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Energy Transduction by Electron Transfer Via a Pyrrolo-Quinoline Quinone-Dependent Glucose Dehydrogenase in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter calcoaceticus* (var. *lwoffi*)

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The coupling of membrane-bound glucose dehydrogenase (EC 1.1.99.17) to the respiratory chain has been studied in whole cells, cell-free extracts, and membrane vesicles of gram-negative bacteria. Several *Escherichia coli* strains synthesized glucose dehydrogenase apoenzyme which could be activated by the prosthetic group pyrrolo-quinoline quinone. The synthesis of the glucose dehydrogenase apoenzyme was independent of the presence of glucose in the growth medium. Membrane vesicles of *E. coli*, grown on glucose or succinate, oxidized glucose to gluconate in the presence of pyrrolo-quinoline quinone. This oxidation led to the generation of a proton motive force which supplied the driving force for uptake of lactose, alanine, and glutamate. Reconstitution of glucose dehydrogenase with limiting amounts of pyrrolo-quinoline quinone allowed manipulation of the rate of electron transfer in membrane vesicles and whole cells. At saturating levels of pyrrolo-quinoline quinone, glucose was the most effective electron donor in *E. coli*, and glucose oxidation supported secondary transport at even higher rates than oxidation of reduced phenazine methosulfate. Apoenzyme of pyrrolo-quinoline quinone-dependent glucose dehydrogenases with similar properties as the *E. coli* enzyme were found in *Acinetobacter calcoaceticus* (var. *lwoffi*) grown aerobically on acetate and in *Pseudomonas aeruginosa* grown anaerobically on glucose and nitrate.

Glucose metabolism in gram-negative bacteria can proceed via phosphorylation to glucose-6-phosphate or by direct oxidation to gluconate, catalyzed by membrane-bound glucose dehydrogenase (EC 1.1.99.17) (4, 27). This enzyme was shown recently to belong to the class of quinoproteins which have pyrrolo-quinoline quinone (PQQ) as the prosthetic group (6, 8). Glucose dehydrogenase is a membrane-bound aldolase dehydrogenase, which transfers electrons from the aldose sugars directly to the electron transport chain. The enzyme has been detected in a wide variety of bacteria, including *Pseudomonas aeruginosa* (32) *Aerobacter aerogenes* (2), *Serratia* sp. (J. H. Duine, personal communication), *Rhodopseudomonas sphaeroides* (35), *Acinetobacter calcoaceticus* (14), and *Klebsiella aerogenes* (34).

The role of glucose dehydrogenase in glucose metabolism has been the subject of a large number of physiological and genetic studies (4, 19, 33, 36, 37). This role differs significantly in different organisms. In *P. aeruginosa* the enzyme is inducible by glucose, gluconate, mannitol, and glycerol (20), whereas in *A. calcoaceticus* the enzyme is synthesized constitutively (5). In *A. calcoaceticus* strains which can grow on glucose, this sugar is completely oxidized by glucose dehydrogenase to gluconate, which is then further metabolized by the Entner-Doudoroff pathway. In strains of this organism which are unable to grow on glucose, glucose metabolism proceeds via glucose dehydrogenase only to gluconic acid (22). *A. calcoaceticus* (var. *lwoffi*) (known as *A. lwoffi*) does not metabolize glucose at all, but nevertheless synthesizes apoglucose dehydrogenase constitutively. The addition of PQQ is required for an active holoenzyme, which oxidizes glucose quantitatively to gluconate (42). Such a noncoordinated synthesis of glucose dehydrogenase protein and its prosthetic group PQQ was also observed in *P. aeruginosa* cultivated anaerobically on glucose with nitrate as electron acceptor (42).

We have observed that *Escherichia coli* also synthesizes an apoglucose dehydrogenase which can be converted with PQQ to an active holoenzyme. These observations confirmed and extended those of Hommes et al. (17), who discovered apoglucose dehydrogenase in K^+-limited cultures of *E. coli* strain C. Collaborative research in both laboratories has shown that many different *E. coli* strains contain apoglucose dehydrogenase. Although a role of glucose dehydrogenase in energy metabolism is likely, its function and contribution to the energy budget of the cell was never substantiated. In this paper we report studies on the functional coupling of the enzyme to electron transfer systems in gram-negative bacteria. The results show that electrons from glucose are transferred to oxygen via the electron transfer chain and that this process leads to the generation of a proton motive force. The observations that the activity of glucose dehydrogenase can be manipulated by the addition of limiting amounts of PQQ suggest a procedure for varying the rate of electon transfer without the use of electron transfer chain inhibitors.

MATERIALS AND METHODS

Microorganisms and growth conditions. *E. coli* ML-35, ML308-225, and B and a typical K-12 strain were grown aerobically at 37°C in shake flask culture on mineral medium

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A (3) with 20 mM glucose or 30 mM succinate as carbon source.

P. aeruginosa PA01 was grown anaerobically on glucose with nitrate as electron acceptor as described previously (42).

A. calcoaceticus (var. *lwoffi*) ATCC 15309 was obtained from the Delft Culture Collection (LMD 73.1). This strain was the original type strain of *A. lwoffi*, an organism which differs from *A. calcoaceticus* by its inability to oxidize aldose sugars. For simplicity we have used the original name in this paper. The organism was grown in acetate-limited cultures as described previously (42).

Preparation of membrane vesicles. Membrane vesicles from *E. coli* were prepared as described by Kaback (23). Membrane vesicles from *P. aeruginosa* PA01 were prepared by a modified procedure of Stinnett et al. (41) and Hoshino and Kageyama (18). Cells from a 20-liter culture were harvested at the end of the exponential-growth phase by centrifugation at room temperature and washed once with 100 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 2.5% KCl and 20% sucrose. The pellet (40 g [wet weight]) was resuspended in 150 ml of the same buffer at 4°C. Lysozyme was added (final concentration, 0.5 mg/ml) to the cell suspension and incubated at 37°C in a rotatory shaker for 30 min. The osmotically fragile rods were harvested by centrifugation at 4°C and carefully resuspended in 30 ml of the above buffer containing 0.1 mg of DNase per ml. Fifty volumes of ice-cold 50 mM potassium phosphate buffer (pH 6.6) containing 10 mM $MgSO_4$ and 0.1 mg of both DNase and RNase per ml was added slowly with stirring. The suspension was centrifuged (4,000 × *g*) at 4°C for 15 min to remove the cell debris and remaining whole cells. Membrane vesicles were collected by centrifugation (30 min, 45,000 × *g*) at 4°C. The pellet was suspended in 50 mM potassium phosphate buffer (pH 6.6) at ca. 10 mg of protein per ml and stored in portions of 0.5 ml in liquid nitrogen.

Preparation of cell-free extracts. Cells were broken by ultrasonic treatment with an MSE 150-W sonifier. Whole cells and debris were removed by centrifugation for 30 min at 40,000 × *g*. The clear supernatants, containing 5 to 9 mg of protein per ml, were used as cell-free extracts.

Agarose gel electrophoresis. Electrophoresis of protein extracts of membrane vesicles on agarose gels was performed as described for the first dimension of crossed immunoelectrophoresis (10, 40) at 2.5 V/cm for 2 h in barbital buffer at pH 8.6. Proteins were extracted by incubating the membrane vesicles for 30 min at 37°C in Tris-chloride (pH 8.6) supplemented with 10 mM sodium-EDTA and 8% Triton X-100.

Polarographic measurements of glucose dehydrogenase activity. The aldose- and PQQ-dependent glucose dehydrogenase activity in whole cells, cell-free extracts, and membrane vesicles was assayed polarographically with a Clark-type oxygen electrode (Yellow Springs Instruments, Inc., Yellow Springs, Ohio) at 30°C in 50 mM potassium phosphate buffer (pH 6.6) containing 10 mM $MgSO_4$. After preincubation of the samples with PQQ (0.2 μM for *A. lwoffi* and 12 μM for *E. coli* and *P. aeruginosa*) for 30 min, the reaction was started by the addition of 20 mM glucose or 20 mM D-xylose. Samples without PQQ served as blanks. When membrane vesicles solubilized with Triton X-100 or cell-free extracts were used, 300 μM phenazine methosulfate (PMS) was added to mediate electron transfer between the dehydrogenase and oxygen.

Measurements of the electrical potential. Electrical potential ($\Delta\psi$) measurements were performed with a tetraphenylphosphonium (TPP^+)-sensitive electrode at 30°C as described (12, 28). The incubation mixtures were gassed with water-saturated oxygen-free nitrogen (16) or air.

Solute transport. Transport of solutes by cells or membrane vesicles was measured at 30°C as described previously (25). Preincubation with PQQ in 50 mM potassium phosphate buffer (pH 6.6) containing 5 mM $MgSO_4$ was carried out for 10 min with membrane vesicles or 1 h with intact cells. In some experiments solute transport, $\Delta\psi$, and the rate of oxygen consumption were measured simultaneously as described previously (12).

Analytical methods. Protein was determined by the method of Lowry et al. (29) with bovine serum albumin as a standard. Glucose and gluconic acid were measured as described previously (42).

Chemicals. Radioactive labeled solutes were obtained from the Radiochemical Centre (Amersham, Buckinghamshire, United Kingdom). They were used at the following specific activities: [1-^{14}C]acetic acid, 2.2 GBq/mmol; L-[*U*-^{14}C]alanine; 5.7 GBq/mmol; L-[*U*-^{14}C]glutamic acid, 10.5 GBq/mmol; and D-glucose-[1-^{14}C]lactose; 0.8 GBq/mmol. 2,7,9-Tricarboxy-1*H*-pyrrolo(2,3,*f*)quinoline-4,5-dione (PQQ) was kindly provided by J. A. Duine of the Department of Biochemistry, University of Technology, Delft, The Netherlands. PQQ is stable only in the fully oxidized form (7). Purified glucose dehydrogenase from *A. calcoaceticus* prepared as previously described (8) was a gift from P. Dokter from the same department.

All other reagents were obtained from commercial sources and were of reagent grade.

RESULTS

Glucose dehydrogenase in cells and membrane vesicles of *E. coli*. Cell-free extracts of various *E. coli* strains contained an inactive glucose dehydrogenase. These extracts oxidized glucose only after preincubation with 12 μM PQQ, the prosthetic group of glucose dehydrogenase. Activities varied from 10 to 40 nmol of O_2 per min per mg of protein.

TABLE 1. Effect of PQQ on the rate of xylose or glucose oxidation in cells or membrane vesicles from three gram-negative bacteria

Organism[a] and strain	Carbon source for growth	Preparation	Oxidation rate[b]	
			With PQQ[c]	Without PQQ
E. coli ML31	Glucose	Cells	118	6
E. coli K-12	Glucose	Cells	125	11
E. coli K-12	Succinate	Cells	204	12
E. coli B	Glucose	Cells	93	7
E. coli B	Succinate	Cells	243	22
E. coli B	Glucose	Vesicles	99	6
E. coli ML308-225	Succinate	Vesicles	170	6
A. lwoffi LMD 73.1	Acetate	Cells	250	15
P. aeruginosa PAO1	Glucose + nitrate	Vesicles	120	5

[a] *E. coli* and *A. lwoffi* strains were grown aerobically and *P. aeruginosa* was grown anaerobically in the presence of nitrate with the indicated carbon source.

[b] The rate of glucose or xylose oxidation was measured with a Clark-type oxygen electrode and is expressed as nanomoles of oxygen consumed per minute per milligram of protein. Xylose was used in whole cells of *E. coli*, whereas glucose was used for the other experiments. Glucose or xylose was added to a final concentration of 20 mM.

[c] PQQ was added at a saturating concentration (0.2 to 12 μM) with 10 min of preincubation.

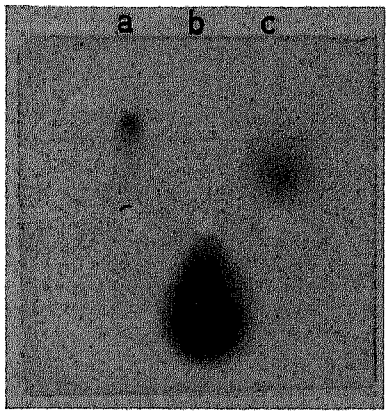

FIG. 1. Agarose electrophoresis of the glucose dehydrogenase from *E. coli*, *A. calcoaceticus*, and *P. aeruginosa*, visualized by zymogram staining. The conditions of electrophoresis were as described in the text. The top of the figure is the side next to the anode. The zymogram staining was performed by incubating the electrophoresis plates for 2 h in 50 mM potassium phosphate buffer supplemented with 20 mM glucose, 15 μM PQQ, and 330 μM tetranitroblue tetrazolium. Lane a, Two hundred micrograms of protein from membrane vesicles from *E. coli* ML308-225 solubilized in 10 mM Tris buffer plus 8% (wt/vol) Triton X-100. Lane b, Twenty-five micrograms of purified glucose dehydrogenase from *A. calcoaceticus* dissolved in 4 μl of electrophoresis buffer. Lane c, One hundred micrograms of protein from membrane vesicles from *P. aeruginosa* dissolved as described for lane a.

Similar observations were made with whole cells of *E. coli* (Table 1). However, PQQ-dependent glucose dehydrogenase activity was masked by the high rate of glucose oxidation observed in the absence of PQQ. This oxidation was due to the presence of a phosphoenolpyruvate-dependent glucose phosphotransferase system or hexose kinase activity, or both (30). The role of PQQ-reconstituted glucose dehydrogenase in glucose oxidation by whole cells of *E. coli* could be demonstrated by using D-xylose as a substrate for glucose dehydrogenase (15). Suspensions of *E. coli* cells grown on succinate or glucose oxidized D-xylose at a low rate. However, after preincubation of cells with 12 μM PQQ, a 10- to 20-fold increase in the rate of xylose-dependent oxygen consumption was measured (Table 1). Similar results were obtained with isolated cytoplasmic membrane vesicles. In contrast to whole cells, membrane vesicles oxidized glucose at a very low rate, but in the presence of PQQ (12 μM) a 15- to 25-fold higher rate of oxygen consumption was observed (Table 1). Further studies with membrane vesicles demonstrated that oxidation of glucose by the PQQ-dependent glucose dehydrogenase was quantitatively accompanied by the formation of gluconate (data not shown).

These results indicate that glucose dehydrogenase is a membrane-bound enzyme in *E. coli*. After extraction from the membrane with Triton X-100, the enzyme remained dependent on PQQ for activity. Protein extracts of membrane vesicles of *E. coli* were subjected to agarose gel electrophoresis. Zymogram staining for glucose dehydrogenase activity with the artificial electron acceptor tetranitroblue tetrazolium occurred only in the presence of PQQ. For comparison the glucose dehydrogenase solubilized from *P. aeruginosa* membrane vesicles and the purified glucose dehydrogenase from *A. calcoaceticus* were also electrophoresed. At pH 8.6 the glucose dehydrogenases of *E. coli* and *P. aeruginosa* were positively charged, whereas the *A. calcoaceticus* enzyme was negatively charged (Fig. 1). The isoelectric points of the enzymes, estimated on the basis of electrophoretic mobilities at different pH values, for the glucose dehydrogenases of *E. coli*, *P. aeruginosa*, and *A. calcoaceticus* were ca. 7.0, 7.5, and 9.0, respectively.

Generation of a proton motive force in *E. coli* by glucose dehydrogenase-linked oxidation of glucose. After activation of glucose dehydrogenase with PQQ, the enzyme was found to be coupled to the proton-translocating electron transport chain. This was evident from the generation of a proton motive force upon glucose oxidation.

The rate of glucose oxidation by membrane vesicles of *E. coli* increased with the concentration of PQQ (Fig. 2). The concentration of PQQ required for half-maximal glucose

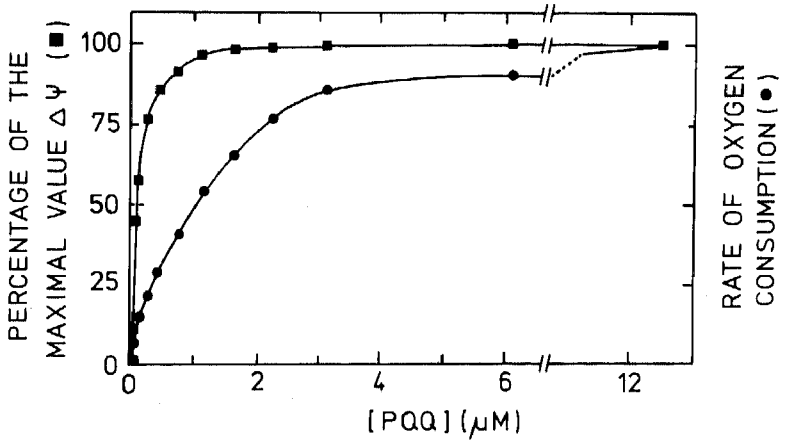

FIG. 2. Rate of oxygen consumption and the generation of $\Delta\psi$ upon addition of glucose in membrane vesicles of *E. coli* ML308-225 as a function of the PQQ concentration. Membrane vesicles (0.7 mg/ml) were suspended in a medium containing 50 mM potassium phosphate, 5 mM $MgSO_4$, 1 μM nigericin, and 3 μM TPP^+ at pH 7.0 and incubated for 10 min at the indicated PQQ concentrations. The $\Delta\Psi$ and the rate of oxygen consumption were measured simultaneously with a TPP^+-sensitive ion-selective electrode and a Clark-type oxygen electrode, respectively. Glucose was added to a final concentration of 20 mM. The 100% values of $\Delta\psi$ and the rate of oxygen consumption after glucose addition were −90 mV and 89 nmol/min per mg of protein, respectively. In the absence of glucose no oxygen consumption or $\Delta\psi$ generation was observed.

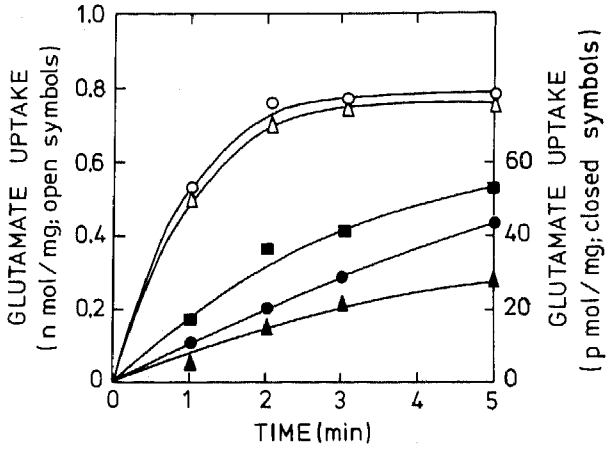

FIG. 3. Uptake of glutamate by membrane vesicles from *E. coli* ML308-225, energized by electron transfer via the PQQ-dependent glucose dehydrogenase. Uptake of [^{14}C]glutamate was assayed as described in the text. The following concentrations were used: 18 mM glucose, 12 mM potassium gluconate, 10 mM potassium-ascorbate, 3 μM PQQ, 100 μM PMS, and 3.5 μM [^{14}-C]glutamate. Symbols: ○, plus glucose and PQQ; △, plus ascorbate and PMS; ■, plus glucose; ●, plus PQQ; and ▲, no additions. Note the different scales at the two vertical axes.

oxidation rates was 1 μM. Upon oxidation of glucose, a $\Delta\psi$, inside negative, was generated across the cytoplasmic membranes of the vesicles (Fig. 2). The steady-state values of the $\Delta\psi$ increased with the PQQ concentration, reaching a maximal value of −90 mV at ca. 2 μ PQQ. The concentration of PQQ required for half-maximal $\Delta\delta\psi$ values was 0.1 μM, which is 10-fold lower than the concentration required for the half-maximal glucose oxidation rate.

Glucose oxidation drives secondary transport in *E. coli*. In the presence of glucose and PQQ, membrane vesicles of *E. coli* accumulated glutamate (Fig. 3), lactose (Table 2), proline and alanine (data not shown) at high rates. Uptake of these solutes was dependent on both preincubation with PQQ and on glucose. Membrane vesicles, not preincubated with PQQ, showed low rates of solute uptake upon addition of glucose (Fig. 3).

Glucose appeared to be an effective energy source for driving secondary solute transport in PQQ-preincubated membrane vesicles of *E. coli* (Fig. 3 and Table 2). The uptake of glutamate was as rapid with glucose-PQQ as with the artificial electron donor system ascorbate-phenazine methosulfate, which up to now has been found to be the most effective electron donor (26). In Table 2 a more detailed comparison is shown for lactose accumulation, energized by the electron donors lactate, glucose-PQQ, and ascorbate-PMS. Ascorbate-PMS and glucose-PQQ were equally effective in generating a $\Delta\psi$ and superior to lactate. Of the three electron donors, glucose was oxidized at the lowest rate. However, the rates of lactose accumulation driven by these electron donors increased in the order of lactate, ascorbate-PMS, and glucose-PQQ.

TABLE 2. Efficiency of different electron donors in energizing lactose transport by membrane vesicles of *E. coli* ML308-225[a]

Electron donor	Respiration rate (nmol of O_2/min per mg of protein)	$\Delta\psi$ (mV)	Initial uptake rate (nmol/min per mg of protein)
D,L-Lactate	284	75	24
Glucose-PQQ	158	92	40
Ascorbate-PMS	578[b]	90	33

[a] The initial rates of lactose uptake, $\Delta\psi$, and oxygen consumption were measured simultaneously in a buffer containing 50 mM potassium phosphate, 5 mM $MgSO_4$, 1 μM nigericin, 3 μM TPP^+, 12 μM PQQ, and 200 μM [^{14}-C]lactose in a volume of 2.6 ml. The electron donors were added to a final concentration of 20 mM. The protein concentration with the electron donors lactate and glucose was 0.7 mg/ml.

[b] The rate of oxygen consumption has not been corrected for autooxidation of reduced PMS.

Glucose dehydrogenase in *P. aeruginosa*. Evidence has been presented that *P. aeruginosa* also contains an apoglucose dehydrogenase when grown anaerobically on glucose and nitrate (42). It has been shown above that the enzyme could be identified in the agarose gel-electrophoresis pattern of solubilized membrane vesicles with PQQ-dependent zymogram staining (Fig. 1). The activity of the enzyme depended on the presence of PQQ, as was apparent from the 24-fold stimulation of the rate of glucose oxidation by membrane vesicles of anaerobically grown cells (Table 1). Also in this organism the PQQ-dependent oxidation of glucose led to the generation of a proton motive force (Fig. 4). In the presence of saturating concentrations of PQQ, glucose oxidation generated a $\Delta\psi$ of −70 mV. In membrane vesicles of *P. aeruginosa* grown anaerobically on glucose and nitrate, nitrate could also serve as electron acceptor with glucose as electron donor. This process also led to the generation of a $\Delta\psi$ (Fig. 4).

Glucose dehydrogenase in *A. lwoffi*. The studies on *E. coli* had been prompted by our earlier observation that *A. lwoffi* contained apoglucose dehydrogenase (42). *A. lwoffi* starved for endogenous energy oxidized glucose at a low rate in the absence of PQQ. The addition of PQQ increased the rate of glucose oxidation ca. 16-fold (Table 1).

These studies were performed in *A. lwoffi* cells which were

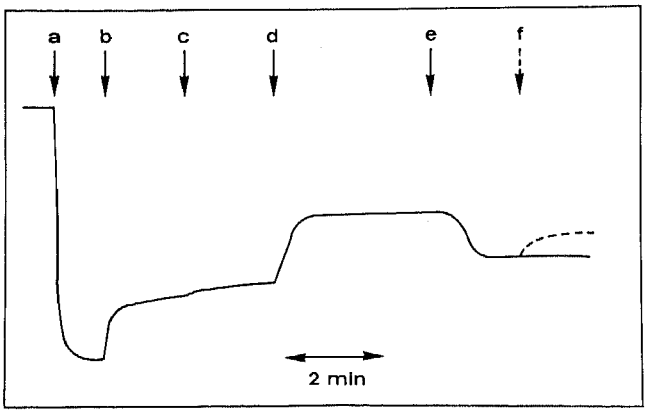

FIG. 4. Uptake of TPP^+ by membrane vesicles from *P. aeruginosa* energized by electron transfer via the PQQ-dependent glucose dehydrogenase. The measurements were performed in a 50 mM potassium phosphate buffer (pH 6.6) containing 10 mM $MgSO_4$ and 2 μM TPP^+. The following additions were made: doubling of the TPP^+ concentration to 4 μM (arrow a; observe response of electrode); membrane vesicles (final concentration, 0.7 mg of protein per ml) (arrow b); glucose (final concentration , 10 mM) (arrow c); 0.3 μM PQQ (arrow d); and stopping of the oxygen supply (arrow e). In a separate experiment during anaerobiosis, choline nitrate (final concentration, 50 mM) was added (arrow f). The choline nitrate stock solution was flushed with nitrogen. An accurate calculation of the generated $\Delta\psi$ was only possible after complete characterization of the TPP^+ binding.

TABLE 3. Acetate uptake by energy-depleted cells of *A. lwoffi* energized by electron transfer via the PQQ-dependent glucose dehydrogenase[a]

PQQ addition	Electron donor	Rate of uptake (nmol/min per mg of protein)
No	None	2
Yes	None	2
Yes	Glucose	61
Yes	Glucose + DCCD	1
Yes	Xylose	58
Yes	Gluconate	1

[a] The uptake of [^{14}C]acetate was linear during the first 4 min. The final concentrations of [^{14}C]acetate, PQQ, glucose, xylose, and potassium gluconate were 34, 2, 18, 18, and 12 μM, respectively. Cells were incubated with DCCD (50 μM) for 15 min.

starved for endogenous energy. The cells were treated with 50 μM dicyclohexylcarbodiimide (DCCD) to inhibit the membrane-bound ATPase and incubated with saturating amounts of nigericin (1 μM) to dissipate the Δ pH. Furthermore, to fully activate glucose dehydrogenase, the cells were preincubated with 2 μM PQQ. In the absence of glucose these cells generated a $\Delta\psi$ of −80 mV, inside negative. This $\Delta\psi$ most probably resulted from the presence of residual endogenous energy. Upon addition of glucose the $\Delta\psi$ increased gradually to a new steady-state level of −135 mV (Fig. 5, curve A). The ability of the cells to maintain this $\Delta\psi$ was dependent on the presence of oxygen, since the $\Delta\psi$ collapsed rapidly when the oxygen supply stopped. When the sequence of additions was reversed, i.e., addition of glucose was followed by oxygen (Fig. 5, curve B), an even higher increase of $\Delta\psi$ was observed. This was due to a lower $\Delta\psi$ under anaerobic conditions. In both experiments (curves A and B) the effects of glucose were strictly dependent on preincubation of the cells with PQQ.

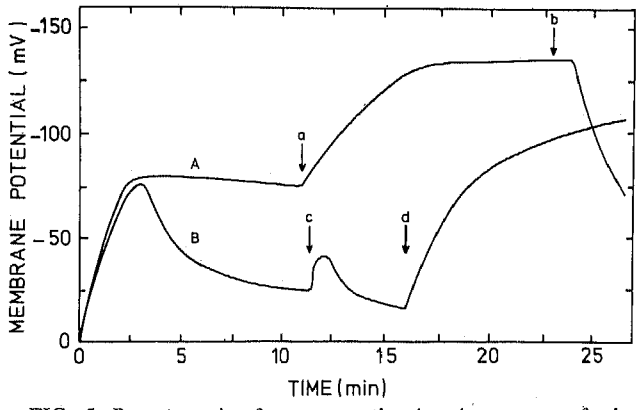

FIG. 5. Proton motive force generation by electron transfer by the PQQ-dependent glucose dehydrogenase in energy-starved cells of *A. lwoffi*. The cells were starved for endogenous metabolic energy as described in the text. Subsequently the cells were incubated with 50 μM DCCD for 15 min. $\Delta\psi$ measurements were performed in a buffer containing 50 mM potassium phosphate, 10 mM $MgSO_4$, 4 μM TPP^+, and 1 μM nigericin at pH 6.6 as described in the text. Curve A, Cells (final concentration, 0.69 mg of protein per ml) were introduced into an aerated vessel, and the following manipulations were done: 18 mM glucose was added (arrow a), and the oxygen supply was terminated by flushing the vessel with nitrogen (arrow b). Curve B, Cells (final concentration, 0.35 mg of protein per ml) were introduced into an anaerobic vessel: 18 mM glucose was added (arrow c), and the cells were supplied with a saturating amount of oxygen (arrow d). The cells were preincubated with 2 μM PQQ.

To investigate whether oxidation of glucose in *A. lwoffi* could supply the energy for solute transport, the uptake of acetate and alanine was studied. Cells of *A. lwoffi* grown with acetate limitation in chemostat culture and starved for 1 h and preincubated with PQQ showed negligible accumulation of acetate. However, in the presence of glucose, acetate was accumulated rapidly (Table 3). In such cells D-xylose could replace glucose as the energy source, but gluconate could not, in agreement with the ability of these cells to oxidize glucose and xylose at high rates (via glucose dehydrogenase) but not gluconate. PQQ and glucose-dependent acetate uptake by intact cells of *A. lwoffi* was completely inhibited by DCCD at low concentrations. L-Alanine accumulation also could be energized by glucose in PQQ-pretreated *A. lwoffi* cells (Fig. 6) but not by gluconate. These results demonstrate that the oxidation of glucose in *A. lwoffi* is an energy-transducing process.

DISCUSSION

During a study of our laboratories on the role of membrane-bound glucose dehydrogenase in energy metabolism of gram-negative bacteria, it was discovered that *P. aeruginosa* and *A. lwoffi* synthesized inactive glucose dehydrogenase which could be fully activated by PQQ. This paper and a recent report by Hommes et al. (17) show that the same is true for a number of *E. coli* strains. It is not clear why these bacteria synthesized inactive glucose dehydrogenase. It has been suggested that PQQ might be found in a sufficiently high concentration in some natural environment and then might be regarded as a vitamin (42).

Our finding that the activity of apoglucose dehydrogenase could be manipulated with the concentration of PQQ opened new possibilities to study the role and contribution of this enzyme in the energy budget of the cell. The demonstration of the same phenomena in membrane vesicles of *E. coli*

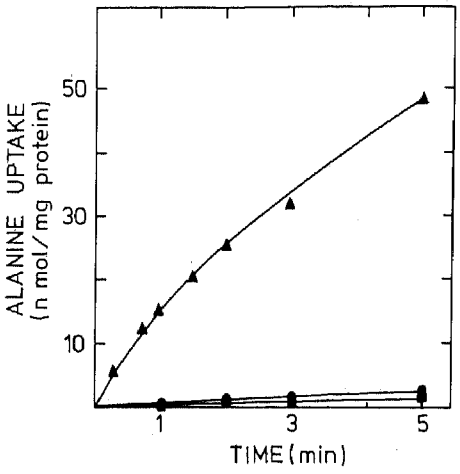

FIG. 6. L-Alanine uptake by energy-starved cells of *A. lwoffi*, energized by electron transfer via the PQQ-dependent glucose dehydrogenase. Cells were starved, and the uptake of L-[^{14}C]alanine was measured as described in the text. Cells were preincubated for 30 min with 2 μM PQQ. L-[^{14}C]alanine was added to a final concentration of 10 μM. Symbols: ▲, 18 mM glucose; □, 12 mM potassium gluconate; ●, no glucose added.

(Table 1) allowed a direct demonstration of the important role of glucose dehydrogenase in the generation of a proton motive force and in transport (Table 2). A few experiments with membrane vesicles of *P. aeruginosa* (Fig. 4) and whole cells of *A. lwoffi* (Table 3) served to show that our results concern a general property of membrane-bound, PQQ-dependent glucose dehydrogenase.

A more detailed analysis of the reconstitution of glucose dehydrogenase activity by PQQ in one of the *E. coli* strains (Table 1) showed that partial restoration of glucose oxidation already had a significant effect on the generation of a proton motive force and on secondary solute transport. Low PQQ concentrations therefore can have an important effect on energy metabolism (30, 32). At saturating PQQ concentrations glucose was the most effective electron donor for energizing secondary transport in membrane vesicles of *E. coli*. Coupling of glucose oxidation to secondary transport was even more efficient than with D-lactate (23), the artificial electron donor reduced phenazine methosulfate (26), or intravesicularly generated NADH (13). The explanation for this observation might be that glucose uptake is not required for the oxidation of this electron donor. Another possible origin for the high efficiency may be the direct interaction between the respiratory chain and secondary transport systems, as has been found in *R. sphaeroides* (11, 22).

The periplasmic localization of the quinoproteins methanol dehydrogenase and methylamine dehydrogenase (24) is in line with observations that electrons from these enzymes are donated to the respiratory chain at the level of cytochrome *c* (1). This fits with the relatively high redox potential of free PQQ (E'_0 = 90 mV) (6). However, *E. coli* and *Acinetobacter* spp. lack cytochrome *c* (21). Various observations suggest a coupling of glucose dehydrogenase at or before cytochrome *b* in these organisms. For example complexes of glucose dehydrogenase with cytochrome *b* are found in the initial stages of purification of glucose dehydrogenase from *A. calcoaceticus* (14). Our results also indicate a coupling of glucose dehydrogenase at or before cytochrome *b* in *P. aeruginosa*, since PQQ-dependent glucose oxidation took place in membrane vesicles of this organism under anaerobic conditions with nitrate as electron acceptor (Fig. 4). No information is available about the involvement of other respiratory chain components such as quinones. In this respect it is relevant that uniquinones, which cannot replace PQQ as prosthetic group (J. A. Duine, personal communication), can act as electron acceptors for the enzyme in vitro (31). Mutants of *E. coli* lacking quinone components of the respiratory chain are now being studied in our laboratories to investigate the role of quinones in glucose dehydrogenase-mediated glucose oxidation.

The contribution of glucose dehydrogenase to the total energy budget differs for the different organisms studied. On one hand most *Acinetobacter* strains metabolize glucose only to gluconate, which cannot be metabolized further. Nevertheless, this single oxidation step leads to a dramatic cell yield increase when cultures growing on acetate are supplied with additional glucose (5). On the other hand, *E. coli* and *P. aeruginosa* can grow on glucose as the sole carbon and energy source. Both organisms can also grow on gluconate as the sole carbon and energy source (4, 9), and oxidation of glucose to gluconate can occur by glucose dehydrogenase located at the outer surface (periplasmic side) (32). Strains deficient in glucose uptake should be able to grow on glucose as the sole carbon and energy source in the presence of PQQ (9, 38, 39). This has indeed been found for an *E. coli* strain lacking a functional PTS (17).

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