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Mechanism of Na^+-Dependent Citrate Transport in *Klebsiella pneumoniae*

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Citrate transport via CitS of *Klebsiella pneumoniae* has been shown to depend on the presence of Na^+. This transport system has been expressed in *Escherichia coli*, and uptake of citrate in *E. coli* membrane vesicles via this uptake system was found to be an electrogenic process, although the pH gradient is the main driving force for citrate uptake (M. E. van der Rest, R. M. Siewe, T. Abee, E. Schwartz, D. Oesterhelt, and W. N. Konings, J. Biol. Chem. 267:8971–8976, 1992). Analysis of the affinity constants for the different citrate species at different pH values of the medium indicates that H-citrate^{2-} is the transported species. Since the electrical potential across the membrane is a driving force for citrate transport, this indicates that transport occurs in symport with at least three monovalent cations. Citrate efflux is stimulated by Na^+ concentrations of up to 5 mM but inhibited by higher Na^+ concentrations. Citrate exchange, however, is stimulated by all Na^+ concentrations, indicating sequential events in which Na^+ binds before citrate for translocation followed by a release of Na^+ after release of citrate. CitS has, at pH 6.0 and in the presence of 5 mM citrate on both sides of the membrane, an apparent affinity (K_{app}) for Na^+ of 200 μM. The Na^+/citrate stoichiometry was found to be 1. It is postulated that H-citrate^{2-} is transported via CitS in symport with one Na^+ and at least two H^+ ions.

Studies of citrate transport in *Klebsiella pneumoniae* have shown the presence of three different transport systems (14). One of these transport systems (CitH) appears to be proton dependent and is functional under aerobic conditions (14). The gene coding for this transport system has been cloned and sequenced previously (16). It has been shown that CitH belongs to the class of sugar transport systems described by Baldwin and Henderson (1). Studies of the mechanism of transport via CitH have shown that H-citrate^{2-} is transported in symport with three protons (15). The second citrate transport system has not been studied in detail but is presumed to be an inducible transport system which functions under aerobic conditions (14). The third citrate transport system of *K. pneumoniae* (CitS), described in the present report, appears to be an Na^+-dependent transport system which functions under anaerobic conditions (3, 14). The genetics of this system have also been studied elsewhere (17), and it was found that this system is related to CitH, although the secondary structures of the proteins, as predicted by hydropathy analysis, appear to differ. Both CitH and CitS have been functionally expressed in *Escherichia coli* (14, 16, 17). *E. coli* does not possess a citrate transport mechanism but is able to use citrate in the citric acid cycle (7, 8); therefore, *E. coli* is an ideal host for the expression of citrate transport systems from other bacteria.

There is some conflict in the literature as to how citrate is transported via CitS. Dimroth and Thomer (3) showed that in whole cells of *K. pneumoniae* grown anaerobically on citrate, citrate transport was Na^+ dependent but also that the proton gradient across the membrane (ΔpH) and the electrical potential across the membrane (ΔΨ) could drive citrate uptake. To separate the citrate transport system from the catalytic machinery of the cell, the citrate carrier was solubilized from the *K. pneumoniae* cell membranes and reconstituted into liposomes (4). In this model system, the accumulation of citrate into proteoliposomes could only be driven by a chemical sodium gradient across the membrane ($\Delta\bar{\mu}_{Na^+}$). This uptake was unaffected by the addition of valinomycin or FCCP (carbonylcyanide *p*-trifluoromethoxyphenylhydrazone) to proteoliposomes containing CitS, suggesting an electroneutral Na^+-citrate symport mechanism. Dimroth and Thomer postulated that the trianionic citrate species is taken up in symport with three Na^+ ions. Transport studies with membrane vesicles of *E. coli* in which CitS was functionally expressed, however, showed that citrate transport was mainly driven by the ΔpH and to a lesser extent by the ΔΨ and the $\Delta\bar{\mu}_{Na^+}$ (17). These data suggest that citrate is translocated across the membrane electrochemically in symport with protons and sodium ions. In the present study, the mechanism of citrate transport via CitS has been analyzed in detail, and the results reveal that H-citrate^{2-} is the transported species which is symported with one Na^+ and at least two H^+ ions.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *citS* gene was derived from genomic DNA of *K. pneumoniae* ATCC 13882. *E. coli* DH1 was used as a host for the plasmid pRS63-12, a derivative of pRS63-2 (17) which functionally expresses CitS.

Media and antibiotics. Luria broth and Luria broth agar (12) were used for routine bacterial growth. The selective medium for citrate utilization was Simmons citrate agar (Difco), supplemented with 4 μg of thiamine per ml and 100 μM isopropyl-β-D-thiogalactopyranoside (IPTG). Ampicillin was used at 100 μg/ml.

Preparation of membrane vesicles. *E. coli* DH1/pRS63-12 was grown to an A_{660} of 0.7 on Luria broth medium containing 100 μg of ampicillin per ml and 1 mM IPTG. Cells were harvested and membrane vesicles were isolated as described by Kaback (5). Membrane vesicles were finally resuspended in 50 mM potassium phosphate (pH 7.0) (9.8 mg of protein

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per ml) and stored in portions of 0.5 ml in liquid nitrogen until use. Protein concentration was measured by the method of Lowry et al. (9).

Solute transport. Solute uptake, driven by a proton motive force (Δp) generated by the oxidation via the respiratory chain of the electron donor system ascorbate-phenazine methosulfate (6), was assayed at 30°C as described previously (15). The uptake experiment was started by the addition of 4.5 μM [1,5-^{14}]citrate.

Citrate counterflow was measured at 20°C. The membrane vesicles were washed twice with 50 mM K-PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)] (pH 6.0) containing 5 mM citrate and different concentrations of NaCl. The membrane vesicle suspension was allowed to equilibrate for 6 h at 20°C. Aliquots of the concentrated suspension (25 mg of protein per ml) were diluted 325-fold in K-PIPES (pH 6.0) containing 4.5 μM [1,5-^{14}C]citrate and different concentrations of NaCl. Valinomycin and nigericin were added to concentrations of 1 μM to prevent the formation of electrical or pH gradients. The degree of uptake was assayed by filtration as described elsewhere (15).

Citrate exchange and efflux were measured at 20°C. Membrane vesicles were washed twice with 50 mM K-PIPES (pH 6.0) containing 5 mM citrate and different concentrations of NaCl. The membrane vesicle suspension was equilibrated for 4 h at 20°C. A concentrated suspension of this mixture (25 mg/ml) was equilibrated for 2 h with 50 mM K-PIPES (pH 6.0) containing 5 mM [1,5-^{14}C]citrate and different concentrations of NaCl. For exchange, the concentrated membrane vesicles were diluted 325-fold in K-PIPES (pH 6.0) containing 5 mM citrate, 1 μM valinomycin, 1 μM nigericin, and different concentrations of NaCl. For efflux, the membrane vesicles were diluted 325-fold in K-PIPES (pH 6.0) containing 1 μM valinomycin, 1 μM nigericin, and different concentrations of NaCl.

The efflux of Na^+ was measured at 20°C. The membrane vesicles were washed twice with 50 mM K-PIPES (pH 6.0) containing 5 mM NaCl in the presence or absence of 5 mM citrate. The membrane vesicle suspension was equilibrated for 4 h at 20°C. A concentrated suspension of this mixture (25 mg/ml) was equilibrated for 2 h with 50 mM K-PIPES (pH 6.0) containing 5 mM ^{22}NaCl in the presence or absence of 5 mM citrate. For efflux, the membrane vesicles were diluted 400-fold in K-PIPES (pH 6.0). ^{22}NaCl activity was measured in a gamma counter (Cobra Auto-gamma counting system model 5002; Packard Instruments). To compare the stoichiometry of Na^+/citrate efflux, membrane vesicles loaded with 5 mM [1,5-^{14}C]citrate in the presence or absence of 5 mM NaCl were diluted 400-fold in 50 mM K-PIPES (pH 6.0). The results of the efflux and exchange experiments were analyzed by subtracting the radioactivity retained on the filter after 60 min of incubation from the amount of radioactivity retained in the vesicles. The logarithms of the percentage of citrate retained in the vesicles were plotted as a function of time.

Kinetic analysis of uptake. For kinetic analysis of Δp-driven citrate uptake, the initial rate of uptake of citrate was determined from the amount of label accumulated during the first 30 s in the presence of 10 mM NaCl. A total of 15 different concentrations of [1,5-^{14}C]citrate, ranging from 0.9 to 204.5 μM, were used to determine the apparent affinity (K_{app}) of CitS for citrate. For kinetic analysis of sodium-dependent citrate efflux, the exchange rate was determined with six datum points during the first 15 s in the presence of 5 mM [1,5-^{14}C]citrate. The concentration of NaCl varied, with seven concentrations ranging from 0.5 to 20 mM. The results were analyzed with an Eadie Hofstee plot.

Calculations. The concentrations of the different citrate species at different pH values are calculated according to the pK_a values of citrate (pK_1, 3.14; pK_2, 4.77; and pK_3, 5.40) as described previously (15).

DNA manipulations. General procedures for cloning and DNA manipulations were essentially performed as described by Maniatis et al. (10). Restriction enzymes and T4 DNA ligase were used as recommended by the manufacturer (Boehringer Mannheim).

Subcloning of the *citS* determinant. In a previous paper, we described the cloning and expression of the sodium-dependent citrate carrier from *K. pneumoniae* (CitS) in *E. coli* (17). The 2.1-kb DNA fragment determining the Cit$^+$ phenotype, located on plasmid pRS63-2, coded not only for the citrate transport protein but also for the γ subunit of the oxaloacetate decarboxylase from *K. pneumoniae* (17). This oxaloacetate decarboxylase is a membrane-bound Na^+ pump that catalyzes the decarboxylation of oxaloacetate to pyruvate (2). The free energy of the decarboxylation reaction is converted into an electrochemical gradient of Na^+. The exact function of the γ subunit is not known, but to prevent interference from this protein in our transport studies, we deleted a large part of this gene from pRS63-2, creating pRS63-12 (results not shown). The level of uptake of citrate in *E. coli* containing this plasmid did not differ significantly from that in *E. coli* containing pRS63-2 (results not shown).

Chemicals. Radioactively labeled solutes were obtained from the Radiochemical Centre (Amersham, Buckinghamshire, United Kingdom) with the following specific activities: [1,5-^{14}C]citrate, 111 mCi/mmol; and ^{22}NaCl, 736 mCi/mmol. All other chemicals were reagent grade and obtained from commercial sources.

RESULTS

pH dependency of citrate transport. The initial rates of citrate uptake and the steady-state accumulation levels in membrane vesicles of *E. coli* DH1/pRS63-12 were found to be markedly influenced by the pH value (Fig. 1). The highest uptake rates were observed at pH 5.5, while at pH 8.0 no uptake of citrate was found. The effect of the pH on citrate transport could be the result of effects on the magnitude of the ΔpH. The ΔpH is maximal at pH 5.0 and decreases to 0 at pH 8.0 (13). However, the effect of the pH on citrate transport could also reflect the availability of the transported citrate species (15). Citrate has pK_a values of 3.14, 4.77, and 5.40. The concentrations of the different citrate species can be calculated at different pH values. At pH 2, the fully protonated citrate species is predominant, and at pH 8, citrate^{3-} is predominant. From our studies of the proton-dependent citrate transport system, CitH, from *K. pneumoniae* (15), it was clear that only H-citrate^{2-} is transported. For the CitS protein, however, a more detailed study of the kinetics of citrate transport is necessary to determine the nature of the transported citrate species.

Kinetic analysis of citrate transport via CitS. The maximum rate of citrate uptake (V_{max}) did not vary significantly between pH 5.5 and 7.0, whereas the K_{app} of the transport system for total citrate increased with increasing pH values (Table 1). Calculation of the K_{app} at various pH values for the different citrate species showed that the K_{app} of H-citrate^{2-} was the least affected between the pH range of 5.5 and 7.0. The K_{app} values of H_3-citrate and H_2-citrate$^-$ were extremely low, while the K_{app} values of all citrate species

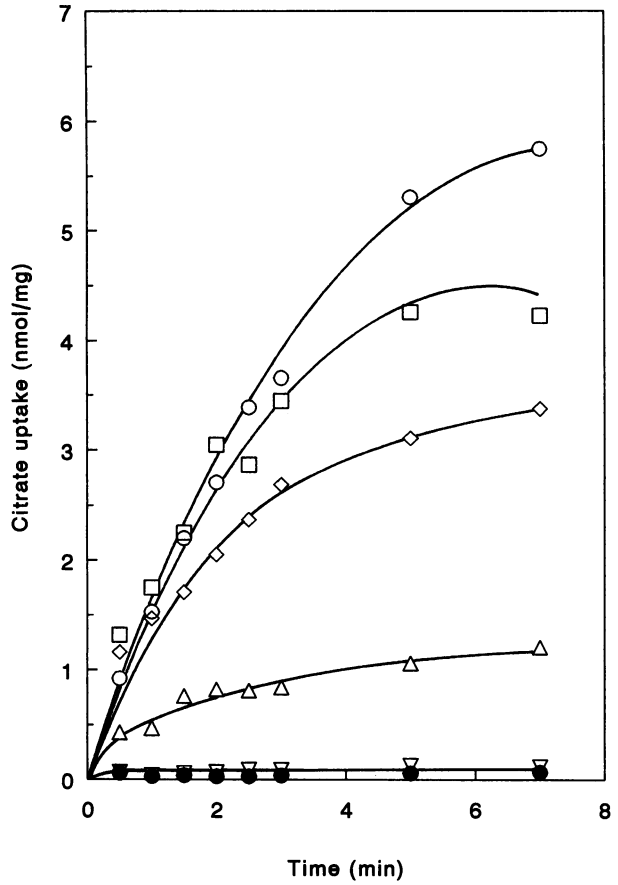

FIG. 1. Effect of external pH on citrate uptake in membrane vesicles of *E. coli* DH1/pRS63-12. Assays were conducted in 20 mM K phosphate, K-MES [2-(*N*-morpholino)ethanosulfonate], and K-PIPES buffer at 30°C. Membrane vesicles were energized by addition of 2 μM PQQ [2,7,9-tricarboxyl-1-*H*-pyrrolo-(2,3*f*)-quinoline-4,5-dione] and 20 mM glucose. Uptake experiments were started by addition of 4.5 μM [1,5-^{14}C]citrate at pH 5.5 (○), 6.0 (□), 6.5 (◇), 7.0 (△), 7.5 (▽), and 8.0 (●).

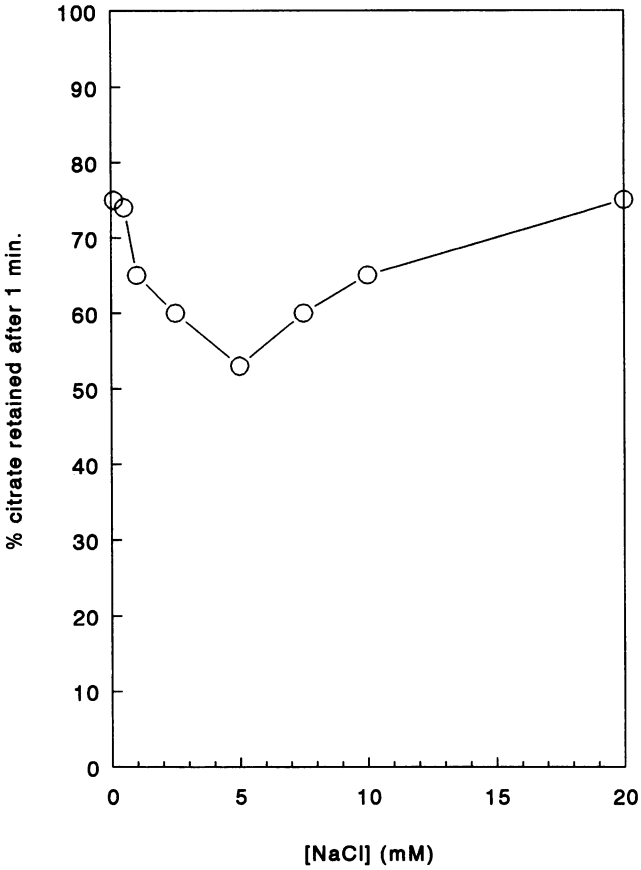

FIG. 2. Na+-dependent citrate efflux. Membrane vesicles were loaded with 5 mM [1,5-^{14}C]citrate. Efflux experiments were started by diluting the membrane vesicles 325-fold in K-PIPES (pH 6.0) at 20°C. NaCl was present, in equal concentrations, on both sides of the membrane at the concentrations indicated. The percentages of citrate retained in the membrane vesicles after 1 min are plotted as functions of the NaCl concentrations.

except H-citrate^{2-} varied drastically over this pH range. Furthermore, uptake of citrate decreased with increasing pH values (Fig. 1), suggesting that citrate^{3-} is not transported by the transport system. These observations indicate that H-citrate^{2-} is the only citrate species transported by CitS. The ΔpH, the ΔΨ, and the $\Delta\bar{\mu}_{Na^+}$ have been found to be driving forces for citrate uptake via CitS (17). This implies that the translocation is electrogenic and that at least three positive ions are translocated in symport with H-citrate^{2-}, of which at least one is a Na^+ ion.

Na^+ dependency of citrate efflux via CitS. To investigate the effect of Na^+ on citrate transport, without the interference of ion gradients, the efflux of citrate was studied in the presence of the ionophores valinomicin and nigericin and at different concentrations of Na^+. A concentrated suspension of membrane vesicles was equilibrated with 5 mM [1,5-^{14}C]citrate, a concentration considerably higher than the K_{app} for transport (see above), and different concentrations of NaCl. This membrane vesicle suspension was diluted in buffer containing the same concentration of NaCl, so that the concentrations of Na^+ on both sides of the membrane were equal. The rate of efflux of citrate increased with increases in the NaCl concentrations of up to 5 mM. Concentrations of NaCl above 5 mM resulted in an inhibition of citrate efflux (Fig. 2). In the absence of NaCl, 25% of the internal citrate was released from the membrane vesicles in 1 min (Fig. 2).

TABLE 1. Effect of external pH on kinetic parameters of citrate transport via CitS

pH	V_{max} (nmol · min^{-1} · mg^{-1})	K_{app} (nM)				
		$Citrate_{total}$	H_3-citrate	H_2-citrate$^-$	H-citrate^{2-}	$Citrate^{3-}$
5.5	8.2	7,200	2	530	2,980	3,700
6.0	8.3	8,500	0.1	100	1,710	6,700
6.5	8.2	12,700	0.007	17	950	11,600
7.0	6.6	30,600	0.0005	4	760	29,800

Na^+ dependency of citrate counterflow. For Na^+-dependent citrate counterflow, membrane vesicles were loaded with 5 mM citrate and different concentrations of NaCl. An aliquot of concentrated membrane vesicles was then diluted in buffer containing 4.5 μM [1,5-^{14}C]citrate and the same concentration of NaCl, so that the concentrations of Na^+ were the same on both sides on the membrane. In the absence of Na^+, counterflow occurs, but at a low rate (Fig. 3). Increasing the NaCl concentration on both sides of the

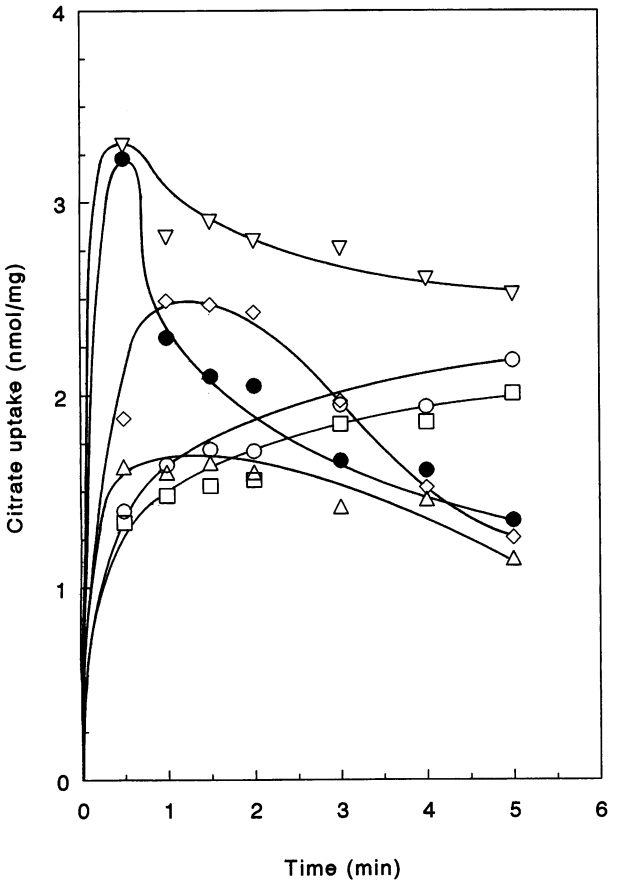

FIG. 3. Na^+-dependent citrate counterflow. The membrane vesicles were loaded with 5 mM citrate. Counterflow experiments were started by diluting the membrane vesicles 325-fold in K-PIPES (pH 6.0), at 20°C, containing 4.5 μM [1,5-^{14}C]citrate. NaCl is present on both sides of the membrane at the following concentrations (millimolar); 0 (□), 0.1 (○), 1 (△), 2.5 (◇), 5 (●), and 7.5 (▽).

membrane results in an increase of the initial rate of counterflow and at the same time in an increase of efflux activity. The initial stage of [1,5-^{14}C]citrate uptake is, after equilibration of the label, followed by efflux of the label. As in the experiment described above, this efflux of label is first stimulated and then inhibited by increases in the concentrations of NaCl on both sides of the membrane.

Na^+ dependency of citrate exchange. During exchange of citrate, essentially the same steps in the transport cycle are studied as during counterflow, except that the concentrations of both citrate and Na^+ are the same on both sides of the membrane. The rates of efflux and influx are equal, but since the radioactive label is present only at the inside of the membrane, only efflux can be measured. This efflux is not obstructed by counteracting ion or substrate gradients; therefore, the initial rates of citrate exchange in the presence of different concentrations of NaCl can be used to determine a K_{app} of CitS for Na^+. Exchange of citrate was found to be a first-order process during the first 30 s. An Eadie Hofstee plot was used to determine the K_{app} of CitS for Na^+ (between 0.1 and 20 mM) on both sides of the membrane (Fig. 4). The slope of the line indicates that CitS has a K_{app} for Na^+ of 200 μM.

Na^+/citrate stoichiometry during cotransport. The rate of Na^+ transport in the presence and absence of citrate was measured by loading the membrane vesicles with 5 mM ^{22}NaCl with or without 5 mM citrate. An aliquot of concentrated membrane vesicles was diluted in buffer, and the rate of ^{22}NaCl loss from the membrane vesicles was measured. The rate of ^{22}NaCl efflux was found to be twice as fast in the presence of citrate as in the absence of citrate (Fig. 5A), and the difference between the two rates was taken to be the rate of CitS-mediated Na^+-citrate cotransport. In an identical experiment, membrane vesicles were loaded with 5 mM [1,5-^{14}C]citrate in the presence and absence of 5 mM NaCl. An aliquot of concentrated membrane vesicles was diluted in buffer. The rate of sodium-stimulated [1,5-^{14}C]citrate efflux was also found to be twice as fast as the rate of efflux in the

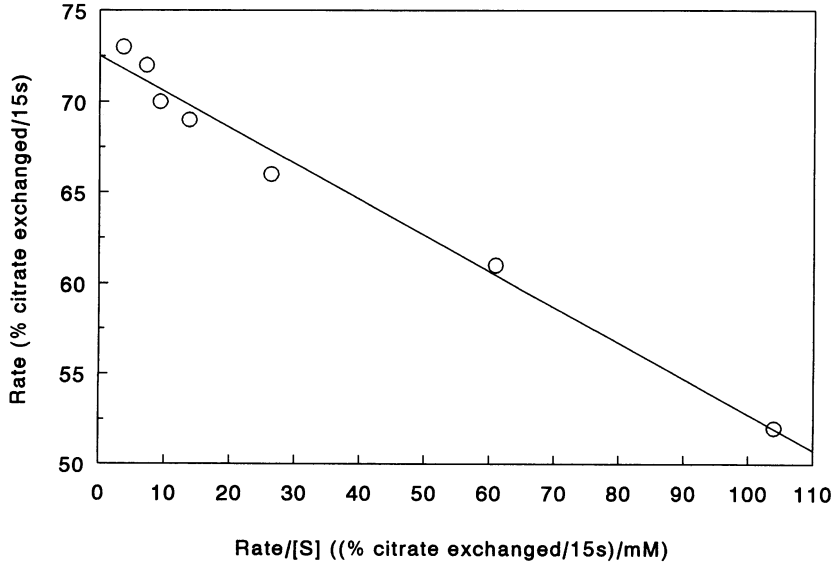

FIG. 4. K_{app} of CitS for Na^+. The rate of citrate exchange was measured as a function of the Na^+ concentration at 20°C. The Na^+ concentration was varied between 0.1 and 20 mM. Membrane vesicles were loaded with 5 mM [1,5-^{14}C]citrate. Exchange experiments were started by diluting the membrane vesicles 325-fold in K-PIPES (pH 6.0) with 5 mM citrate. The rate of initial exchange is plotted with an Eadie Hofstee plot.

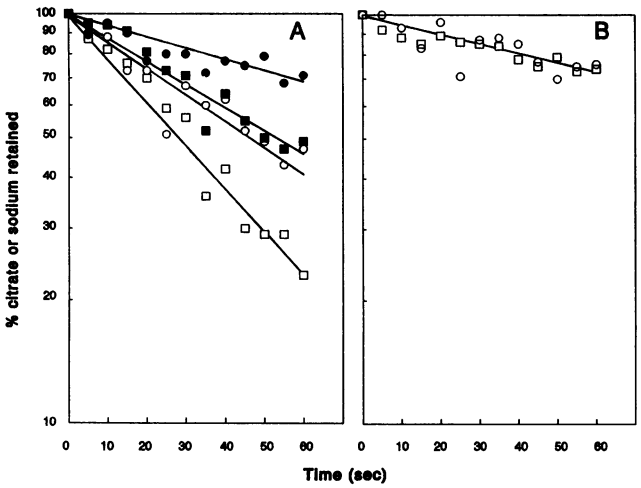

FIG. 5. Citrate- and/or Na^+-mediated efflux via CitS. Membrane vesicles were loaded with 5 mM [1,5-^{14}C]citrate in the presence or absence of 5 mM NaCl or with 5 mM ^{22}NaCl in the presence or absence of 5 mM citrate. The membrane vesicles were diluted 400-fold in K-PIPES (pH 6.0) at 20°C. (A) [1,5-^{14}C]citrate efflux (●), Na^+-stimulated [1,5-^{14}C]citrate efflux (○), ^{22}NaCl efflux (■), and citrate-stimulated ^{22}NaCl efflux (□); (B) CitS-mediated, Na^+-stimulated [1,5-^{14}C]citrate efflux (○) and CitS-mediated, citrate-stimulated ^{22}NaCl efflux (□).

absence of Na^+ (Fig. 5A). The difference between the two rates was taken to represent the rate of CitS-mediated Na^+-citrate cotransport. The carrier-mediated, sodium-stimulated citrate efflux and carrier-mediated, citrate-stimulated Na^+ efflux rates are compared in Fig. 5B. These results clearly suggest that the stoichiometry of Na^+/citrate transport is 1, indicating that only one Na^+ ion is transported in symport with one citrate ion.

DISCUSSION

In *K. pneumoniae*, the anaerobic fermentation of citrate is induced by citrate and involves two key reactions: (i) the citrate lyase reaction cleaving citrate into oxaloacetate and acetate and (ii) the oxaloacetate decarboxylase reaction catalyzing the decarboxylation of oxaloacetate to pyruvate and CO_2. The oxaloacetate decarboxylase was found by Dimroth (2) to be a biotin-containing membrane protein consisting of three subunits and acting as an Na^+ pump. The Na^+ gradient thus generated could be involved in citrate uptake via a Na^+-citrate symport mechanism. It has already been firmly established that Na^+ is cotransported with citrate via CitS, but recently it was shown that protons are also cotransported with citrate via CitS (17). Therefore, a detailed analysis of Na^+-H^+-citrate cotransport is of special interest to establish the bioenergetic links in the citrate fermentation pathway of *K. pneumoniae*. The uptake of citrate decreased dramatically with an increase in the pH of 5.5 to 8.0 (Fig. 1). The K_{app} of the transport system was found to be essentially constant for H-citrate^{2-}, in the range of 3 to 0.8 μM at different pH values, indicating that this species is recognized and transported by CitS. This resembles the uptake of citrate via CitH of *K. pneumoniae*, which also recognizes and transports H-citrate^{2-} (15). In a previous study, it was found that uptake of citrate was mainly driven by the ΔpH and also, although to a lesser extent, by the ΔΨ and the $\Delta\bar{\mu}_{Na^+}$ (17). This indicates that H-citrate^{2-} is transported with at least three positive ions.

To study the uptake of citrate via CitS in more detail, the rates of efflux, exchange, and counterflow of citrate were determined. In Fig. 6, a simplified model is given of translocation of citrate and Na^+ via CitS, which most simply explains the results obtained. This symmetric model assumes a sequential binding and release of the cosubstrates to and from the carrier to the inner or outer surface of the membrane. Protons are not discussed in this model because no extensive experiments were performed to establish the effect of protons on the transport of citrate. The experiments were performed with equal concentrations of NaCl on both sides of the membrane and in the presence of the ionophores valinomycin and nigericin. In this way, the Na^+-dependent citrate uptake could be studied without the imposition of ion gradients. In the efflux process of citrate, at least four steps can be distinguished: (i) binding of citrate and cotransported ions (Na^+ and/or H^+) to the carrier on the inner surface of the membrane (order unspecified), (ii) reorientation of the

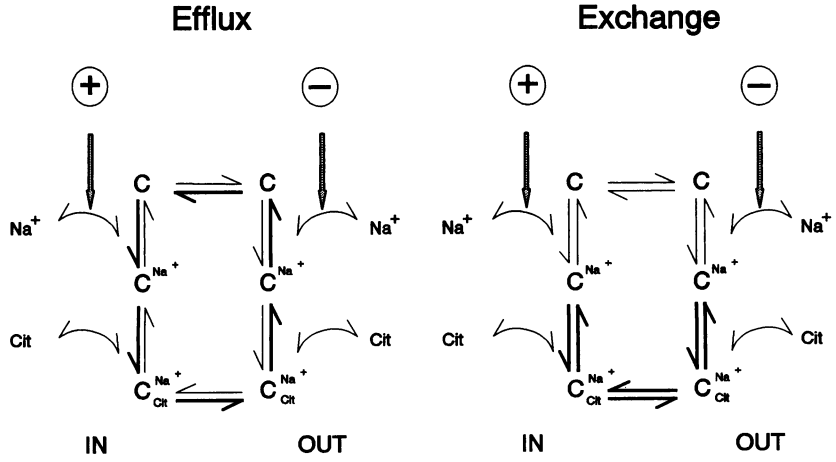

FIG. 6. Schematic representation of reactions involved in citrate exchange and efflux. C, CitS; Cit, the substrate (citrate); ⊕, the stimulation of binding of Na^+ to the carrier, on the inside of the membrane, by high Na^+ concentrations; ⊖, the inhibition of Na^+ release from the carrier, on the outside of the membrane, by high Na^+ concentrations; boldface arrows, the reactions involved in the efflux or exchange reaction; in and out, the inside and the outside of the membrane, respectively.

citrate-Na^+-H^+ carrier complex across the membrane, (iii) release of citrate and cotransported ions from the carrier on the outside of the membrane, and (iv) reorientation of the free carrier to the inner surface of the membrane. Counterflow and exchange of citrate can be described as consisting of at least six different steps in the transport cycle: (i) binding of citrate and cotransported ions (Na^+ and/or H^+) to the carrier on the inner surface of the membrane, (ii) reorientation of the citrate-Na^+-H^+ carrier complex across the membrane, (iii) release of citrate and possibly Na^+ or H^+ from the carrier on the outside of the membrane, (iv) rebinding of [1,5-^{14}C]citrate to the carrier, (v) reorientation of the [1,5-^{14}C]citrate-Na^+-H^+ carrier complex to the inner surface of the membrane, and (vi) release of [1,5-^{14}C]citrate on the inner surface of the membrane.

During efflux, an increase in the Na^+ concentration on the inside of the membrane results in an increase in the Na^+-citrate-carrier complex, thereby stimulating the reorientation of the complex to the outside. On the outside, high Na^+ concentrations prevent the release of Na^+ from the Na^+-carrier complex. This prevents the unloaded carrier from returning to the inside of the membrane, thus inhibiting the efflux process. The efflux observed in the absence of Na^+ (Fig. 2) could be due to small amounts of Na^+ in the reaction medium. The efflux experiments do not supply information about the sequence of release of Na^+ and citrate from the carrier on the outside of the membrane. From the exchange experiments, however, we learn that exchange is not inhibited by high concentrations of NaCl on the outside, indicating that citrate is first released on the outside, after which the Na^+ ion is released. The counterflow experiments can be explained by a very fast equilibration of the radioactive label flowing from the outside to the inside. On the inside, labeled citrate is diluted by the large concentration of nonlabeled citrate. Subsequent efflux of label has the same properties as in normal efflux experiments. The net rate of efflux is always higher than the net rate of influx, since the citrate gradient is directed outward. The counterflow data are consistent with the model proposed. The data presented in Fig. 5 indicate that the Na^+/citrate stoichiometry is 1. To obtain electrogenic transport of citrate, more than one proton has to be translocated. This corresponds with the observation that the ΔpH is more effective as a driving force than the $\Delta\bar{\mu}_{Na^+}$ (17). We have shown that H-citrate^{2-} is translocated via CitS. Citrate transport via CitS, therefore, occurs via a unique system that transports citrate in symport with one Na^+ ion and two protons.

During anaerobic growth of *K. pneumoniae* on citrate, the oxaloacetate decarboxylase exports 2 Na^+ ions per citrate molecule out of the cell (2). The generated Na^+ gradient is not sufficient to drive uphill transport of H-citrate^{2-} into the cell. Carrier-mediated excretion of metabolic end products (11) can additionally provide the pH gradient necessary for citrate transport. Fermentation of citrate yields stoichiometric amounts of acetate, formate, and bicarbonate, which, if exported from the cell in symport with protons, will generate a pH gradient. In this way, the metabolic energy obtained from citrate metabolism can fully be used for biosynthesis and cell growth.

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