Glucose-dehydrogenase-mediated Solute Transport and ATP Synthesis in *Acinetobacter calcoaceticus*

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Evidence is presented that in *Acinetobacter calcoaceticus* oxidation of glucose to gluconate by the periplasmic quinoprotein glucose dehydrogenase (EC 1.1.99.17) leads to energy conservation. Membrane vesicles prepared from cells grown in carbon-limited chemostat culture exhibited (1) a high rate of glucose-dependent oxygen consumption and gluconate production, (2) glucose-mediated cytochrome reduction, (3) uncoupler sensitive, glucose-dependent generation of a membrane potential and (4) glucose-driven accumulation of amino acids. Furthermore, oxidation of glucose to gluconate by whole cells was associated with ATP synthesis. These results confirm and extend previous observations that periplasmic glucose oxidation can act as a driving force for energy-requiring processes. It is therefore concluded that the incomplete oxidation of glucose by bacteria may serve as an auxiliary energy-generating system.

INTRODUCTION

Bacterial glucose metabolism can proceed via initial phosphorylation of the substrate or by direct oxidation to gluconate. This latter reaction is catalysed by a membrane-bound glucose dehydrogenase (GDH, EC 1.1.99.17). The enzyme is located on the periplasmic side of the cell membrane, which implies that the oxidation is an extracellular event (Dawes, 1981; Dokter *et al.*, 1986). Frequently the turnover capacity of the glucose-oxidizing system exceeds that of gluconate uptake and metabolism. Thus, in the presence of excess glucose, gluconic acid is accumulated in the medium, a phenomenon already described by Bouteux (1880).

Dalby & Blackwood (1955) first established the importance of GDH in the production of gluconate by bacteria with '*Aerobacter aerogenes*' (*Klebsiella pneumoniae*). The characteristics of GDH from '*Bacterium anitratum*' (*Acinetobacter calcoaceticus*) were extensively studied by Hauge (1960). The nature of the unusual cofactor of the enzyme was, however, established only recently: the enzyme belongs to the class of quinoproteins which have pyrrolo-quinoline quinone (PQQ) as the prosthetic group (Duine *et al.*, 1986). GDH is coupled to the electron transport chain at the level of cytochrome *b* (Hauge, 1960; Beardmore-Gray & Anthony, 1986).

GDH has been detected in a wide range of Gram-negative bacteria of families such as *Enterobacteriaceae*, *Pseudomonadaceae*, *Rhizobiaceae* and *Neisseriaceae* (Ameyama *et al.*, 1985; van Schie *et al.*, 1985, 1987). The *in vivo* role of GDH has been the subject of many physiological

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Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; GDH, glucose dehydrogenase; PMS, phenazine methosulphate; PQQ, pyrrolo-quinoline quinone; SF6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidene-malonitrile; TPP, tetraphenylphosphonium.

and genetic studies with a variety of organisms. Invariably, however, such studies have been carried out with bacteria which possess, in addition to GDH, alternative mechanisms of glucose metabolism. This seriously hinders the interpretation of results obtained with *in vivo* studies. In view of this complication we decided to study the energetics of glucose oxidation by GDH in *A. calcoaceticus* LMD 79.41. This organism constitutively synthesizes GDH (Visser *et al.*, 1985) but is unable to grow on glucose or gluconate as the sole carbon source (Juni, 1978; van Schie, 1987). Preliminary results showed that in membrane vesicles of *A. calcoaceticus* glucose oxidation leads to energy conservation (Pronk *et al.*, 1985). This paper presents a more detailed study on the *in vitro* energetics of aldose oxidation in *A. calcoaceticus* LMD 79.41.

METHODS

Organism and growth. *Acinetobacter calcoaceticus* LMD 79.41 was obtained from the culture collection of the Laboratory of Microbiology and Enzymology, Delft. The organism was grown carbon-limited in an Applikon laboratory fermenter (working volume 1 litre) at a dilution rate of 0.15 h^{-1} and at 30°C . In order to prevent foaming, aeration was accomplished by vortex mixing. The dissolved O_2 tension was kept constant at 40% of air saturation by controlling the stirring rate. The pH was controlled at 7.0. The mineral medium was prepared according to van Schie *et al.* (1984). Sodium acetate (30 mM), alanine (20 mM) or sodium *p*-hydroxybenzoate (10 mM) were added as carbon sources.

Preparation of membrane vesicles. The procedure of Stinnett *et al.* (1973) was slightly modified. Cells from a carbon-limited continuous culture were harvested by centrifugation (10000 *g*, 10 min). The pellet was resuspended in an ice-cold solution containing 20% (w/v) sucrose, 2.5% (w/v) LiCl, 0.1% lysozyme, and 10 mM-potassium phosphate, pH 7.0/10 mM-MgSO₄, to a density of 2.5 g dry wt l⁻¹. The suspension was heated to 30°C and then incubated for 90 min on a rotatory shaker at that temperature. The osmotically fragile spheroplasts were collected by centrifugation (10000 *g*, 10 min) and the pellet was resuspended by means of a hypodermic syringe (fitted with a needle of 1.5 mm diameter) in the smallest volume possible of the above buffer without lysozyme. This suspension was slowly added to 50 vols ice-cold lysis buffer (10 mM-potassium phosphate, pH 6.6/1 mM-MgSO₄) under vigorous stirring. DNAase and RNAase were added to a final concentration of 20 µg ml⁻¹ each and the mixture was incubated at 30°C for 30 min. During this incubation the light-scattering of the cell suspension decreased due to cell lysis. This suspension was then centrifuged at 40000 *g* for 10 min at 4°C and the pellet was resuspended in 50 ml ice-cold buffer containing 100 mM-potassium phosphate, pH 6.6/10 mM-MgSO₄. Whole cells and large fragments were removed by centrifugation at 3000 *g* for 10 min. The supernatant was carefully decanted and the centrifugation step was repeated. The supernatant, which was devoid of whole cells as judged by phase contrast microscopy, was centrifuged (40000 *g*, 10 min) to collect membrane vesicles. The pellet was washed twice with the same buffer and the final pellet was resuspended to a final concentration of 1–5 mg membrane protein ml⁻¹. Vesicles were stored in liquid nitrogen.

Solute transport. With some batches of vesicles, anomalously low rates of alanine uptake were obtained, which could be enhanced by the addition of *N,N'*-dicyclohexylcarbodiimide (DCCD) (see Results). Such preparations were discarded. Uptake assays were performed at 30°C in small test tubes (internal diameter 9 mm, length 30 mm). Membrane vesicle suspensions were rapidly thawed and diluted in a buffer containing 100 mM-potassium phosphate, pH 6.6/10 mM-MgSO₄ to give a final concentration of 0.2–0.5 mg membrane protein ml⁻¹. When intact cells were used the final concentration was 0.02–0.06 mg dry wt ml⁻¹. The reaction mixture was aerated by rapid stirring with magnetic, Teflon coated, bars (length 7 mm, diameter 2 mm). The final volume of the reaction mixture after addition of electron donor and ¹⁴C-labelled solute was 100 µl. Membrane vesicles were preincubated with the appropriate electron donor for 1 min after which the uptake experiments were started by the addition of the ¹⁴C-labelled substrate (to a final concentration of 10 µM) with a Hamilton microsyringe. At the desired time the uptake reaction was terminated by dilution of the reaction mixture with 2 ml 100 mM-LiCl and filtration through nitrocellulose filters (20 mm diameter, 0.45 µm pore size, Schleicher & Schüll). After washing with 2 ml 100 mM-LiCl the radioactivity on the filters was counted in Scintillator 299 (Packard) using a Beckman 3801 scintillation counter (NEN).

Measurement of respiratory capacity. Respiration rates of whole cells and membrane vesicles were assayed polarographically with a Clark-type oxygen electrode (Yellow Springs Instruments). Vesicles were diluted in 100 mM-potassium phosphate buffer (pH 6.6)/10 mM-MgSO₄. Cells from carbon-limited chemostat cultures were assayed directly, without dilution, in the culture fluid. All determinations were carried out at 30°C and calculations were made on the basis of an oxygen concentration at that temperature of 225 nmol O₂ ml⁻¹.

Measurement of membrane potential. The membrane potential was determined using a tetraphenyl phosphonium⁺ (TPP⁺)-selective electrode as described previously (van Schie *et al.*, 1985); correction for probe-binding to the vesicle membranes was made according to Lolkema *et al.* (1982).

Electron microscopy. Vesicles were prefixed with 3% (v/v) glutaraldehyde and 1% (w/v) tannic acid in 0.1 M-

cacodylate buffer pH 7.2 for 1 h, washed five times with the same buffer and fixed with 1% (w/v) osmium tetroxide in 0.1 M-cacodylate buffer pH 7.2, containing 10 mM-CaCl₂ for 17 h at room temperature. After fixation the vesicles were stained with 1.5% (w/v) aqueous uranyl acetate during dehydration at the 50% (v/v) ethanol step and finally embedded in Spurr resin. Ultra-thin sections, post-stained with uranylacetate and Reynolds lead citrate, were examined in a Philips EM 201 electron microscope.

Cytochrome analysis. Absorption difference spectra of membrane vesicles were recorded with a Beckman UV 5260 spectrophotometer at room temperature. The protein concentration was 1.7 mg ml⁻¹ and glucose (40 mM) was added as electron donor; for complete reduction a few grains of dithionite were added. The cytochrome *b* content was estimated from the difference in absorption between 560 and 575 nm, assuming an absorption coefficient of 22 mm⁻¹ cm⁻¹ (Jones & Poole, 1985).

Measurement of ATP synthesis. Intracellular ATP levels were measured in chemostat cultured cells. Samples were starved for 2–3 h to deplete the ATP pool and diluted 100-fold in 0.9% NaCl. ATP was measured using a commercially available luciferin–luciferase bioluminescence assay system (M 2010 A Biocounter, LUMAC/3M).

Analytical assays. A Beckman model 915B Tocamaster total organic carbon analyser was used to determine the cellular carbon content. Bacterial dry weight was calculated assuming a carbon content of 50%. Protein was measured by the Lowry method with bovine serum albumin as standard.

Chemicals. Lysozyme, DNAase and RNAase were obtained from Sigma. [¹⁴C]Alanine (170 mCi mmol⁻¹, 6.29 GBq mmol⁻¹); [¹⁴C]glutamic acid (125 mCi mmol⁻¹, 4.63 GBq mmol⁻¹); [¹⁴C]glycine (117 mCi mmol⁻¹, 4.33 GBq mmol⁻¹); [¹⁴C]leucine (125 mCi mmol⁻¹, 4.63 GBq mmol⁻¹) and [¹⁴C]acetic acid (60 mCi mmol⁻¹, 2.22 GBq mmol⁻¹) were obtained from NEN. [¹⁴C]Gluconic acid (240 mCi mmol⁻¹, 8.89 GBq mmol⁻¹) was obtained from CEA, Service des molécules marquées. The uncoupler 3,5-di-tert-butyl-4-hydroxybenzylidene-malonitrile (SF6847) was a gift of Dr Y. Nishizawa (Sumitomo Chemical Industry, Osaka, Japan).

RESULTS

Preparation and respiratory characterization of membrane vesicles

The technique of Stinnett *et al.* (1973) for the preparation of membrane vesicles of *Pseudomonas aeruginosa* proved to be applicable to *A. calcoaceticus*. Routinely 40–60 mg membrane protein was obtained from 1 g cells (dry wt). Membrane vesicles were free of whole

Fig. 1. Electron micrograph of a thin section of membrane vesicles of *A. calcoaceticus*, prepared from an acetate-limited chemostat culture. Bar, 0.50 μ m.

Fig. 2

Fig. 3

Fig. 2. Inhibition of glucose oxidation in membrane vesicles prepared from acetate-limited chemostat cultures of *A. calcoaceticus* by cyanide. The rate of glucose oxidation is expressed as a percentage of the value without inhibitor [$1900 \text{ nmol O}_2 \text{ min}^{-1} (\text{mg protein})^{-1}$].

Fig. 3. Uptake of [^{14}C]alanine by membrane vesicles from *A. calcoaceticus*. The organism was grown in the chemostat under limitation of different carbon and energy sources. Solute transport was energized by electron transfer via PQQ-dependent glucose dehydrogenase. Alanine uptake in the absence of an electron donor was negligible. Vesicles were isolated from cells grown on acetate (○), *p*-hydroxybenzoate (△), and alanine (□).

Table 1. *Respiration rates of whole cells and membrane vesicles of A. calcoaceticus*

A. calcoaceticus preparations were obtained from continuous cultures grown at a dilution rate of 0.15 h^{-1} and at 30°C . Unless indicated otherwise, cells were cultured under acetate limitation (reservoir substrate concentration 30 mM).

Electron donor	Concn (mM)	Oxidation rate [$\text{nmol O}_2 \text{ min}^{-1} (\text{mg protein})^{-1}$]	
		Whole cells	Membrane vesicles
Acetate	5	510	0
Glucose	20	540	1957
Xylose	20	530	1941
Malate	10	185	84
Succinate	10	210	79
NADH	10	ND	1220
Gluconate	20	0	0
Glucose*	20	1088	3300

ND, Not determined.

* Cells grown in alanine-limited chemostat culture (reservoir substrate concentration = 20 mM).

cells or protoplasts, as judged by phase contrast microscopy. Electron micrographs of thin sections of a preparation of the membrane vesicles revealed vesicular structures, without recognizable cytoplasmic constituents (Fig. 1). Furthermore, absence of whole cells was also indicated by the negligible rate of acetate oxidation by vesicles prepared from acetate-grown cells (Table 1). Vesicles showed a much higher specific rate of glucose oxidation than whole cells. The reverse was found for malate and succinate oxidation.

GDH activity was always enriched 3- to 6-fold in the membrane fraction (Table 1). The isolation method proved to be very reproducible with respect to the respiratory activities of the vesicles. In five separate isolations from acetate-limited chemostat cultures the specific rate of glucose oxidation of vesicles differed by less than 3%. The orientation of the vesicles was not investigated in detail, but it seems likely that their orientation was right side out. This can be concluded from their bioenergetic parameters (see below) and from the fact that glucose, which

Table 2. Initial rate of solute uptake by membrane vesicles and whole cells from acetate-limited chemostat cultures of *A. calcoaceticus*, energized by electron transfer via the PQQ-dependent GDH

Amino acid	Initial uptake rate [nmol min ⁻¹ (mg protein) ⁻¹]	
	Cells	Membrane vesicles
Alanine	12	2.5
Glycine	ND	1.0
Leucine	ND	0.6
Glutamate	ND	0.8
Acetate	40	0.05
Gluconate	0.8	0.25

ND, Not determined

Table 3. Effect of various electron donors on alanine transport in vesicles obtained from acetate-limited cells of *A. calcoaceticus*

Electron donor	Concn (mM)	Initial uptake rate	
		[nmol min ⁻¹ (mg membrane protein) ⁻¹]	
Glucose	20	1.3	
Xylose	20	1.2	
PMS-ascorbate	0.2/20	1.35	
NADH	10	0	
Gluconate	20	0	
δ -Gluconolactone	20	0	
Malate	10	0.4	

was not taken up by whole cells and vesicles (data not shown), was oxidized by cells and vesicles with nearly the same affinity constant (1.1 and 0.8 mM respectively).

Various respiratory chain inhibitors were tested for their effect on the glucose-dependent O₂ consumption of vesicles. Glucose oxidation was partly sensitive to KCN. Low concentrations (less than 0.1 mM) caused 60% inhibition (Fig. 2), a value which hardly increased with increasing KCN up to 1 mM. Glucose oxidation was insensitive to antimycin A (8 μ M). Addition of rotenone (30 μ M) or myxothiazole (4 μ M) resulted in a partial reduction of glucose-dependent O₂ uptake (27 and 50% inhibition, respectively). Glucose oxidation was very sensitive to DCCD. Addition of 22 μ M DCCD inhibited glucose oxidation by 50%.

Active uptake of solutes by membrane vesicles

Electron transfer from glucose to O₂ via GDH energized the transport of several amino acids. In vesicles from acetate-grown cells alanine was accumulated with the highest initial rate (Table 2) and therefore this amino acid was used in further experiments. Unlike whole cells, membrane vesicles did not accumulate acetate.

Membrane vesicles, prepared from chemostat cells grown on different carbon sources, were all transport-active, albeit that initial uptake rates and plateau levels of accumulated alanine differed. Initial uptake rates were highest for vesicles prepared from alanine-limited cells [20 nmol alanine min⁻¹ (mg membrane protein)⁻¹, Fig. 3]. The same was found for whole cells: alanine-grown cells transported alanine more rapidly than acetate-grown cells [80 and 12 nmol alanine min⁻¹ (mg dry wt)⁻¹, respectively].

The effect of various electron donors on alanine transport was studied in membrane vesicles prepared from acetate-grown cells (Table 3). Xylose and the artificial electron donor system phenazine methosulphate (PMS) plus ascorbate were as effective as glucose in energizing transport. Malate-driven alanine uptake was lower than with aldoses as electron donor. Gluconate and gluconolactone were not able to energize transport (Table 3). NADH, which in contrast to gluconate was rapidly oxidized by vesicles (Table 1), was also ineffective. Although,

Fig. 4. Analysis of respiratory components present in membrane vesicles isolated from acetate grown *A. calcoaceticus*. The base-line (1) was obtained by scanning aerated membranes ($1.7 \text{ mg protein ml}^{-1}$) present in sample and reference cuvettes. Glucose (40 mM) was added to the sample cuvette and the difference spectrum was recorded (2). Complete reduction of the membranes in the sample cuvette was obtained by adding dithionite (3).

as mentioned above, the glucose-oxidizing capacity of vesicles from acetate-grown cells was the same in different batches, this did not hold for the rate of alanine transport. Occasionally preparations were obtained which exhibited uptake rates 2- to 5-fold lower than usual. Addition of low concentrations of DCCD ($50 \mu\text{M}$) restored uptake to normal rates. The cause of the deviating behaviour of such preparations is unknown. In most preparations (7 out of 10) the high rate of alanine transport was not stimulated by addition of DCCD. In such vesicles alanine was accumulated 500- to 1000-fold assuming a specific volume of $2 \mu\text{l (mg membrane protein)}^{-1}$ (Kaback & Barnes 1971), which implies an alanine gradient of 150–180 mV.

Coupling of glucose dehydrogenase to the electron transport chain

Membrane vesicles prepared from acetate-grown cells were examined for the presence of cytochromes by oxidized minus reduced difference spectra. Reduction with glucose revealed the presence of a *b*-type cytochrome with peaks at 565 and 529 nm, and a broad Soret peak with a maximum at 428 nm (Fig. 4). As compared to dithionite about 70% of the cytochromes were reduced with glucose as electron donor. From the dithionite reduced minus oxidized difference spectrum a cytochrome *b* concentration of $0.3 \text{ nmol (mg membrane protein)}^{-1}$ was calculated.

Generation of a membrane potential

Since active transport can be energized by the oxidation of glucose, electron transfer from glucose to O_2 via the respiratory chain should generate a transmembrane electrochemical potential ($\Delta\psi$, interior negative and/or ΔpH , interior alkaline). Measurements with a TPP^+ -selective electrode showed a glucose- and xylose-driven $\Delta\psi$ formation of about 110 mV. Addition of the uncoupler SF6847 at a concentration of $500 \mu\text{M}$ completely abolished glucose-driven $\Delta\psi$ generation. The concentration of SF6847 required to reduce $\Delta\psi$ by 50% was approximately $30 \mu\text{M}$.

Fig. 5. ATP synthesis in whole cells of *A. calcoaceticus*. Cells grown in acetate-limited continuous culture were starved to deplete the intracellular ATP pool. When endogenous respiration was zero and the cytoplasmic ATP concentration was low, the formation of ATP was measured as a function of time after addition of 20 mM-glucose (○), 20 mM-xylose (■), 5 mM-acetate (▲) or 20 mM-gluconate (●).

ATP synthesis by whole cells

From the *in vitro* experiments described above it is clear that in *A. calcoaceticus* the oxidation of glucose to gluconate is associated with the formation of a proton motive force that can energize active solute transport. Additional evidence for GDH-mediated energy conservation was sought in the measurement of ATP synthesis by whole cells. *A. calcoaceticus* is well suited for this type of experiment since addition of glucose to whole cells results in a stoichiometric oxidation of the sugar according to the equation $1 \text{ glucose} + \frac{1}{2} \text{O}_2 \rightarrow 1 \text{ gluconate}$ (van Schie *et al.*, 1984).

Cells of an acetate-limited continuous culture were starved for 2–5 h to lower the steady state concentration of intracellular ATP from 16 to 2 nmol ATP (mg dry wt)⁻¹. Addition of non-oxidizable substrates such as gluconate, lactose or alanine did not affect the ATP level in these cells. Addition of glucose, xylose or 2-deoxyglucose (all substrates of GDH), however, resulted in a rapid increase of the intracellular ATP concentration (Fig. 5). Addition of acetate also resulted in ATP synthesis, although at a lower rate than with glucose.

DISCUSSION

The present investigation clearly demonstrates that the oxidation of glucose to gluconate by the quinoprotein GDH can energize the transport of a number of amino acids in *A. calcoaceticus* LMD 79.41. These results confirm and extend earlier observations of GDH-mediated transport of solutes in membrane vesicles from *P. aeruginosa* (Stinnett *et al.*, 1973) and *Escherichia coli* (van Schie *et al.*, 1985).

Vesicles were prepared from cells grown under well-defined environmental conditions. Chemostat cultivation in general is well-suited for the study of the relationship between the physiological status of a cell and solute transport, but is seldom used as such. The method described here is also applicable to chemostat-grown *E. coli* B (B. J. van Schie, unpublished data). In addition, energization of active solute transport via periplasmic glucose oxidation with GDH is an ideal physiological system, since the dehydrogenase is directly accessible from the external medium. The activity of glucose dehydrogenase in the membrane vesicles was very high, the oxidation rate of glucose being 10–20 times faster than that of malate or succinate. Rates of O₂ consumption with the latter two substrates were in the same order of magnitude as those reported for membrane vesicles of other organisms (Konings, 1977). Membrane vesicles

isolated from alanine-grown cells transported alanine with an initial uptake rate that was five times higher than that of acetate-grown cells, probably as a result of the presence of a higher concentration of transport carriers for alanine in the membrane.

Although vesicles usually exhibited high rates of glucose oxidation and alanine transport, occasionally the transport activity was low. In such preparations the ATPase complex had probably been damaged during isolation: addition of the ATPase inhibitor DCCD restored $\Delta\psi$ generation and transport activity to the usual value, probably by preventing ATPase-associated proton leakage. A similar phenomenon (stimulation of active transport by DCCD) has also been observed with ATPase mutants of *E. coli* (van Thienen & Postma, 1973). It is noteworthy that the stimulation of active transport by DCCD in certain vesicle preparations occurred despite the fact that DCCD inhibited glucose oxidation. This finding is consistent with the observation of van Schie *et al.* (1985) that $\Delta\psi$ generation and energization of active transport can function optimally even at submaximal respiration rates.

Electron transport from GDH proceeds via cytochrome *b* (Hauge, 1960; Beardmore-Gray & Anthony, 1986). In *A. calcoaceticus* terminal oxidation may proceed via cytochrome *o* or cytochrome *d*. The level of these cytochromes in the membrane is dependent upon the O_2 concentration in the growth medium (Ensley & Finnerty, 1980). Cytochrome *d* levels are known to increase with decreasing dissolved O_2 concentration and cytochrome *d* is less sensitive to cyanide than cytochrome *o* (Jones & Poole, 1985). Since in acetate-grown cells addition of 0.1 mM-KCN resulted in only partial inhibition of glucose oxidation (Fig. 2), it can be concluded that electrons from GDH can be transferred not only to cytochrome *o*, but also to a relatively cyanide insensitive terminal cytochrome oxidase, probably cytochrome *d*.

Analysis of intracellular ATP levels in starved cells of *A. calcoaceticus* revealed an efficient coupling between glucose oxidation and ATP synthesis. Oxidative phosphorylation energized by glucose oxidation via a membrane-bound GDH has been demonstrated previously in *Gluconobacter liquefaciens* (Stouthamer, 1962). The rate of build-up of the ATP pool with glucose as a substrate was much faster than with acetate, although the rate of O_2 consumption with both substrates was comparable. The difference is most likely due to the fact that glucose is oxidized in the periplasm, whereas acetate must be taken up and transformed to acetyl-CoA at the expense of ATP before energy can be produced. In addition acetate, in contrast to glucose, allows biosynthesis, which will drain the ATP pool.

The results demonstrate that the oxidation of glucose via GDH can be effectively used for the energization of cellular energy-consuming processes. It is therefore not surprising that during growth of *A. calcoaceticus* in chemostat cultures on mixtures of acetate and glucose the cell yield is much higher than during growth on acetate alone (van Schie *et al.*, 1987). Taken together these results provide strong evidence for the physiological importance of GDH in the energy budget of *A. calcoaceticus*. In view of previous results (van Schie *et al.*, 1985) it seems highly probable that the same applies to the physiological role of GDH in a large variety of other bacteria.

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REFERENCES

- AMEYAMA, M., NONOBE, M., HAYASHI, M., SHIN-AGAWA, E., MATSUSHITA, K. & ADACHI, O. (1985). Mode of binding of pyrroloquinoline quinone to apoglucose dehydrogenase. *Agricultural and Biological Chemistry* **49**, 1227-1231.
- BEARDMORE-GRAY, M. & ANTHONY, C. (1986). The oxidation of glucose by *Acinetobacter calcoaceticus*: interaction of the quinoprotein glucose dehydrogenase with the electron transport chain. *Journal of General Microbiology* **132**, 1257-1268.
- BOUTROUX, L. (1880). Sur une fermentation nouvelle du glucose. *Comptes rendus hebdomadaires des séances de l'Académie des sciences* **91**, 236-238.
- DALBY, A. & BLACKWOOD, A. C. (1955). Oxidation of sugars by an enzyme preparation from *Aerobacter aerogenes*. *Canadian Journal of Microbiology* **1**, 733-742.
- DAWES, E. A. (1981). Carbon metabolism. In *Continuous Culture of Cells*, vol. 2, pp. 1-38. Edited by P. H. Calcott. Boca Raton, Florida: CRC Press.

- DOKTER, P., FRANK, J. Z. & DUINE, J. A. (1986). Purification and characterization of quinoprotein glucose dehydrogenase from *Acinetobacter calcoaceticus* LMD 79.41. *Biochemical Journal* **239**, 163–167.
- DUINE, J. A., FRANK, J. Z. & JONGEJAN, J. A. (1986). PQQ and quinoprotein enzymes in microbial oxidations. *FEMS Microbiology Reviews* **32**, 165–178.
- ENSLEY, B. D. & FINNERTY, W. R. (1980). Influences of growth substrates and oxygen on the electron transport system in *Acinetobacter* sp. H01–N. *Journal of Bacteriology* **142**, 859–868.
- HAUGE, J. G. (1960). Purification and properties of glucose dehydrogenase and cytochrome *b* from *Bacterium anitratum*. *Biochimica et biophysica acta* **45**, 250–262.
- JONES, C. W. & POOLE, R. K. (1985). The analysis of cytochromes. *Methods in Microbiology* **18**, 285–328.
- JUNI, E. (1978). Genetics and physiology of *Acinetobacter*. *Annual Review of Microbiology* **32**, 349–371.
- KABACK, H. R. & BARNES, E. M. (1971). Mechanism of active transport in isolated membrane vesicles. II. The mechanism of energy coupling between D-lactic dehydrogenase and β -galactoside transport in membrane preparations from *Escherichia coli*. *Journal of Biological Chemistry* **246**, 5523–5531.
- KONINGS, W. N. (1977). Active transport of solutes in bacterial membrane vesicles. *Advances in Microbial Physiology* **15**, 175–251.
- LOLKEMA, J. S., HELLINGWERF, K. J. & KONINGS, W. N. (1982). The effect of 'probe binding' on the quantitative determination of the proton motive force in bacteria. *Biochimica et biophysica acta* **681**, 85–94.
- PRONK, J. T., VAN SCHIE, B. J., VAN DIJKEN, J. P. & KUENEN, J. G. (1985). Energization of solute transport by PQQ-dependent glucose dehydrogenase in membrane vesicles of *Acinetobacter* species. *Antonie van Leeuwenhoek* **51**, 560.
- VAN SCHIE, B. J. (1987). *The physiological function of gluconic acid production in Acinetobacter species and other Gram-negative bacteria*. PhD thesis, Delft University of Technology, The Netherlands.
- VAN SCHIE, B. J., VAN DIJKEN, J. P. & KUENEN, J. G. (1984). Non-coordinated synthesis of glucose dehydrogenase and its prosthetic group PQQ in *Acinetobacter* and *Pseudomonas* species. *FEMS Microbiology Letters* **24**, 133–138.
- VAN SCHIE, B. J., HELLINGWERF, K. J., VAN DIJKEN, J. P., ELFERINK, M. G. L., VAN DIJL, J. M., KUENEN, J. G. & KONINGS, W. N. (1985). Energy transduction by electron transfer via a pyrrolo-quinoline quinone dependent glucose dehydrogenase in *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* (var. *lwoffi*). *Journal of Bacteriology* **163**, 493–499.
- VAN SCHIE, B. J., DE MOOY, O. H., LINTON, J. D., VAN DIJKEN, J. P. & KUENEN, J. G. (1987). PQQ-dependent production of gluconic acid by *Acinetobacter*, *Agrobacterium* and *Rhizobium* species. *Journal of General Microbiology* **133**, 867–875.
- STINNETT, J. D., GUYMON, L. F. & EAGON, R. G. (1973). A novel technique for the preparation of transport-active membrane vesicles from *Pseudomonas aeruginosa*: observations on gluconate transport. *Biochemical and Biophysical Research Communications* **52**, 284–290.
- STOUTHAMER, A. H. (1962). Energy production in *Gluconobacter liquefaciens*. *Biochimica et biophysica acta* **56**, 19–32.
- VAN THIENEN, G. & POSTMA, P. W. (1973). Coupling between energy conservation and active transport of serine in *Escherichia coli*. *Biochimica et biophysica acta* **323**, 429–440.
- VISSER, W., VAN SCHIE, B. J., DE BONT, J. A. M., VAN DIJKEN, J. P. & KUENEN, J. G. (1985). Continuous culture studies on the regulation of PQQ-dependent glucose dehydrogenase in *Acinetobacter calcoaceticus*. *Antonie van Leeuwenhoek* **51**, 563.