PROTON-MOTIVE FORCE IN THE OBLIGATELY ANAEROBIC BACTERIUM *CLOSTRIDIUM PASTEURIANUM*: A ROLE IN GALACTOSE AND GLUCONATE UPTAKE

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1. Introduction

Comparatively few studies of transport mechanisms have been undertaken with obligately anaerobic bacteria, and what little is known of carbohydrate accumulation in species of *Clostridium* suggests the involvement of phosphoenolpyruvate (PEP)-dependent phosphotransferase systems [1–5]. However, it has recently been reported that growing *Clostridium pasteurianum* maintains a pH gradient (interior alkaline) 'essential for the growing cell as it may be required for substrate accumulation and other types of transport processes' [6].

In this communication we report that two substrates fermented by *Cl. pasteurianum* and utilised as sole sources of carbon and energy for its growth, are indeed accumulated by inducible proton symport mechanisms driven by the proton-motive force (interior electronegative and alkaline). Galactose uptake is accomplished by electrogenic transport (motivated by the membrane potential, $\Delta\psi$ and/or the transmembrane pH gradient Δ pH), whilst gluconate uptake is an electroneutral process (motivated by the Δ pH).

2. Experimental procedures

Clostridium pasteurianum was supplied as ATCC 6013 by Mrs Winifred Ego, University of Hawaii, USA, and maintained as previously described [7]. Cultures were grown anaerobically at 37°C in the defined glucose (4%)-ammonium salts and vitamins medium of Robson et al. [8], with the glucose being replaced when required by D-galactose or sodium D-gluconate. Total cell counts were made microscopically using an improved Neubauer Counting Chamber. Exponential phase cultures were harvested by centrifugation and the organisms anaerobically resuspended in 50 mM potassium phosphate buffer pH 7.0, to a density of about 2×10^8 organisms/ml. The suspension was poised at an appropriately low E_h by the incorporation of 0.1% of a suitable reductant; ascorbate (adjusted to pH 7) was routinely employed. The anaerobic cell suspension was briefly stored under N_2 plus CO_2 (95:5 v/v) at 4°C, but immediately before use was warmed to 37°C while being sparged with O_2-free argon. Energy-depleted cells were prepared by anaerobic preincubation in reduced buffer for 3 to 4 h at 37°C. The organisms were then harvested and anaerobically resuspended in either 25 mM sodium phosphate buffer pH 7, or the glycylglycine buffer mixture of Harold and Baarda [9].

2.1. *Uptake of radioactively-labelled compounds*

A quantity (4 ml) of a pre-warmed suspension of organisms, was anaerobically transferred with immediate mixing, to a tube (0.6 cm diam.) continuously flushed with argon and containing 0.5 ml of [1-^{14}C]-galactose, or [U-^{14}C] gluconate (each 0.75 μmol of 3 μCi/μmol). During incubation at 37°C, samples (0.5 ml) were removed at intervals and filtered through Millipore membranes (0.8 μm pore size; 2.5 cm diam.). These were immediately washed with 10 ml of distilled water, dried, placed in vials containing 10 ml

of a toluene: PPO mixture (4.8 g of 2,5-diphenyloxazole in 1 litre of toluene), and their radioactivity measured in a Beckman LS-200B spectrometer.

2.2. *Chemicals*

Tetrachlorosalicylanilide (TCS) was the kind gift of Dr Colin Jones, University of Leicester, UK. Carbonylcyanide *m*-chlorophenylhydrazone (CCCP), *N,N'*-dicyclohexylcarbodiimide (DCCD) and valinomycin were purchased from Sigma London Chemical Co. Ltd. Radioactively-labelled compounds were obtained from the Radiochemical Centre, Amersham, UK.

3. Results

The uptake systems responsible for galactose and gluconate transport into *Cl. pasteurianum,* were not present in organisms grown on glucose, but were independently and inducibly synthesised by cultures grown on galactose and gluconate respectively, as sole sources of carbon and energy. The induced synthesis of neither transport system was repressed by inclusion in the growth medium of an equimolar concentration of glucose (table 1).

Uptake by *Cl. pasteurianum* of glucose and its non-metabolised derivative α-methylglucoside, is accomplished by a PEP-dependent phosphotransferase system (Booth and Morris, to be published). Yet gluconate is known to be metabolised by *Cl. pasteurianum* without prior phosphorylation [10], and, using toluenised cells, we obtained no evidence of PEP-dependent phosphorylation of galactose. Over 50% of the radioactively-labelled galactose or gluconate accumulated by whole organisms was immediately exchangeable with the corresponding, unlabelled compound when this was added in excess to the cell suspension.

3.1. *Transport by proton symport*

While accumulation of α-methylglucoside was not affected by the proton conductors TCS and CCCP, or

Table 1
Uptake of α-methylglucoside, galactose and gluconate by *Cl. pasteurianum* grown on different carbon sources

	Rate of uptake (pmol/min per 10^8 organisms)		
Growth substrate	[U-^{14}C]α-methylglucoside	[1-^{14}C]galactose	[U-^{14}C]gluconate
Glucose	598	nil	nil
Galactose	571	98	nil
Galactose plus glucose	930	117	nil
Gluconate	636	nil	36
Gluconate plus glucose	945	nil	56

Table 2
Effects of inhibitors on uptake by *Cl. pasteurianum* of α-methylglucoside, galactose and gluconate

	Rate of uptake (as % of rate found in absence of inhibitor)		
Inhibitor[a]	[U-^{14}C]α-methylglucoside	[1-^{14}C]galactose	[U-^{14}C]gluconate
TCS (5 μM)	100	9	2
CCCP (25 μM)	100	6	18
DCCD (10 μM)	109	nil	1

[a]Added in ethanolic solution (0.5% ethanol final conc.).

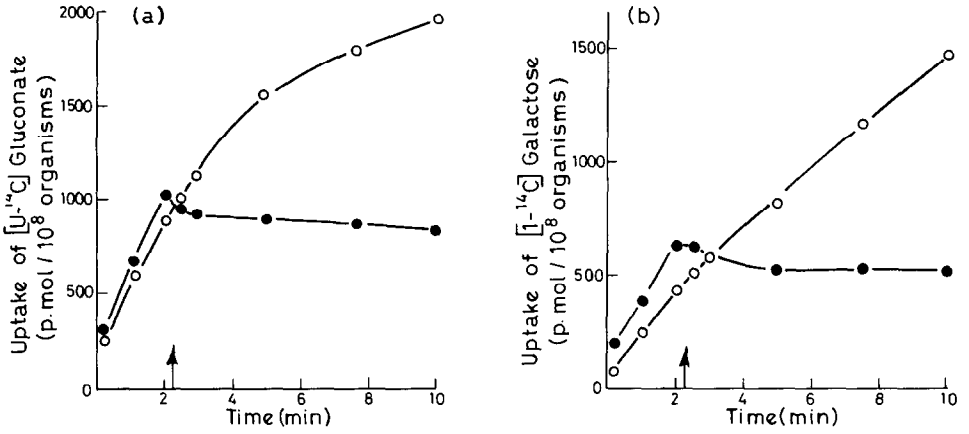

Fig.1. Inhibition by the proton conductor TCS of the uptake of (a) gluconate and (b) galactose into pre-induced *Cl. pasteurianum*. Uptake of the radioactively-labelled substrates was followed in the absence (○) and presence (●) of 5 μM TCS added (arrowed) after approx. 2 min incubation at 37°C.

by the membrane ATPase inhibitor DCCD, these compounds were potent inhibitors of both galactose and gluconate uptake by pre-induced organisms (table 2 and fig.1). This suggests that galactose and gluconate are actively transported into *Cl. pasteurianum* by proton symport powered by an ATPase-sustained protonmotiveforce. In support of this conclusion, alkalinisation of the medium was observed when galactose or gluconate were supplied to suitably grown, starved cells suspended in a lightly buffered glycylglycine medium pH 6.9.

According to the chemiosmotic theory [11–13], transport by proton symport would mean that electroneutral influx of gluconate should occur in response to a pH gradient (interior alkaline), and electrogenic influx of galactose in response either to such a pH gradient or to a membrane potential (interior negative).

3.2. *Galactose and gluconate uptake motivated by a pH gradient*

A favourable (if transient) pH gradient can be established by quickly increasing the (external) proton acti-

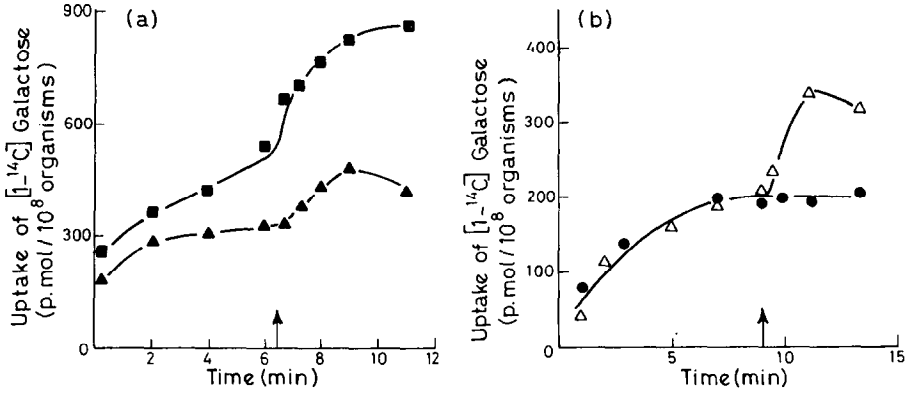

Fig.2. Uptake of [1-^{14}C]galactose by energy-starved *Cl. pasteurianum* following creation of (a) a membrane potential (interior negative) and (b) a pH gradient (interior alkaline). (a) Valinomycin (10 μg/ml) was added at approx. 6 min (arrowed) to energy-starved, galactose grown cells in 25 mM sodium phosphate buffer of pH 7.1 (▲) or pH 6.2 (■). (b) HCl was added at 9 min (arrowed) to similar cell suspensions as used in (a), causing an abrupt decrease in the (external) pH from pH 7.1 to pH 6.2 in the absence (△) or presence (●) of 5 μM TCS.

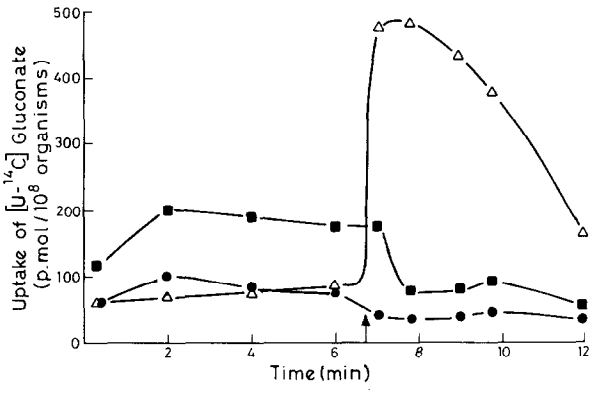

Fig.3. Uptake of [U-^{14}C]gluconate by energy-starved *Cl. pasteurianum* motivated by Δ pH but not by Δψ. Gluconate grown, energy-starved cells suspended in 25 mM sodium phosphate buffer were treated as follows: (i) HCl was added at approx. 7 min (arrowed) to cause a pH shift from pH 7.0 to 6.2 in the absence (△) or presence (●) of 5 μM TCS. (ii) Valinomycin (10 μg/ml) was added at approx. 7 min (arrowed) to a suspension at pH 6.2 (■).

vity in a suspension of energy-starved bacteria [14]. Immediate accumulation of galactose or gluconate was provoked by the abrupt creation of such a Δ pH in pre-induced, starved cells of *Cl. pasteurianum*. In each case this influx of substrate did not occur in the presence of the proton conductor TCS (fig.2 and 3).

3.3. *Galactose (but not gluconate) uptake motivated by the membrane potential*

An appropriate membrane potential (interior negative) may briefly be created by exposure to valinomycin of K^+-replete, energy-starved bacteria suspended in a medium of low K^+ activity [14,15]. Uptake of galactose but not of gluconate was observed, when this procedure was adopted with pre-induced, starved *Cl. pasteurianum* in 25 mM sodium phosphate buffer pH 6.2 (fig.2 and 3).

4. Discussion

Several excellent studies of anaerobic, energy coupled, nutrient transport have been made with obligately fermentative species of *Streptococcus* (e.g. [12,14]), but there is some suspicion that such organisms might be the descendants of facultative aerobes which, by 'retrogressive evolution', lost their former capacity to undertake electron transport-linked ATP synthesis [6]. Thus the possession by *Cl. pasteurianum* of a DCCD-sensitive membrane ATPase [6,16] which mediates proton translocation [6], is of possible phylogenetic significance, since this organism's antecedents were very likely obligate anaerobes which had never acquired the means of oxidative phosphorylation [6,17].

The protonmotive force generated in *Cl. pasteurianum* by ATP consumption, undoubtedly mediates ionic regulation, but is is also harnessed as in some other bacteria [9,18–20] to the active transport of galactose and gluconate (and possibly other nutrients). By this means, the range of fermentable substrates available to *Cl. pasteurianum* is extended beyond those carbohydrates utilised by its limited array of constitutive, PEP-dependent phosphotransferase systems.

Acknowledgement

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References

[1] Roseman, S. (1972) in: Metabolic Pathways (Greenberg, D. M., ed.), 3rd Edn. pp. 41–89, Academic Press, New York.
[2] Kornberg, H. L. (1973) Proc. Roy. Soc. Ser. B 183, 105–123.
[3] Groves, D. J. and Gronlund, A. F. (1969) J. Bacteriol. 100, 1256–1263.
[4] Patni, N. J. and Alexander, J. K. (1971) J. Bacteriol. 105, 226–231.
[5] Hugo, H. v. and Gottschalk, G. (1974) FEBS Lett. 46, 106–109.
[6] Riebeling, V., Thauer, R. K. and Jungermann, K. (1975) Eur. J. Biochem. 55, 445–453.
[7] Mackey, B. M. and Morris, J. G. (1971) J. Gen. Microbiol. 66, 1–13.
[8] Robson, R. L., Robson, R. M. and Morris J. G. (1974) Biochem. J. 144, 503–511.
[9] Harold, F. M. and Baarda, J. R. (1968) J. Bacteriol. 96, 2025–2034.
[10] Bender, R., Andreesen, J. R. and Gottschalk, G. (1971) J. Bacteriol. 107, 570–573.
[11] Mitchell, P. (1963) Biochem. Soc. Symp. 22, 142–168.
[12] Harold, F. M. (1972) Bacteriol. Rev. 36, 172–230.

[13] Hamilton, W. A. (1975) Adv. Microbial Physiol. 12, 1–53.
[14] Kashket, H. R. and Wilson, T. H. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2866–2869.
[15] Niven, D. F., Jeacocke, R. E. and Hamilton, W. A. (1973) FEBS Lett. 29, 248–252.
[16] Clarke, D. J. and Morris, J. G. (1975) Biochem. Soc, Trans. 3, 389–391.
[17] Morris, J. G. (1975) Adv. Microbial Physiol. 12, 169–246.
[18] Henderson, P. J. F. and Skinner, A. (1974) Biochem. Soc. Trans. 2, 543–545.
[19] Essenberg, R. C. and Kornberg, H. L. (1975) J. Biol. Chem. 250, 939–945.
[20] Robin, A. and Kepes, A. (1973) FEBS Lett. 36, 133–136.