Glucose Transport in Stationary-Phase Cultures of an Asporogenous Strain of *Bacillus licheniformis*

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The sporulation-deficient industrial organism *Bacillus licheniformis* HWL10 possesses two distinct glucose transport systems in log-phase cells, a glucose phosphotransferase system (PTS) and a non-PTS mechanism. The strain continues to take up glucose at a significant though reduced rate during prolonged stationary-phase incubation, but only the PTS is active.

Bacillus licheniformis is an important industrial microorganism which is used extensively for the production of extracellular enzymes (3). In an earlier report we established the presence of two distinct glucose transport systems in a laboratory strain of this organism (9). One of these is a phosphotransferase system (PTS), and the other is dependent on either the proton motive force or ATP. Under the appropriate conditions, the operations of the two systems can be demonstrated by using specific inhibitors. Thus, in the presence of an uncoupling agent, the uptake of glucose does not occur via the non-PTS mechanism. The glucose analog 2-deoxyglucose (2DG), which is a substrate only of the non-PTS mechanism, also selectively inhibits glucose uptake by this route. The residual uptake in the presence of either inhibitor is due to the glucose PTS. The earlier studies were restricted to actively growing cells, as this bacterium sporulates efficiently and completely. In industrial situations, however, cultures are generally grown for several days to maximize product accumulation. There is nothing known regarding the functioning of the two transport systems during this prolonged growth.

We recently reported the construction of a genetically engineered strain of *B. licheniformis* which carries a defined deletion in the *spoIIAC* gene (4). This deletion prevents the synthesis of the product of the *spoIIAC* gene, the sigma factor σ^F , which is necessary for the expression of essential sporulation-specific genes (6). The mutant, *B. licheniformis* HWL10, is totally asporogenous but retains important industrial characteristics. In particular, the strain remains viable for long periods without forming spores and hence offers a unique environment in which to study stationary-phase metabolism. We wished to characterize the strain further and in particular to examine glucose transport during prolonged growth of this industrially important organism.

Glucose uptake by stationary-phase cells. Whole cells of *B. licheniformis* HWL10 were prepared from cultures grown for either 12, 48, or 72 h in Spizizen salts minimal medium (1) with glucose as the sole carbon source, and they were assayed for glucose uptake as described previously (9). The accumulation of [¹⁴C]glucose by resting whole cells was monitored in the presence and absence of a 100-fold excess of 2DG. Glucose uptake by cells from 12-h cultures was reduced by approximately 50% in the presence of 2DG (Fig. 1A), which is in

agreement with results obtained with log-phase cells of our laboratory strain (9). However, when we assayed cells which had been prepared from cultures grown for 48 or 72 h, we found that although glucose was accumulated to a significant extent by these cells, uptake was not inhibited by excess 2DG (Fig. 1B and C). This implies that in late-stationary-phase cells, glucose is transported largely, if not exclusively, via a mechanism for which 2DG is not a substrate. Significantly, the extent of glucose uptake in both the 48- and 72-h cells was reduced relative to that of the 12-h cells, and uptake was equivalent to that seen for 12-h cells taking up glucose in the presence of excess 2DG (Fig. 1).

The uncoupling agent tetrachlorosalicylanilide (TCS) abolishes the proton gradient and lowers the intracellular ATP content (2, 10). We therefore monitored glucose uptake in the presence and absence of 5 μ g of TCS (a gift from I. R. Booth, University of Aberdeen) per ml by whole cells of *B. licheniformis* HWL10 grown for 12, 48, or 72 h. With whole cells from 12-h cultures, glucose uptake was sensitive to the effects of TCS and was significantly reduced by the uncoupler (Fig. 2A). This implies that glucose is transported at least in part by an energy-dependent mechanism driven by either the proton motive force or ATP. Similar results were previously obtained with log-phase cells of our laboratory strain (9). However, with whole cells prepared from both the 48- and 72-h cultures, we could find no evidence of sensitivity to TCS. Glucose was accumulated to the same extent after 48 and 72 h, and uptake was not reduced in the presence of TCS (Fig. 2B and C). The fact that glucose uptake in late-stationary-phase cells is not sensitive to the effects of the uncoupler indicates that the non-PTS transport mechanism is not operational in these cells, and hence uptake must occur via the glucose PTS.

Glucose PTS activity. The presence of PTS activity can be readily revealed as phosphoenolpyruvate (PEP)-dependent sugar phosphorylation in cell-free extracts. The extracts were prepared by the method of Mitchell and Booth (5), as described by Tangney et al. (10), from cultures which had been grown in Spizizen salts minimal medium with glucose as the sole carbon source. Cultures were grown for either 12 or 64 h. Extracts were assayed for PEP-dependent phosphorylation of [¹⁴C]glucose, as described previously (9). In the absence of a high-energy phosphate donor, there was no detectable phosphorylation of glucose. However, PEP stimulated phosphorylation both in extracts from cultures grown for 12 h (Fig. 3A) and in extracts from 64-h cultures (Fig. 3B), demonstrating the presence of a functional glucose PTS throughout the growth of this organism. The presence of a 100-fold excess of 2DG did

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FIG. 1. The effects of 2DG on glucose uptake by *B. licheniformis* HWL10. Whole-cell suspensions were prepared from cultures grown for 12 h (A), 48 h (B), or 72 h (C) on glucose as the sole carbon source. Accumulation of [14 C]glucose (0.2 mM final concentration) was monitored in the presence (■) or absence (▲) of 20 mM 2DG.

not inhibit glucose phosphorylation in extracts prepared from cultures at either stage of growth, confirming that it is not a substrate for the glucose PTS (Fig. 3), which is consistent with earlier results (9).

The most reasonable interpretation of the data presented here is that during the initial growth of *B. licheniformis* HWL10 there are two glucose transport systems in operation, as was seen previously for *B. licheniformis* NCIB 6346 (9). The non-PTS system is sensitive to TCS and can transport 2DG, and therefore the total glucose uptake by cells from actively dividing cultures is significantly reduced in the presence of either of these inhibitors. The fact that neither TCS nor 2DG has any effect on uptake in late-stationary-phase cells implies that this

mechanism of glucose transport is not in operation but that glucose is still transported via the PTS. Furthermore, a measure of the relative contribution of the PTS to total glucose uptake in actively dividing cells is the amount of glucose taken up by cells in the presence of either TCS or 2DG. It is therefore quite significant that the reduced rate of glucose uptake observed in late-stationary-phase cells is the same as that for cells from 12-h cultures in the presence of either inhibitor (Fig. 1 and 2). We therefore conclude that while two transport systems for glucose operate early during growth, the PTS is the predominant if not the sole mechanism for the transport of glucose during prolonged growth of *B. licheniformis*. A functional PTS was demonstrated in extracts prepared from late-

FIG. 2. Effects of TCS on glucose uptake by *B. licheniformis* HWL10. Whole-cell suspensions were prepared from cultures grown for 12 h (A), 48 h (B), or 72 h (C) on glucose as the sole carbon source. Accumulation of [14 C]glucose (0.2 mM final concentration) was followed in the presence (●) or absence (▲) of 5 μ g of TCS per ml.

FIG. 3. PTS activity in cell-free extracts of *B. licheniformis* HWL10. Extracts of *B. licheniformis* were prepared from cultures which had been grown for 12 h (A) or 64 h (B) on glucose as the sole carbon source. Extracts were assayed for phosphorylation of [14 C]glucose (0.2 mM final concentration). The assay mixtures contained the following additions: none (●), 1 mM PEP (■), or 1 mM PEP and 20 mM 2DG (▲).

stationary-phase cultures (Fig. 3); this was the first demonstration of any transport system at such a late stage of cultivation.

One trivial explanation as to why the non-PTS transport system is absent in late-stationary-phase cells would be that the mutant is energy depleted in the later stages of growth. This, however, is unlikely since maltose, which is accumulated by a proton symport mechanism in *B. licheniformis* (10), is readily taken up by the mutant even after 72 h of growth (data not shown), implying that a proton motive force must be present. It is more probable that the lack of the non-PTS transport system in late-stationary-phase cells is a consequence of the regulation of glucose transport mechanisms within the cell. It is tempting to speculate that the glucose PTS may be responsible for the inhibition of the alternative transport system. In *Bacillus subtilis*, for example, it has been shown that the glucose PTS can inhibit the uptake of both maltose and glycerol, neither of which is transported by a PTS in this organism (7, 8). Such regulation is an important consideration in terms of catabolite repression of enzyme synthesis, which is particularly relevant to an industrial microorganism. In fact, we have observed catabolite repression of enzyme activity in 72-h cultures of *B. licheniformis* HWL10 (unpublished data). It would be interesting to create a mutant lacking glucose PTS activity to examine the effect on sugar uptake and catabolite repression in stationary-phase cultures. In summary, this is the first investigation into sugar transport in late-stationary-phase cultures, and we have demonstrated that glucose transport remains active dur-

ing prolonged culture incubation but is restricted to a PTS mechanism of uptake.

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