells grown on lysine?

At high substrate concentrations (high growth rates), facilitated diffusion appeared to be the dominant method of both lysine and arginine uptake (Fig. 2c). However, as the available substrate declined (low growth rates), more of the arginine was taken up by the high-affinity, active transport system. Since more energy was required for transport at low substrate concentrations, less energy (approximately 40% less) was available for growth-related functions. With lysine, facilitated diffusion was always the dominant transport mechanism and the nongrowth energy requirement (maintenance) did not increase.

The effect of pH on ruminal amino acid degradation has not been examined in a systematic fashion, but Erfle et al. (10) noted that the ammonia production of mixed continuous cultures decreased when the pH was lowered. Previous work indicated that strain SR was able to grow only at near-neutral pH (5), and this pH sensitivity is consistent with the effect of pH on the arginine carrier. Because lysine is often the first limiting amino acid, there has been considerable interest in the ruminal protection of this amino acid (18, 19). SR was the only monensin-sensitive ruminal bacterium which could ferment lysine at a high rate (5, 14), but SR had a poor affinity for lysine. Because the K_s was high, one might conclude that monensin would protect lysine only at high lysine concentrations. However, it should be realized that K_s/V_{max} is a better index of the utilization rate than K_s alone and that SR has a very high V_{max} for lysine.

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