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Succinate Uptake and Related Proton Movements in *Escherichia coli* K12

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1. The apparent K_m values for succinate uptake by whole cells of *Escherichia coli* K12 depend on pH in the range 6.5–7.4. 2. Uptake of succinate in lightly buffered medium is accompanied by proton uptake. 3. The apparent K_m values for succinate uptake and for succinate-induced proton uptake are similar. 4. Approximately two protons enter the cell with each succinate molecule. 5. The pattern of inhibition of succinate uptake is similar to that of succinate-induced proton uptake. 6. Uptake of fumarate and malate, which share the succinate-transport system, is also accompanied by the uptake of approximately two protons per molecule of fumarate or malate. 7. Uptake of aspartate by the dicarboxylic acid-transport system is accompanied by the uptake of approximately two protons per molecule of aspartate. 8. It is concluded that uptake of dicarboxylic acids by the dicarboxylic acid-transport system is obligatorily coupled to proton uptake such that succinate, malate and fumarate are taken up in electroneutral form and aspartate is taken up in cationic form. 9. These results are consistent with, though they do not definitely prove, the energization of succinate uptake by the ΔpH.

The active transport of a substance into a cell against an electrochemical potential gradient is an endergonic process requiring coupling to an exergonic reaction. The nature of this coupling is in dispute. The chemiosmotic hypothesis (Mitchell, 1961) as applied to active transport in bacteria, suggests that a protonmotive force consisting of a pH gradient ($Z\Delta pH$) and a membrane potential ($\Delta\Psi$) may energize active transport of various substances in several ways. Cationic substances may accumulate in response to $\Delta\Psi$ interior negative. Neutral and anionic substances may be symported with protons and thus accumulate in response to the protonmotive force and ΔpH respectively (Mitchell, 1967; Harold, 1974).

A considerable number of experimental observations have been made which are compatible with these suggestions. For example it has been shown that in *Staphylococcus aureus* basic, neutral and acidic amino acids accumulate in response to $\Delta\Psi$, protonmotive force and ΔpH respectively (Niven & Hamilton, 1974) and that in *Streptococcus faecalis* thiomethyl galactoside is accumulated in response to protonmotive force (Kashket & Wilson, 1973). In *Escherichia coli* lactose uptake is accompanied by proton uptake (West & Mitchell, 1973) and gluconate uptake is accompanied by alkalinization of the medium (Robin & Kepes, 1973). In eukaryotes hexose uptake and hexose-induced proton uptake in *Chlorella vulgaris* have similar properties (Komor & Tanner, 1974).

Escherichia coli K12 has an inducible transport system for succinate and other dicarboxylic acids which is saturable, specific and capable of concentrative uptake (Lo *et al.*, 1972).

When aerobic succinate-grown cells of *E. coli* are given small amounts of glucose, rapid proton extrusion occurs, followed by a slower rise in pH. We observed that addition of succinate during this phase of rising pH increased the rate of proton uptake. The present study was undertaken to characterize the succinate-induced proton uptake and relate it to succinate transport.

Materials and Methods

Materials

E. coli K12 strain AN259 (*ArgH*$^-$, *entA*$^-$), used in all the experiments reported here, was described elsewhere (Butlin *et al.*, 1973) and was provided by Dr. G. B. Cox. [2,3-^{14}C]Succinic acid (5mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and was diluted appropriately with carrier potassium succinate for use. Carbonyl cyanide *m*-chlorophenylhydrazone was obtained from Calbiochem, Carlingford, N.S.W., Australia. All other chemicals used were of reagent grade.

Nitrogen was passed before use through two scrubbing towers containing 5% (w/v) pyrogallol in 14M-KOH, and then through water. Air was passed

through 0.5M-KOH, 0.25M-H_2SO_4 and water, in that order.

Growth of cells

Cells were grown at pH 7.0 in half-strength medium 56 (Monod *et al.*, 1951), supplemented with 2mM-L-arginine, 10μM-2,3-dihydroxybenzoic acid, 0.2μM-thiamin and the appropriate carbon source at growth-limiting concentrations (5mM-glucose or 16mM-succinate) so as to arrest growth at the late-exponential phase. All supplements were autoclaved separately and added as sterile solutions. Cells were grown at 37°C overnight aerobically in 250ml of medium in 1-litre flasks shaken at 300rev./min in an NBS gyrotory shaker.

Measurement of succinate uptake in phosphate buffer

The medium used for measurement of succinate uptake (uptake medium) was the same as growth medium but contained 20mM-glucose as carbon source and was adjusted to the required pH with KOH. Cells were harvested by centrifugation, washed twice with uptake medium, resuspended in uptake medium to an E_{660} of 0.3–0.5, then stored on ice until required.

The cell suspension (2.5ml) was shaken at 300rev./min at 37°C in a New Brunswick Metabolyte water bath for 6min in 25ml Pyrex flasks. Uptake was initiated by adding [^{14}C]succinate to the required concentration. Samples of the cell suspension (0.5ml) were withdrawn at the appropriate times after the start of uptake, and were filtered through Gelman Metricel GA6 filters (pore size 0.45μm). The filters were then washed, dried, dissolved in scintillant, and counted for radioactivity in a Packard liquid-scintillation spectrometer as described elsewhere (Rosenberg *et al.*, 1975).

Standard counts for radioactivity were carried out each day on the stock solutions. Control filtrations (without cells) were performed daily to correct for background radiation and non-specific adsorption of radioactive material to the filters. Such non-specific adsorption was small.

Corrected values of counts of cellular radioactivity are expressed as nmol of dicarboxylic acid taken up/mg dry wt. of bacterial mass. Cell densities were measured as described elsewhere (Rosenberg *et al.*, 1975).

Measurement of succinate uptake and succinate-induced proton uptake in lightly buffered medium

Cells were harvested by centrifugation, washed twice with 100mM-KCl–2mM-glycylglycine, pH 7.0 (West & Mitchell, 1973) (lightly buffered medium), resuspended in the same medium at an E_{660} of 1–1.5 (0.45–0.6mg dry wt./ml) and stored on ice until required.

The cell suspension (2.5ml) was incubated at 37°C in a water-jacketed capped 5ml-capacity glass cell containing a Philips C14/20 glass electrode connected to a Rikadenki TCO 2001 recorder via a Radiometer pH26 pH-meter. The cell suspension was stirred by a stream of moist prewarmed air or nitrogen for aerobic and anaerobic incubations respectively. KSCN (50mM) and specific growth supplements in the same concentrations as used in growth were added to the cell suspension at the start of incubation. In measurements of anaerobic proton movement and succinate uptake, 5mM-KCN was also added at the start of incubation.

For measurement of the kinetic parameters of succinate uptake and succinate-induced proton uptake, 2.5ml of cell suspension was equilibrated at the required pH by the addition of small amounts of KOH or HCl. Addition of glucose at this stage caused a reproducible fall in external pH followed by a gradual pH increase as protons return into the cells (Fig. 2). When the pH rose to a chosen value, the potassium salt of the dicarboxylic acid or aspartate was added and the induced proton movements were recorded. (Dicarboxylate and aspartate caused no detectable pH change when added to lightly buffered, cell-free medium at the pH values used.) Samples (0.2ml) were withdrawn and filtered as described above. Experimental conditions, with respect to initial pH (before glucose addition), cell density and the amount of glucose added, were chosen such that the desired pH was obtained just after the peak of proton extrusion and such that succinate uptake was linear with time over the first 30s of uptake. These precise conditions are given in the legends to the Tables and Figures.

Cell densities were measured as described above after a fivefold dilution with buffer. pH changes were converted into nmol of H^+ by using an experimentally determined conversion factor derived from the addition of known amounts of HCl to the cell suspension.

Extraction and chromatography of 'pool' material

For identification of 'pool' materials, 10ml of cell suspension in uptake medium was incubated for 1min with 20μM-potassium [^{14}C]succinate, filtered and washed as described above. The filters were then treated as described by Kay & Kornberg (1971). Tricarboxylic acid-cycle intermediates were identified by t.l.c. (Myers & Huang, 1966).

Radioactive components were eluted with scintillant and counted for radioactivity in a Packard liquid-scintillation spectrometer. Appropriate standards were used to determine quenching.

Measurement of oxygen uptake

Succinate-grown cells were prepared in lightly buffered medium as described above. Cells (2.5ml) were incubated at 30°C and oxygen uptake was

measured polarographically by using a Titron oxygen electrode. Additions were made as shown in Fig. 4.

Results

pH dependence of succinate uptake

The variation with pH of the kinetic parameters of succinate uptake by *E. coli* is shown in Table 1. In each experiment at least two readings were taken for each point and a computer program for linear regression of reciprocals was used to obtain the best fit of the experimental data to a double-reciprocal plot.

K_m and $V_{max.}$ values from two separate experiments are shown together with standard errors. The apparent K_m for succinate transport depends on the pH of the medium in which the cells are suspended, and the $V_{max.}$ is constant over the pH range tested.

Energy dependence of succinate uptake

Succinate uptake during the first 30s was shown to be markedly enhanced by the addition of D-glucose (Fig. 1).

Succinate-induced proton entry into the cells

The addition of 50nmol of D-glucose to aerobic cells caused a rapid fall in pH, corresponding to the release of about 100nmol of H^+. This was followed by a slower increase in pH. Additions of potassium salts of aspartate and dicarboxylic acids were made at this stage at the appropriate pH and were found to cause marked acceleration of proton uptake (Fig. 2).

With anaerobic cells, the rate of acidification of the medium on addition of glucose was much slower than with aerobic cells, and no subsequent rise of pH was observed. At that stage succinate which had been bubbled with nitrogen for 15min was added to the appropriate concentration.

Table 1. *K_m and $V_{max.}$ values for succinate uptake in phosphate-buffered medium at various pH values*

Cells were grown on succinate. Initial rates of aerobic succinate uptake were measured in uptake medium as described in the Materials and Methods section and used in double-reciprocal plots to give the K_m and $V_{max.}$ values shown.

pH	K_m for succinate uptake (μM)	$V_{max.}$ for succinate uptake (nmol/min per mg dry wt.)
6.5	12.1±1.4	56±10
	11.1±0.6	56±6
6.8	12.1±0.7	50±5
	12.7±0.9	51±6
7.1	15.4±0.6	55±5
	15.7±0.9	52±6
7.4	19.5±1.6	51±12
	20.0±0.2	56±0

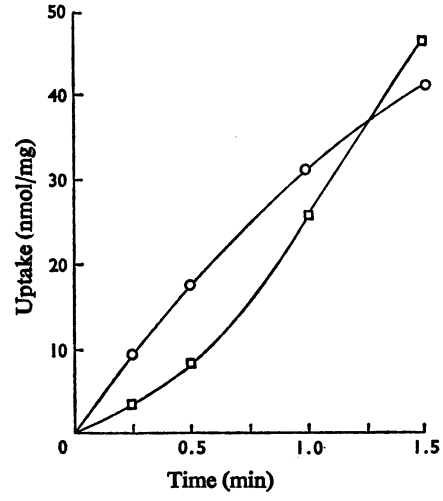

Fig. 1. *Dependence of succinate uptake in phosphate-buffered medium on added energy source*

Cells were grown on succinate. Initial rates of aerobic succinate uptake were measured in uptake medium as described in the Materials and Methods section. Cells at pH7.0 and E_{660} 0.3 were incubated with nothing (□) or 20mM-D-glucose (○) for 6min before uptake was initiated by the addition of 20μM-[^{14}C]succinate.

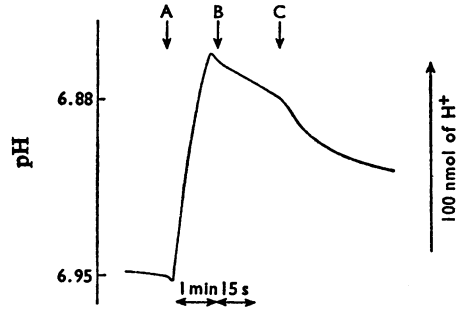

Fig. 2. *Succinate-induced proton uptake in lightly buffered medium*

Cells were grown on succinate and assayed aerobically in lightly buffered medium as described in the Materials and Methods section. Glucose and succinate were added to 20μM final concentration at the times and pH values shown. A, Glucose addition; B, change of time-scale; C, succinate addition.

To determine the relationship between proton uptake induced by dicarboxylic acids and aspartate, and the uptake of these acids, we determined the kinetic parameters of succinate-induced proton movement and compared them with those for

succinate uptake by the same cells. A double-reciprocal plot of one such determination is shown in Fig. 3.

Apparent K_m and $V_{max.}$ values from all determinations are shown in Table 2, and the apparent K_m values for succinate-induced proton uptake and for succinate uptake are similar (Table 2 and Fig. 3).

The K_m values found in the present study for both succinate uptake and succinate-induced proton uptake are in good agreement with previously reported values for succinate uptake in whole cells of *E. coli* (Murakawa *et al.*, 1972) and in membrane vesicles of *E. coli* (Rayman *et al.*, 1972).

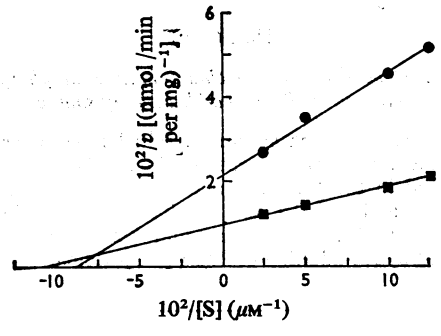

Fig. 3. *K_m and $V_{max.}$ determination of succinate uptake and succinate-induced proton uptake in lightly buffered medium*

Cells were grown on succinate, and were prepared and assayed in lightly buffered medium as described in the Materials and Methods section. Cells at an E_{660} of 1.5 were equilibrated at pH6.95 and 50nmol of D-glucose was added. Additions of succinate were made at pH6.88 during the phase of alkalinization of the medium. Initial rates of succinate uptake (●) and succinate-induced proton uptake (■) were calculated and used in a double-reciprocal plot as shown.

Stoicheiometry of succinate-induced proton uptake

The number of protons taken up per succinate molecule, obtained by a comparison of the relevant $V_{max.}$ values (Table 2), varies between 1.8 and 2.2.

Effect of inhibitors

The pattern of inhibition of succinate uptake and of succinate-induced proton uptake is similar (Table 3).

Effect of uncoupler and of non-radioactive succinate on preloaded cells

Carbonyl cyanide *m*-chlorophenylhydrazone (40μM) added 60s after the addition of succinate to succinate-grown cells suspended in uptake medium caused efflux of approx. 40% of the accumulated radioactive material within 15s. Addition of unlabelled succinate (0.5mM) caused a similar efflux, though at a rate slightly slower than with the uncoupler (Fig. 4). This failure to cause complete efflux is due to the occurrence of considerable metabolism of succinate during the experiment (see below).

Effect of uncouplers on succinate metabolism

Addition of succinate to succinate-grown cells in lightly buffered medium stimulated the rate of oxygen consumption after a slight lag. Addition of carbonyl cyanide *m*-chlorophenylhydrazone (20μM) after succinate addition did not inhibit the rate of oxygen consumption (Fig. 5*a*). Addition of the uncoupler before succinate, however, abolished the succinate stimulation of oxygen consumption (Fig. 5*b*).

Specificity of the succinate-transport system

Fumarate and malate share the succinate-transport system (Kay & Kornberg, 1971).

In succinate-grown cells, fumarate and malate inhibited succinate uptake (Table 4) and induced proton uptake, in the ratio of approximately two protons taken up per molecule of fumarate or malate (Table 5).

Table 2. *K_m and $V_{max.}$ determinations of succinate uptake and succinate-induced proton uptake in lightly buffered medium*

Cells were grown on succinate. Initial rates of uptake were measured in lightly buffered medium as described in the Materials and Methods section and used in double-reciprocal plots to give the K_m and $V_{max.}$ values shown. For experiments at pH6.64, cells at an E_{660} of 1.0 were equilibrated at pH6.72, and 33nmol of D-glucose was added. For experiments at pH6.88, cells at an E_{660} of 1.5 were equilibrated at pH6.95, and 50nmol of D-glucose was added.

pH	K_m for succinate uptake (μM)	$V_{max.}$ for succinate uptake (nmol/min per mg dry wt.)	K_m for succinate-induced proton uptake (μM)	$V_{max.}$ for succinate-induced proton uptake (nmol/min per mg dry wt.)	H^+/succinate ($V_{max.}$ ratio)
6.88	11.4±2.0	46±3	9.3±0.6	100±8	2.2
	13.0±2.0	50±7	12.0±1.0	100±10	2.0
	12.7±0.7	46±3	10.8±0.3	83±7	1.8
6.64	8.3±1.4	50±6	8.0±0.1	100±0	2.0
	9.8±1.3	52±6	8.9±0.3	110±11	2.1

Table 3. *Effects of metabolic inhibitors and anaerobiosis on succinate uptake and succinate-induced proton uptake in lightly buffered medium*

Cells were preincubated with inhibitors for 5min in lightly buffered medium at 37°C and assayed as described in the Materials and Methods section. Cells at an E_{660} of 1.5 were equilibrated at pH6.95 and 50nmol of D-glucose was added. Succinate was added at pH6.88. Data for succinate uptake are initial rates of succinate uptake caused by the addition of 40μM-[^{14}C]succinate, expressed as nmol of succinate taken up/min per mg of dry wt. of bacterial mass. Data for succinate-induced proton uptake are initial rates of proton uptake caused by the addition of 40μM-succinate, expressed as nmol of H^+ taken up/min per mg dry wt. of bacterial mass. Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol.

Addition	Concentration (μM)	Succinate uptake	Inhibition (%)	Succinate-induced proton uptake	Inhibition (%)
None	—	48	—	100	—
CCCP	0.65	27.2	43	64.5	35.5
	2	6.0	87	18.6	81.4
	8	0.4	100	0	100
DNP	40	30.8	35	71.0	29.0
	100	20.0	59	39.2	60.8
$AgNO_3$	60	0	100	0	100
Anaerobiosis	—	0.8	98	0	100

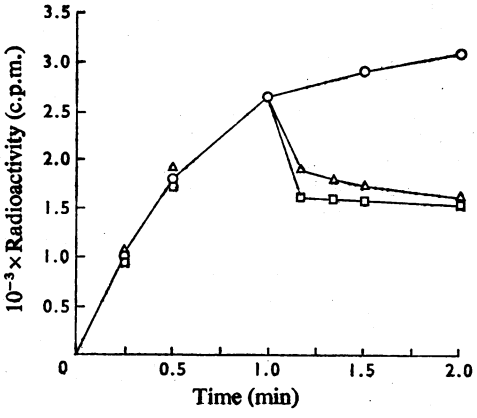

Fig. 4. *Effect of uncoupler and of non-radioactive succinate on pre-loaded cells in phosphate-buffered medium*

Cells were grown on succinate and assayed aerobically in uptake medium as described in the Materials and Methods section. Cells at pH7.0 and E_{660} 0.3 were incubated for 6min with 20mM-D-glucose before uptake was initiated by the addition of 20μM-[^{14}C]succinate. Carbonyl cyanide *m*-chlorophenylhydrazone (40μM; □), [^{12}C]-succinate (0.5mM; △), or nothing (○) was added 60s after the initiation of uptake.

High concentrations of aspartate also inhibit succinate uptake (Table 4) and induce proton uptake. However, in succinate-grown cells the stoicheiometry of proton uptake is found to depend on the concentration of aspartate (Table 5). It has been shown that aspartate is taken up by two different systems in *E. coli* K12. One system is constitutive, specific for aspartate uptake and has a high affinity and low $V_{max.}$. The second system is inducible, shared with dicarboxylic acids and has a low affinity and high $V_{max.}$ for aspartate uptake (Kay, 1971). The results in Table 5 with glucose-grown cells show that in these cells aspartate is taken up well but the other dicarboxylic acids are not, suggesting that in glucose-grown cells expression of the dicarboxylate-transport system is repressed. This is in accordance with previous observations (Kay & Kornberg, 1971). Values for aspartate uptake in glucose-grown cells have been subtracted from those for aspartate uptake in succinate-grown cells to give a measure of aspartate uptake by the dicarboxylate-transport system in succinate-grown cells. This corrected value for aspartate uptake has been used to calculate a corrected stoicheiometry of protons taken up per aspartate molecule. The corrected stoicheiometry is found to be approximately two.

None of the carboxylic acids tested had any detectable effect on proton uptake in glucose-grown cells.

Identification of 'pool' materials

T.l.c. in two solvent systems showed that after 1min of succinate uptake approx. 54% of the radioactive 'pool' material was aspartate, approx. 33% was other amino acids, and approx. 2% was succinate.

Discussion

The results presented here highlight several properties of succinate uptake and their relationship to induced proton movements. First, the apparent K_m

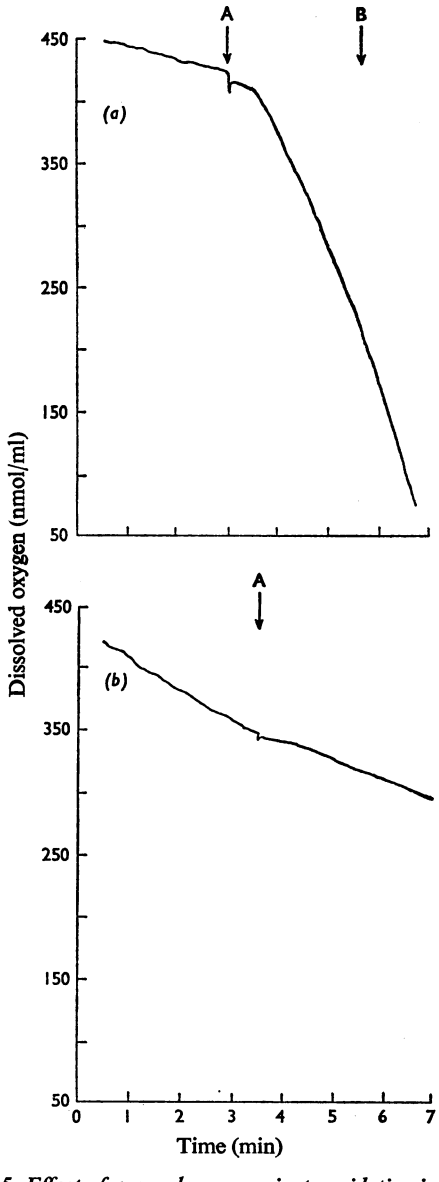

Fig. 5. *Effect of uncoupler on succinate oxidation in lightly buffered medium*

Succinate-grown cells (2.5ml) in lightly buffered medium were incubated for 2min at 30°C either in the absence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (Fig. 5*a*), or in the presence of 10μM-uncoupler (Fig. 5*b*) in an oxygen-electrode cell. Additions were made as shown. Fig. 5(*a*): A, succinate (0.5mM); B, uncoupler (20μM). Fig. 5(*b*): A, succinate (0.5mM).

of succinate transport was found to depend on the pH of the external medium in the range pH6.5–7.4. Addition of 20mM-KCl had no effect on uptake (result not shown). Such dependence on pH might arise from the co-transport of succinate with one or more protons if the transport system is not saturated with respect to protons in the pH range tested. Secondly, in lightly buffered medium, addition of succinate prompted proton uptake that displayed an apparent K_m similar to that for succinate uptake. The effects of metabolic inhibitors and anaerobiosis on succinate uptake and on succinate-induced proton uptake were similar. Our results suggest that two protons are taken up per succinate molecule.

Table 4. *Effect of fumarate, malate and aspartate on succinate uptake in phosphate-buffered medium*

Cells were grown on succinate, and were prepared and assayed aerobically in uptake medium as described in the Materials and Methods section after 5min preincubation with the compounds listed below. Uptake was initiated by the addition of [^{14}C]succinate to a final concentration of 20μM.

Addition	Concentration (mM)	Succinate uptake (nmol/min per mg)	Inhibition (%)
None	—	39.1	—
Fumarate	0.5	2.2	94
Malate	5	0.6	98
Aspartate	20	11.3	71

In succinate-grown cells, fumarate and malate, which share the succinate-transport system, also induce proton uptake in the ratio of two protons per carboxylic acid molecule.

In glucose-grown cells, where the dicarboxylate-transport system is not expressed, neither fumarate nor malate nor succinate is taken up, and none of the three acids induce proton uptake.

Uptake of aspartate is more complex. It is taken up by glucose-grown cells where this uptake is not accompanied by proton uptake. In succinate-grown cells aspartate uptake is faster than in glucose-grown cells and here it is accompanied by proton uptake. However, in succinate-grown cells, the number of protons taken up per aspartate molecule increases as the concentration of aspartate increases (Table 5). These findings may be explained by the presence of two uptake systems for aspartate in *E. coli* K12, namely a high-affinity, low-$V_{max.}$ constitutive system specific for aspartate, and an inducible system of low affinity shared with dicarboxylic acids (Kay, 1971).

Aspartate does not induce proton uptake in glucose-grown cells where only the constitutive system for aspartate uptake is present, suggesting that uptake of aspartate by this system is not accompanied by proton uptake. Both systems operate in succinate-grown cells, and increasing concentrations of aspartate would raise the flux through the low-affinity inducible system, where protons are taken up with the

Table 5. *Effect of fumarate, malate and aspartate on proton uptake in lightly buffered medium*

Cells were grown on succinate or glucose, and were prepared and assayed aerobically in lightly buffered medium as described in the Materials and Methods section. Cells at an E_{660} of 1.5 were equilibrated at pH 6.95, then 50 nmol of D-glucose was added and additions of the test substances were made at pH 6.88. Abbreviation: ND, not detectable. Results are expressed as nmol/min per mg dry wt.

				Succinate-grown cells			
	Final concentration	Glucose-grown cells				H^+/dicarboxylate molecule	
Addition	(μM)	Induced proton uptake	Uptake	Induced proton uptake	Uptake	Uncorrected	Corrected
Succinate	20	ND	2.4	59	30.8	1.9	—
Malate	10	—	—	18	9.2	2.0	—
	20	—	—	35	15.2	2.3	—
	40	ND	2.0	42	20.3	2.1	—
Fumarate	4	—	—	22	12.5	1.8	—
	10	—	—	44	25.4	1.7	—
	20	—	—	52	32.0	1.6	—
	100	ND	2.4	70	37.6	1.9	—
Aspartate	500	ND	7.6	20	17.0	1.2	2.1
	1250	ND	10.0	33	26.4	1.3	2.0
	2500	ND	11.0	42	29.2	1.4	2.3

aspartate. This would be observed as an increasing ratio (approaching two) of protons to aspartate taken up. Assuming that the constitutive system for aspartate operates equally in glucose-grown cells and in succinate-grown cells, a corrected value for aspartate uptake in succinate-grown cells by the inducible dicarboxylate-transport system may be obtained, and a corrected ratio of protons taken up per aspartate molecule taken up by the dicarboxylate-transport system may be calculated (Table 5). This corrected ratio was found to be approximately two.

Thus uptake of dicarboxylate ions by the dicarboxylate-transport system is invariably accompanied by the uptake of two protons (or the extrusion of two OH^- ions) per molecule. This pH change could hardly be attributed to metabolism of succinate (rather than to uptake as such) since suspensions of *E. coli* K12 equilibrated anaerobically with succinate extrude 2.42 protons per oxygen atom following an oxygen pulse (Lawford & Haddock, 1973). Thus oxidation of succinate causes acidification of the medium. In our experiments succinate-induced alkalinization of the medium is maximal immediately after succinate addition, when transport must precede metabolism. Finally we found that the non-metabolizable dicarboxylic acid, D-tartrate, inhibited both succinate uptake and succinate-induced proton uptake in *E. coli* and itself caused an alkalinization of the medium when added to succinate-grown cells, but not when added to glucose-grown cells (S. J. Gutowski, unpublished work). Thus the obligatory linkage of dicarboxylate uptake to proton uptake strongly suggests that succinate, fumarate and malate are taken up in electroneutral form and aspartate is taken up as a cation by the dicarboxylate-transport system.

The chemiosmotic hypothesis suggests that co-transport of protons with a substance is important in the energization of the active transport of that substance (Mitchell, 1967). Succinate has been shown to be actively transported in mutants defective in succinate metabolism (Murakawa *et al.*, 1972) and in vesicles prepared from such mutants (Rayman *et al.*, 1972). In our own studies, results from t.l.c. show that succinate is very quickly metabolized under the conditions used. However, assuming a volume of 1 μl of cell water per 10^9 cells, it can be calculated from these results that succinate is concentrated at least sixfold. This value is likely to be an underestimate, since after filtration and during the preparation of the cell extract, considerable metabolism of succinate can still occur. Further, uptake of succinate was stimulated by an added energy source and was sensitive to low concentrations of uncoupler. Since the uncoupler inhibits succinate oxidation when added before but not after succinate, it is concluded that it inhibits succinate entry into cells but not its subsequent metabolism. The uncoupler also causes efflux of previously accumulated radioactive material. Thus it appears likely that, under the conditions used in the present study, succinate is actively transported until an equilibrium between metabolism and transport is established, and though other explanations are possible, the results described in the present paper are most readily explained by postulating that active transport of succinate is energized by the ΔpH via a symport with protons.

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