Short Communications

The Role of Multivalent Cations in the Uptake and Oxidation of Glucose by *Pseudomonas fluorescens*

By CYNTHIA A. WALKER and NORMAN N. DURHAM

Department of Microbiology, Oklahoma State University, Stillwater, Okla. 74074, U.S.A.

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The effects of Mg^{2+} , Mn^{2+} , Ca^{2+} and Fe^{3+} on the uptake and oxidation of glucose in induced and non-induced *Pseudomonas fluorescens* cells were studied. Mg^{2+} is the most effective single metal ion in the uptake of glucose by the cell, and it functions in membrane stabilization and enzymic activity. Ca^{2+} will substitute for Mg^{2+} , but Mn^{2+} and Fe^{3+} will not.

Multivalent cations affect the transport and oxidation of substrates by micro-organisms. Eagon & Asbell (1969) reported that the uptake and oxidation of glucose, citrate and alanine by *Pseudomonas aeruginosa* was inhibited by Zn^{2+} . In the same study Mg^{2+} reversed the inhibition by Zn^{2+} when the system was self-buffered, and the authors suggested that Zn^{2+} acted at the membrane. Kepes & Cohen (1962) noted that Mg^{2+} stimulated tryptophan transport in *Escherichia coli*; and, in a personal communication to Eagon & Asbell (1969), Eicholz stated that Zn^{2+} , Fe^{3+} , Co^{2+} and Ca^{2+} inhibited binding of glucose to isolated intestinal brush borders, whereas Mg^{2+} and Mn^{2+} did not.

Walker & Durham (1972) noted that cells of *Pseudomonas fluorescens* NND grown in glucose or succinate as the sole source of carbon and energy showed great differences in sensitivity to actinomycin D. Glucose-grown cells were not sensitive to the antibiotic and readily oxidized glucose, whereas cells grown in succinate were readily inhibited by actinomycin D and utilized glucose only after a period of induction. The difference in sensitivity was attributed to different permeability traits of the cells as influenced by the lipopolysaccharide composition of the cell envelope. The succinate-grown cells readily bound and accumulated actinomycin D, whereas glucose-grown cells did not. Since cations are involved in cellular permeability, Mg^{2+} , Mn^{2+} , Ca^{2+} and Fe^{3+} (which comprise the mineral salts solution added to the synthetic salts medium) were studied to determine whether these metal ions influenced the uptake and metabolism of glucose by cells grown in the two different substrates.

Experimental

The cells were grown in a synthetic salts medium containing 0.42% K_2HPO_4 , 0.32% KH_2PO_4 , 0.2% NH_4Cl and 0.2% $NaCl$. The carbon source, glucose or succinate, was added in a final concentration of

0.2% and the pH was adjusted to 7.0. Glucose was sterilized separately and added aseptically. A sterile mineral salt solution containing Mg^{2+} , Mn^{2+} , Ca^{2+} and Fe^{2+} was added in the indicated concentrations (Table 1) just before use.

O_2 uptake by washed-cell suspensions was measured in a Warburg constant-volume respirometer at 37°C. The centre well contained 0.2 ml of 20% (w/v) KOH for CO_2 absorption and the side arms contained 2.8 mmol of glucose and the indicated metal ion(s) (Table 1). The cells were grown in the indicated carbon source, washed and suspended in a minimal-salts buffer (Durham & Keudell, 1969) to $E_{540} = 0.80$ and 2.1 ml was added to each flask. The total liquid volume in the flask was 2.7 ml.

The uptake of [^{14}C]glucose by induced (glucose-grown) or non-induced (succinate-grown) cells was used to measure the induction of the glucose-transport system and augment the results obtained in manometric studies. Toluene-treated cells, in which the permeability barrier is disrupted, were used in respirometric studies to evaluate the oxidative enzymes and substantiate the results on transport enzymes. Thus uptake and oxidation rates could be disassociated and both apparently must be induced.

Studies in this laboratory have established that *Ps. fluorescens* NND cells grown in succinate–salts minimal medium are unable to transport or oxidize glucose. Toluene-treated succinate-grown cells did not readily metabolize glucose, and chloramphenicol inhibited the induction of glucose transport; this suggests that enzymes for both these processes must be induced. Toluene-treated glucose-grown cells readily oxidize glucose at the same rate as do non-toluene-treated cells.

Results and discussion

Results indicated that, of the four metal ions tested (Mg^{2+} , Mn^{2+} , Ca^{2+} and Fe^{3+}), Mg^{2+} showed the highest rate of O_2 uptake in succinate-grown cells

Table 1. Induction time and glucose-oxidation rate of succinate- and glucose-grown *Ps. fluorescens* cells

The rate of oxidation was calculated from the linear region of the plot for O_2 uptake over a 10 min period after induction. Induction time represents the first deviation from the linear basal oxidative rate. Final concentrations of metal ions were as follows: Mg^{2+} , $20\mu M$; Mn^{2+} , $0.6\mu M$; Ca^{2+} , $3.7\mu M$; Fe^{3+} , $3.4\mu M$. The trace-mineral solution contained all cations in the indicated concentrations. O_2 consumptions were calculated as mol of O_2 consumed/mol of glucose.

Addition	Succinate-grown (non-induced) cells			Glucose-grown (induced) cells	
	Rate of oxidation (μl of O_2 /min)	Induction to glucose (min)	O_2 consumption (mol of O_2 /mol of glucose)	Rate of oxidation (μl of O_2 /min)	O_2 consumption (mol of O_2 /mol of glucose)
Water	2.7	131	2.94	6.8	2.44
Mg^{2+}	5.5	88	2.36	7.2	2.49
Mn^{2+}	4.2	108	2.21	6.4	1.84
Ca^{2+}	3.7	87	2.68	7.0	2.13
Fe^{3+}	4.4	114	2.60	7.2	2.06
Trace minerals	6.0	110	2.14	7.2	1.86

Fig. 1. Effects of metal ions on the uptake of glucose by (a) non-induced succinate-grown *Ps. fluorescens* cells and (b) induced glucose-grown *Ps. fluorescens* cells

(a) $[1-^{14}C]$ Glucose ($0.05\mu Ci/ml$) and unlabelled glucose ($2.8mmol$) were added to all flasks. ●, Trace-mineral solution; ▲, Mg^{2+} ($20\mu M$) or Ca^{2+} ($3.7\mu M$); ■, water, Mn^{2+} ($0.6\mu M$) or Fe^{3+} ($3.4\mu M$). (b) $[1-^{14}C]$ Glucose ($0.05\mu Ci/ml$) and unlabelled glucose ($2.8mmol$) were added to all flasks. ●, Trace-mineral solution; ▲, Mg^{2+} , Mn^{2+} , Ca^{2+} or Fe^{3+} ; ■, water.

after induction when compared with the water control. The rate with Mg^{2+} was approximately equivalent to that with the complete trace-mineral solution. In addition, Mg^{2+} shortened the time required

for induction to glucose (Table 1) in succinate-grown cells. In the presence of Mn^{2+} , Ca^{2+} or Fe^{3+} the succinate-grown cells also showed an increased rate of oxidation over the water control, but the rate

was not equivalent to the combination of cations (trace minerals) or to Mg^{2+} . Ca^{2+} shortened the induction time (when compared with the trace-mineral control), but Mn^{2+} and Fe^{3+} did not. The rates of oxidation in glucose-grown cells were similar, with the exception that the rate obtained with Mn^{2+} was consistently lower than in the water system containing no cations (Table 1). In contrast Mn^{2+} did not lower the oxidative rate below that obtained with the water control of non-induced cells, although the rate was far below that for Mg^{2+} . Mn^{2+} also decreased the total O_2 consumed/mol of glucose in both sets of cells. These findings indicate that Mn^{2+} may be acting at a rate-limiting step in glucose metabolism. [$1-^{14}C$]Glucose-uptake studies were performed to augment the results obtained from Warburg respirometry. Cells grown either in glucose or succinate were suspended in 20 ml of minimal-salts buffer containing 2.8 mmol of glucose as carrier, and the appropriate concentration of metal ion or trace-mineral solution was added. After equilibration to $37^\circ C$ [$1-^{14}C$]glucose (specific radioactivity 3.0 mCi/mmol; New England Nuclear, Boston, Mass., U.S.A.) was added and samples were removed at intervals, filtered and washed on Millipore filters (0.45 μm pore size) with minimal-salts buffer.

With non-induced succinate-grown cells both Mg^{2+} and Ca^{2+} promoted more rapid induction of uptake of radioactive glucose, whereas Fe^{3+} and Mn^{2+} did not. Neither, however, stimulated uptake to the same extent as did the complete trace-mineral solution (Fig. 1a). These results augment the results obtained in manometric studies, where both of these cations shortened the induction lag.

A combination of the two metal ions, Mg^{2+} and Ca^{2+} , does not produce an additive effect, and it can be inferred from this that the two metal ions are probably acting at the same site in induction of the uptake mechanism. However, the combination of Mn^{2+} , Ca^{2+} and Mg^{2+} produces a stimulatory effect, which approximately doubles the amount of [^{14}C]glucose that the cells will accumulate in the presence of individual cations and shortens the induction time. These results suggest that a co-operative effect is occurring. Mn^{2+} alone will not shorten the time of induction, but it may be involved in some aspect of metabolism apart from the induction of an active-transport system.

Induced cells showed a uniform uptake pattern regardless of the metal ion present (Fig. 1b). No single cation stimulated uptake to the same extent as did the complete trace-mineral solution. This indicates that more than a single metal ion is involved in achieving uptake at maximum efficiency in the induced-transport system and augments the findings obtained with the non-induced cells.

These results establish that Mg^{2+} stimulates both the respiratory rate and the induction of uptake with succinate-grown cells when glucose is the substrate. Mn^{2+} does not enhance the induction of uptake and may slightly depress the rate of oxidation in the induced system. Ca^{2+} and Fe^{3+} will stimulate induction over that obtained with the water control, but at the same time cause only a slight enhancement of respiratory rate.

It has been suggested by several researchers (Lusk *et al.*, 1968; Indge, 1968; Webb, 1968) that cations, particularly Mg^{2+} and Ca^{2+} , may play a role in membrane stabilization. Mg^{2+} and Ca^{2+} are known to be present in the membrane and wall of bacterial cells. Brown & Melling (1969) reported that growth of *Ps. aeruginosa* under Mg^{2+} and Ca^{2+} -limiting conditions caused a loss in sensitivity to EDTA, and they suggest that absence of these metal ions may greatly influence the molecular structure of the envelope. Thus the general role for a cation in uptake and oxidation may be as a stabilizing agent involved in the maintenance of the correct tertiary or quaternary structure of the proteins that function in transport. Mg^{2+} also serves as the cofactor involved in the first enzymic step of glucose oxidation.

Our results suggest that this specific requirement, along with the general requirement for a membrane-stabilizing agent, makes Mg^{2+} the most effective single metal ion in the uptake and oxidation of glucose. Induction of the respiratory and transport system for glucose in *Ps. fluorescens* will occur in the absence of trace minerals, but only after an extended period of time do they become detectable. The results indicate that Mg^{2+} acts at the cell envelope to hold the transport and respiratory proteins in the proper conformation for accumulation of glucose by the cell, and Ca^{2+} will substitute in the absence of Mg^{2+} , but Mn^{2+} and Fe^{3+} will not effectively replace Mg^{2+} .

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