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COUPLING BETWEEN ENERGY CONSERVATION AND ACTIVE TRANSPORT OF SERINE IN *ESCHERICHIA COLI*

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SUMMARY

1. The transport of serine was studied in membrane vesicles and intact cells of *Escherichia coli* K12, making use of mutants defective in oxidative phosphorylation.

2. Membrane vesicles isolated according to the method of Kaback consist of a heterogeneous population of rightside-in and inside-out membranes, using the ATPase enzyme as a marker of membrane polarity.

3. Membrane vesicles isolated from mutants defective in oxidative phosphorylation do not accumulate serine in the presence of an oxidizable substrate, in contrast to wild-type vesicles. *N,N'*-dicyclohexylcarbodiimide, an inhibitor of *E. coli* ATPase, can restore oxidation-driven transport in these mutants.

4. ATP is able to stimulate serine transport in membrane vesicles isolated from wild-type *E. coli* cells in contrast to membrane vesicles from an $ATPase^-$ mutant.

5. It is concluded that either electron transport or ATP can energize active transport of serine in intact cells and membrane vesicles of *E. coli*, presumably by generation of a high-energy state or membrane potential.

INTRODUCTION

Several hypotheses have been put forward for the mechanism of coupling between energy conservation and active transport in bacteria. Apart from phosphoenolpyruvate (which has been shown to be the energy-donor for the transport of several sugars *via* the phosphotransferase system[1]), ATP[2], a membrane potential or pH gradient[3–7] and a high-energy state of the membrane[8] have been implicated in the concentrative uptake of sugars and amino acids in bacteria.

In addition, Kaback and co-workers[9,10] have suggested that active transport, as measured in bacterial membrane vesicles of *Escherichia coli*, is coupled directly and exclusively to the oxidation–reduction of the solute carriers which are located in the respiratory chain, preferentially in the span between D-lactate dehydrogenase

Abbreviations: PMS, phenazine methosulphate; DCCD, *N,N'*-dicyclohexyl carbodiimide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; ACMA, 9-amino-6-chloro-2-methoxy acridine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

and cytochrome *b*. Oxidative phosphorylation is not involved in this process according to the authors[11].

Several observations, however, militate against this hypothesis of Kaback. First, sugars and amino acids can be accumulated anaerobically in *E. coli*[5,8], the process being sensitive to uncouplers (carbonyl cyanide *m*-chlorophenylhydrazone, CCCP) and inhibitors (*N,N'*-dicyclohexyl carbodiimide, DCCD) of oxidative phosphorylation. Second, Simoni and Shallenberger[12] have described a mutant of *E. coli*, deficient in ATPase, which cannot accumulate amino acids although D-lactate oxidation is not impaired. Prezioso *et al.*[11], on the other hand, report uptake in another mutant of *E. coli*, isolated by Gibson and co-workers[13], which is also deficient in ATPase activity. Shairer and Haddock[14] have shown that in wild-type *E. coli* sugar transport can proceed in the absence of oxidation, while an ATPase-less mutant is unable to utilize ATP formed in glycolysis for solute transport. Thirdly, compounds known to collapse a pH-gradient or membrane potential such as uncouplers of oxidative phosphorylation or valinomycin *plus* potassium, inhibit transport in bacterial vesicles without affecting D-lactate oxidation (*cf.* ref. 9). Lastly, the transport of some sugars is shown to be associated with a proton[4] or potassium[6] movement.

It is the objective of this paper to show that oxidative phosphorylation (although not necessarily ATP *per se*) is involved in active transport both in intact cells and membrane vesicles. Using different mutants of *E. coli*, deficient in oxidative phosphorylation[15–17], it can be shown that active transport, normally absent in membrane vesicles of these mutants, can be restored by recoupling oxidation and energy conservation with DCCD, without affecting oxidation. This is similar to restoration of energy-dependent processes in submitochondrial particles by the inhibitors of oxidative phosphorylation, oligomycin[40] and DCCD[37] (*cf.* also ref. 17). Furthermore, ATP is able to drive active transport in membrane vesicles, provided ATP can reach the inside of the vesicles.

METHODS

Preparation of cells, sonicated particles and membrane vesicles

E. coli strain A_{428} (wild type) and mutants N_{144} and K_{11} were grown and harvested as described elsewhere[16,17]. Particles, obtained by sonication of intact cells were prepared as described in ref. 17. Membrane vesicles, obtained by lysis of protoplasts, were prepared exactly as described by Kaback[18] and stored in liquid nitrogen. To remove the ATPase, membrane vesicles or sonicated particles were washed twice in a medium containing 1 mM Tris–HCl, pH 7.5, and centrifuged for 2.5 min at $100000 \times g$. The pellet was resuspended in 1 mM Tris–HCl.

Transport assay with intact cells

Cells, suspended in 50 mM potassium phosphate (pH 7.5), 15 mM $(NH_4)_2SO_4$ and 0.5 mM $MgSO_4$ after harvesting and washing for 5 min were incubated at 25 °C in a medium containing approximately 0.5 mg protein/ml, 50 mM potassium phosphate, 15 mM $(NH_4)_2SO_4$, 0.5 mM $MgCl_2$ and 80 μg/ml chloramphenicol and if present 5 mM glucose, final pH 7.5. Air was bubbled through to keep the solution aerobic. The reaction was started by the addition of L-[U-^{14}C]serine to

a final concentration of 15 μM. The reaction was terminated by filtration of 0.5-ml aliquots through a millipore filter (pore size, 0.45 μm) and washing twice with 2.5 ml of the incubation medium without glucose and chloramphenicol. After drying, filters were counted in a Nuclear Chicago liquid scintillation counter as described elsewhere[19]. If the effect of inhibitors or uncouplers was tested, these compounds were added 2 min before the serine.

Transport assay with membrane vesicles

To measure transport in membrane vesicles, 1–3 mg protein/ml was incubated for 5 min at 25 °C in a medium containing 50 mM potassium phosphate and 10 mM $MgSO_4$, final pH 6.6. Water-saturated oxygen was bubbled through to keep the solution aerobic[20]. The reaction was started by the addition of the oxidizable substrate, usually 40 mM ascorbate and 100 μM PMS (final concentration), and L-[U-^{14}C]serine to a final concentration of 15 μM. The reaction was terminated by diluting 0.1-ml aliquots in 5 ml 100 mM LiCl, filtering through millipore filters (0.45 μm) and washing once with 5 ml 100 mM LiCl. After drying the filters, the radioactivity was counted as described above. If the effect of inhibitors and uncouplers was tested, these compounds were added 2 min before the labelled amino acid. When the transport was measured with ATP as an energy donor, vesicles were washed first in a low salt medium to remove the ATPase activity (see ref. 17). After preincubation of the vesicles for 30 min at 0 °C with 100 mM ATP, the reaction was started by the addition of the preincubated vesicles to the reaction medium containing 50 mM potassium phosphate, 10 mM $MgSO_4$ and 15 μM L-[U-^{14}C]-serine, final pH 6.6. Alternatively, the reaction was started by adding 200 μl washed vesicles to an equal volume 0.2 M ATP and immediately thereafter 200 μl of the reaction medium, containing 150 mM potassium phosphate, 30 mM $MgSO_4$ and 45 μM L-[U-^{14}C]serine, final pH 6.6, as described for phosphoenolpyruvate by Kaback[21].

ATPase and oxidative capacity of membrane vesicles and sonicated particles

Oxidation velocity of membrane vesicles and sonicated particles was determined polarographically in a medium containing 25 mM Tris–HCl, 5 mM KCl and 1 mM $MgCl_2$, final pH 7.4 at 37 °C or in 50 mM potassium phosphate and 10 mM $MgSO_4$, final pH 6.6 at 25 °C. NADH, succinate, D- and L-lactate were added at final concentrations of 0.7 mM, 6 mM, 6 mM and 6 mM, respectively.

ATPase activity was measured in a medium containing 50 mM Tris–HCl, 2.5 mM $MgCl_2$, 5 mM ATP and 0.05–0.1 mg protein/ml, final pH 7.8, T=37 °C. The reaction was started by addition of the ATP and terminated by adding trichloroacetic acid to a final concentration of 5%. P_i was determined according to Fiske and SubbaRow as described by Sumner[22].

9-Amino-6-chloro-2-methoxy acridine (ACMA) fluorescence

ACMA fluorescence was measured as described in ref. 17.

Special chemicals

L-[U-^{14}C]serine was obtained from the Radiochemical Centre, Amersham. DCCD was obtained from Koch-Light Laboratories. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was a gift of Dr P. Heytler.

RESULTS AND DISCUSSION

Orientation of bacterial membrane vesicles

The validity of the conclusions drawn by Kaback and co-workers[9,23] concerning the different efficiencies with which substrates can couple oxidation and transport depends on the implicit assumption that all substrates, including ATP, have the same capacity to gain access to the various dehydrogenases or the ATPase, all of which are located at the inner side of the plasma membrane.

We have used the ATPase as a marker for the orientation of the plasma membrane since it is located at the inner side of the plasma membrane in intact cells and can be removed from the membrane upon washing in a low salt medium[24,17] when exposed to the outside, as is the case in sonicated particles. Table I shows

TABLE I

ATPase ACTIVITY IN MEMBRANE VESICLES AND SONICATED PARTICLES OF *E. COLI*

The ATPase activity of membrane vesicles and sonicated particles was determined as described in Methods. For the treatment with Triton X-100 protein was preincubated for 5 min with 0.2% Triton X-100. After the preincubation, the membrane protein was diluted in the standard ATPase mixture without Triton X-100. The ATPase reaction was started by the addition of ATP to the reaction medium.

	ATPase (nmoles/min per mg protein)
Membrane vesicles	620
Washed membrane vesicles	169
Membrane vesicles + Triton X-100	1308
Washed membrane vesicles + Triton X-100	1080
Sonicated particles	692
Washed sonicated particles	70
Sonicated particles + Triton X-100	679
Washed sonicated particles + Triton X-100	65

the results of a typical experiment, in which membrane vesicles, obtained by lysis according to the method of Kaback, and particles obtained by sonication of intact cells both lose most of their ATPase activity upon washing in a low salt medium. However, subsequent addition of Triton X-100 unmasks another high ATPase activity in bacterial membrane vesicles, but not in sonicated particles. Addition of Triton X-100 to non-washed membrane vesicles leads to an ATPase activity which is approximately the sum of the activities in non-washed membrane vesicles (without Triton X-100) and washed membrane vesicles treated with Triton X-100. The ATPase activity found by us in membrane vesicles of *E. coli* K12 is somewhat higher than that reported for *E. coli* ML 308-225 (ref. 25) but approximately equal to the values reported by Prezioso *et al.*[11] for *E. coli* K12. The fact that this ATPase activity is inhibited by 50 μM DCCD for 70–80% and that other phosphate esters

such as glucose 6-phosphate, glycerolphosphate and 4-nitrophenyl phosphate are not hydrolysed, suggest that this reaction is not due to an aspecific phosphatase.

We conclude from these results that the ATPase of non-washed membrane vesicles represents the inside-out population, while the rightside-in population is represented by the ATPase activity, unmasked in washed membrane vesicles treated with Triton X-100. This result explains also why vesicles do not show oxidative phosphorylation[8]. ADP cannot reach the coupling factor which is located at the inner side of membrane vesicles. The particles with the ATPase at the outside have a very low P/O ratio, which will be further underestimated by the oxygen uptake of the rightside-in vesicles.

As we have shown elsewhere[17], the fluorescence of the fluorescent acridine dye, 9-amino-6-chloro-2-methoxyacridine (ACMA) is quenched by particles (obtained by sonication of *E. coli*) catalyzing oxidation or ATP hydrolysis. This quenching of the fluorescence is dependent on the presence of the ATPase.

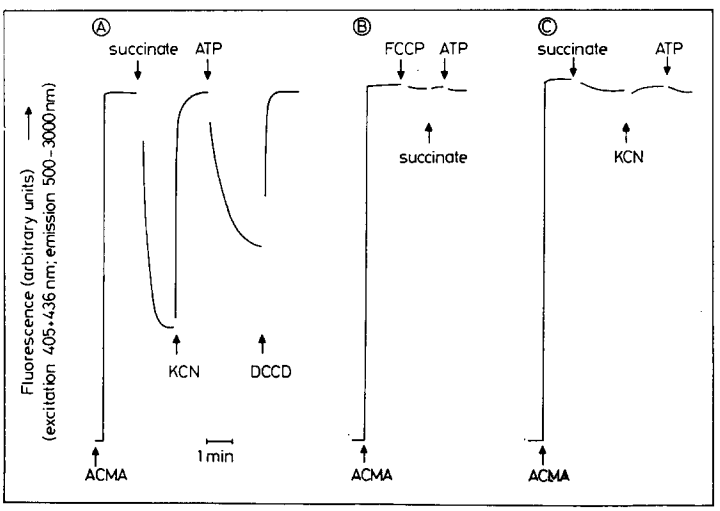

Fig. 1. ACMA fluorescence in washed and non-washed membrane vesicles of *E. coli* A_{428} upon energization. Vesicles were suspended in a medium containing 50 mM Tris–HCl and 2.5 mM $MgCl_2$, pH 7.5. ACMA was added to a final concentration of 1 μM. (A) Membrane vesicles (0.03 mg protein/ml). Succinate, KCN, ATP and DCCD were added to final concentrations of 6 mM, 3 mM, 0.5 mM and 60 μM, respectively. (B) Membrane vesicles (0.03 mg protein/ml). FCCP, succinate and ATP were added to final concentrations of 5 μM, 6 mM and 0.5 mM, respectively. (C) Washed membrane vesicles (0.02 mg protein/ml). Succinate, KCN and ATP were added to final concentrations of 6 mM, 3 mM and 0.5 mM, respectively.

Fig. 1 shows that membrane vesicles also exhibit ACMA fluorescence quenching, both during succinate oxidation and ATP hydrolysis. Upon washing, this fluorescence quenching is abolished, showing that the rightside-in oriented vesicles do not contribute to the process, since only the ATPase oriented to the outside can be removed (see also Table I). After preincubation of the washed vesicles with DCCD, partial restoration (20–40%) of the respiration-induced ACMA fluorescence quenching is found, as reported also for sonicated particles[17] (not shown).

TABLE II

K_m VALUES FOR UPTAKE AND OXIDATION OF SUCCINATE IN SONICATED PARTICLES, MEMBRANE VESICLES AND INTACT CELLS OF *E. COLI*

The oxidation velocity was determined as described in Methods. The uptake of [U-^{14}C]succinate in intact cells was determined as described in ref. 19. Values without a reference were determined in this study. K_m values were determined graphically from Lineweaver–Burk plots.

System	K_m (μM)	
	Oxidation	*Uptake*
Intact cells	20–30	10–20 (ref. 26)
		30 (ref. 28)
		20–30
Membrane vesicles	340	5 (ref. 29)
	350 (ref. 29)	11 (ref. 27)
Sonicated particles	400	—

Another indication that membrane vesicles obtained by lysis of *E. coli* contain a considerable percentage of inverted membranes, *i.e.* with a membrane orientation opposite to the plasma membrane in intact cells, may be derived from the experiments described in Table II. In this table we have collected the K_m values for uptake and oxidation of succinate in intact cells, membrane vesicles and sonicated particles of *E. coli* K12, using data from Lo *et al.*[26], Rayman *et al.*[27], Kay and Kornberg[28], Matin and Konings[29] and ourselves. In intact cells the K_m values obtained by measuring substrate uptake and oxidation are approximately the same. Membrane vesicles have the same K_m for succinate uptake as intact cells, but have the same K_m for succinate oxidation as sonicated particles, presumably the K_m of succinate dehydrogenase.

These results demonstrate clearly that membrane vesicles obtained by lysis are a heterogeneous population, consisting of rightside-in and inside-out vesicles. These conclusions can also be inferred from the results obtained by Salton and co-workers[30,31] who showed using iodination, inhibition by antibody and ferritin labeling, that the ATPase in lysed protoplasts of *Micrococcus lysodeikticus* can react with these compounds, while no reaction is observed in intact protoplasts.

Lombardi and Kaback[10] concluded recently from a comparison of transport in intact cells and membrane vesicles of *E. coli* that membrane vesicles retained approximately 70% or more of the transport capacity of the intact cells, and consequently that it was unlikely that a significant fraction of the membrane vesicles was inverted. However, using their own data (see Tables III and VI and Fig. 3 of ref. 10), we calculate that maximally 10% of the transport capacity of intact cells is retained in their membrane vesicles, suggesting that perhaps a considerable part of the membrane vesicles does not catalyse respiration-linked transport or is inverted, as suggested earlier by Kepes[32], Roseman[1] and Mitchell[33].

Uptake of amino acids by membrane vesicles

To gain more insight in the coupling between energy conservation and active transport we have investigated the transport of amino acids such as serine in mem-

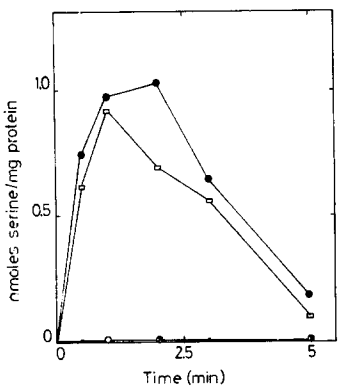

Fig. 2. Uptake of serine in membrane vesicles of *E. coli* A_{428}. Transport of labeled serine was measured as described in Methods, using 100 μM PMS and 40 mM ascorbate as an electron donor. FCCP and DCCD, if present, were added to a final concentration of 20 μM and 60 μM, respectively. ○——○, no addition; ●——●, PMS *plus* ascorbate; ×——×, PMS, ascorbate + FCCP; □——□, PMS, ascorbate + DCCD.

brane vesicles from wild type and mutants of *E. coli* K12, defective in oxidative phosphorylation.

Fig. 2 shows that membrane vesicles prepared from A_{428} (wild type) can accumulate serine with phenazine methosulphate (PMS) *plus* ascorbate as an energy donor. D-Lactate, L-lactate and succinate could replace PMS–ascorbate although they are less effective. The uptake is sensitive to the uncoupler FCCP but is not inhibited by the inhibitor of the ATPase, DCCD, as shown in Fig. 2. Since the oxidation rate of PMS + ascorbate in membrane vesicles of A_{428} is approximately 7 μat 0/min per mg protein at 25 °C while the medium contained 1.65 mg protein/ml,

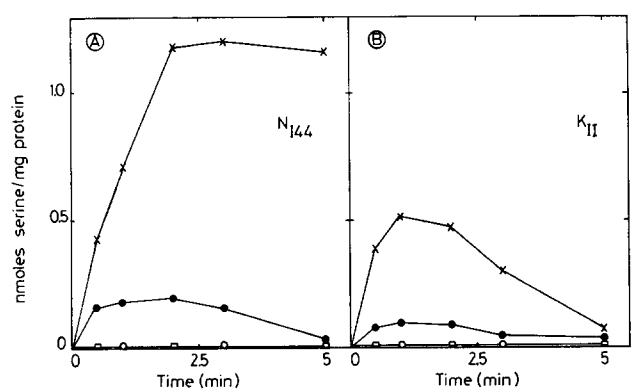

Fig. 3. Uptake of serine in membrane vesicles of mutants N_{I44} and K_{I1}. Transport of labeled serine was measured as described in Methods, using 100 μM PMS and 40 mM ascorbate as an electron donor. DCCD, if present, was added to a final concentration of 60 μM. FCCP was added to a final concentration of 20 μM. (A) Mutant N_{I44}. ○——○, no addition; ●——●, PMS + ascorbate; ×——×, PMS, ascorbate + DCCD; □——□, PMS, ascorbate, DCCD + FCCP. (B) Mutant K_{I1}. ○——○, no addition; ●——●, PMS + ascorbate; ×——×, PMS, ascorbate + DCCD; □——□, PMS, ascorbate, DCCD + FCCP.

it can be calculated that the ascorbate is consumed in approximately 3 min. This may explain the decrease in serine accumulation after 2 min, an observation also made by Konings and Freese[20] in *Bacillus subtilis*. This result is in contrast to those reported by Kaback and Barnes[39] who found that inhibition by oxamate of D-lactate oxidation at the level of the dehydrogenase, which is equivalent to exhaustion of the substrate, leads to a retention of the accumulated amino acids.

Fig. 3 shows the uptake of serine in two different mutants of *E. coli*, defective in oxidative phosphorylation[15–17]. Both in the ATPase$^-$ mutant (N_{144}) and in the ATPase$^+$ mutant (K_{11}) uptake is inhibited almost completely. These results are similar to those of Simoni and Shallenberger[12] but differ from those reported by Prezioso *et al.*[11]. The latter authors used the uncA mutant isolated by Gibson and co-workers[13,34]. However, Hong and Kaback[25] also described an electron transfer coupling mutant (etc$^-$) which was defective in D-lactate stimulated transport. This mutant is possibly similar to B_{V4} or K_{11} described earlier[16,17] or the uncB mutant described by Cox *et al.*[35], since it contains a normal ATPase activity.

These results suggest that the bacterial coupling factor, the ATPase, is closely connected with transport, in contrast to the conclusion drawn by Kaback and co-workers[11]. This involvement of oxidative phosphorylation is borne out, furthermore, by the observation that DCCD is able to restore serine transport in vesicles of these mutants when PMS *plus* ascorbate is the energy donor, as shown in Fig. 3. This stimulation by DCCD is abolished by the uncoupler FCCP. These results correlate nicely with our earlier observations[17] that DCCD can restore respiration-induced quenching of ACMA fluorescence in mutant particles and in wild-type particles devoid of their ATPase.

Finally, Fig. 4 shows that also ATP is able to stimulate serine transport in wild type membrane vesicles, in contrast to earlier reports[10,8,20]. (Note, however,

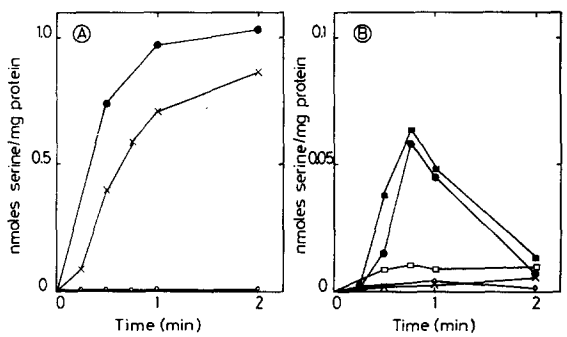

Fig. 4. Stimulation of serine transport in *E. coli* A_{428} by ATP. Transport of serine was measured as described in Methods. (A) Washed and non-washed membrane vesicles were assayed with 100 μM PMS and 40 mM ascorbate as electron donor. ○———○, no addition; ●———●, non-washed membrane vesicles with PMS + ascorbate; ×———×, membrane vesicles washed in a low salt medium with PMS + ascorbate as substrate. (B) Washed membrane vesicles were assayed with ATP as an energy source for transport. ○———○, no addition or with 100 mM ADP. ●———●, + 100 mM ATP shocked into the vesicles as described in ref. 21; ■———■, membrane vesicles preincubated for 30 min at 0 °C in 100 mM ATP. ×———×, membrane vesicles shocked with 100 mM ATP *plus* 40 μM FCCP. □———□, + 100 mM ATP shocked into vesicles as described in ref. 21 in the presence of 60 μM DCCD.

the difference in scale). Since we showed in an earlier section that ATP cannot reach the ATPase in rightside-in vesicles, necessary to provide energy for serine accumulation, we either shocked 100 mM ATP into the vesicles as has been reported for phosphoenolpyruvate by Kaback[21] or preincubated membrane vesicles for 30 min at 0 °C with 100 mM ATP. In both cases the membrane vesicles were washed first in low salt medium to remove the ATPase activity associated with the inside-out particles (see Table I). The experiment shows in addition (Fig. 4A) that washing of the ATPase activity does not lower significantly the capacity to accumulate serine as found also by Hirata *et al.*[36] in *Mycobacterium phlei*, which was expected since the rightside-in vesicles do not lose their ATPase. FCCP and DCCD inhibit the ATP-driven serine accumulation, while ADP gives no stimulation, as shown in Fig. 4B. We could not find any stimulation by ATP in mutant vesicles.

It is inferred from these results that oxidation of various substrates, if they have access to their respective dehydrogenases or the respiratory chain, can generate a high-energy state, possibly a membrane potential[33]. This energized state can drive ATP synthesis, the energy-linked transhydrogenase[15] or active transport. Mutants defective in oxidative phosphorylation are partly uncoupled, probably similar to the situation in submitochondrial particles[37]. Inhibitors of oxidative phosphorylation such as DCCD in bacteria[17] and oligomycin in mitochondria[40] can recouple oxidation and energy-linked processes (for instance active transport) by decreasing the rate of dissipation of the high-energy state or membrane potential *via* leakage (*cf.* refs 41 and 42). It should be stressed that DCCD has no effect on the oxidation velocity, ruling out a direct effect on the oxidation–reduction of the electron carriers, according to Kaback the driving force for active transport. Furthermore, ATP can energize active transport when the coupling factor is present as is the case in the wild-type membrane vesicles. The accumulation of serine driven by the hydrolysis of ATP is small compared to uptake as a result of PMS–ascorbate oxidation, but this is understandable since only a small amount of ATP can be contained within the vesicles (100 nmoles/mg protein on the assumption that 1 mg vesicle protein encloses 1 μl water). Furthermore, only part of this ATP can be effectively hydrolyzed since ADP is a strong inhibitor of the ATPase[38]. Since the ATPase activity at 25 °C is 100–200 nmoles ATP hydrolyzed/min per mg protein, the reaction will be terminated within 1 min.

Uptake of serine by intact cells

Since the results obtained with isolated membrane vesicles should be comparable to those with intact cells, on the assumption that the same mechanism for transport operates in both systems, we have measured serine uptake also in whole cells of wild type and mutants of *E. coli* K12. A more extensive report on these results will be published elsewhere.

Fig. 5 shows that parental cells (A_{428}) are able to accumulate serine both aerobically and in the presence of KCN, suggesting that both oxidative energy and glycolytic ATP can serve as an energy source for active transport in *E. coli*, a conclusion reached earlier by Schairer and Haddock[14] and Klein and Boyer[8]. Mutants of *E. coli* defective in oxidative phosphorylation such as N_{I44} and K_{I1} (not shown) can accumulate serine aerobically in the presence of glucose but KCN inhibits this

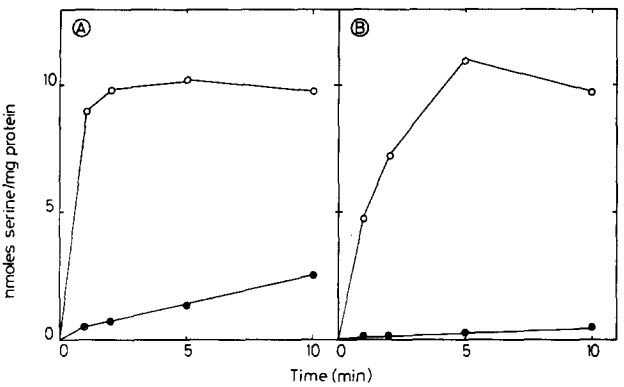

Fig. 5. Serine uptake in intact cells of wild type and mutant of *E. coli* K12. Serine uptake was measured as described in Methods in the presence of 6 mM glucose. (A) A_{428}; (B) N_{I44}; ○——○, no inhibitor. ●——●, *plus* 10 mM KCN.

process almost completely probably due to the fact that the absence of the ATPase (N_{I44}) or poor coupling between the ATPase and the plasma membrane (K_{I1}) prevents conversion of glycolytic ATP in a membrane potential or high-energy state. DCCD at a concentration of 60 μM has no effect on the aerobic serine uptake either in wild-type or in mutants, but FCCP inhibits (not shown). However, serine uptake in wild-type cells in the presence of KCN is inhibited by DCCD.

Final conclusions

The results described in this paper suggest strongly that oxidative phosphorylation is involved in active transport of serine both in intact cells and membrane vesicles of *E. coli*. We want to stress that although we speak about oxidative phosphorylation, we do not necessarily imply the involvement of ATP *per se*, but use this term to describe all reactions leading to synthesis of ATP, driven by respiratory-chain activity. We believe, that our results lead inevitably to the conclusion that active transport of serine and other amino acids or sugars is driven by the same high-energy state or membrane potential which drives ATP synthesis, reversal of electron transfer, the energy-linked transhydrogenase, cation uptake or leads to quenching of acridine fluorescence. Respiratory-chain activity *per se* is not required to drive active transport but leads to generation (for instance) of a membrane potential or pH gradient which drives transport mediated by a H^+-solute symport. This is also borne out by the observations of Pavlasova and Harold[5] and Klein and Boyer[8] that *E. coli* can accumulate sugars and amino acids under anaerobic conditions, in which case the energy is provided *via* ATP, generated in glycolysis. Mutants defective in oxidative phosphorylation are able to concentrate solutes according to this proposal when the membrane is not too leaky to protons, a condition which is probably promoted by DCCD.

The finding that membrane vesicles constitute a heterogeneous population of both rightside-in and inside-out particles and that oxidative phosphorylation is involved in active transport can explain many of the unexpected results found by Kaback, such as the inhibition by uncouplers and valinomycin, the differential

capacities of substrates to stimulate transport, and the apparent lack of ATP to stimulate transport.

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