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Listeria monocytogenes Scott A Transports Glucose by High-Affinity and Low-Affinity Glucose Transport Systems†‡

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***Listeria monocytogenes* transported glucose by a high-affinity phosphoenolpyruvate-dependent phosphotransferase system and a low-affinity proton motive force-mediated system. The low-affinity system ($K_m = 2.9$ mM) was inhibited by 2-deoxyglucose and 6-deoxyglucose, whereas the high-affinity system ($K_m = 0.11$ mM) was inhibited by 2-deoxyglucose and mannose but not 6-deoxyglucose. Cells and vesicles artificially energized with valinomycin transported glucose or 2-deoxyglucose at rates greater than those of de-energized cells, indicating that a membrane potential could drive uptake by the low-affinity system.**

Despite the role of *Listeria monocytogenes* in causing food-borne disease and product recalls (5), only recently has there been much interest in studying the transport processes used by *L. monocytogenes* to accumulate carbohydrates and other essential nutrients. Recently, it was reported that transport of essential di- and tripeptides by *L. monocytogenes* occurs via a proton motive force (PMF)-dependent system (20), whereas uptake of the osmolyte carnitine is driven directly by ATP (21). Another study demonstrated the existence of a fructose-specific phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS)-mediated transport system in *L. monocytogenes* (10). How *L. monocytogenes* transports sugars is particularly important because this facultative, nonrespiring organism depends on carbohydrates for its primary energy source (15). Glucose is reported to be its preferred carbohydrate (12). An early report (22) implicated a role for a high-velocity glucose uptake system during growth of *L. monocytogenes* at a low temperature (10°C), a characteristic that may contribute to its success as a food-borne pathogen.

Recently, evidence for two glucose transport systems in *L. monocytogenes* Scott A, a PEP-PTS and a non-PTS that appeared to be fueled by the PMF, was provided (2). Interestingly, both systems were inhibited by the bacteriocin pediocin JD (2). In this study, we have measured glucose uptake in cells and vesicles. The data support the hypothesis that two glucose transport systems are present in *L. monocytogenes* Scott A, a low-affinity PMF-driven system and a high-affinity PTS.

MATERIALS AND METHODS

Organism and growth conditions. *L. monocytogenes* Scott A was grown and maintained in tryptic soy broth containing 0.5% yeast extract (Difco Laboratories, Detroit, Mich.), as described previously (1).

Sugar uptake assays. Log-phase cells were harvested by centrifugation (10,000 × *g* for 10 min) and washed twice with 50 mM sodium phosphate buffer (pH 6.5). For kinetic and initial rate experiments, cells were resuspended in phosphate buffer and 50 μl (0.05 mg of cell protein) was added to triplicate reaction mixtures containing [¹⁴C]glucose (Sigma Radiochemicals, St. Louis, Mo.), at concentrations ranging from 0.05 to 20 mM. After 5 s of incubation at 21°C, a phosphate solution containing 5 ml of ice-cold 0.5 M glucose was added and the entire mixture was filtered through 0.45-μm-pore-size polyethersulfone filter membranes (Gelman Sciences, Ann Arbor, Mich.). The filters were washed

twice with 5 ml of the buffer-glucose quench solution and transferred to scintillation vials containing 4 ml of Ultima Gold scintillation cocktail (Packard Instrument Co., Meriden, Conn.), and the radioactivity was determined by liquid scintillation counting (model 3801; Beckman Instruments, Fullerton, Calif.). All counts were corrected for nonspecific binding. Kinetic plots were obtained from the average of three independent determinations. For most concentrations, rates were linear for 0.5 min (data not shown).

Other glucose and 2-deoxyglucose uptake experiments were performed using log-phase cells, which were harvested and resuspended in 100 mM sodium or potassium phosphate buffer (pH 6.5) to approximately 0.5 mg of protein/ml. Reactions were started by addition of [¹⁴C]glucose or [³H]2-deoxyglucose (Sigma Radiochemicals) at 0.5 or 15 mM. The mixtures were incubated at 21°C, and 50-μl samples were removed at intervals, rapidly filtered under a vacuum, and washed with 4 ml of cold buffer. Radioactivity was determined as described above.

Vesicle formation, electron microscopy, and sugar uptake. Membrane vesicles were prepared from 1 liter of log-phase cells by the osmotic lysis procedures of Russell et al. (14) and Otto et al. (11). Briefly, cells were centrifuged, resuspended in 30 ml of 20 mM sodium maleate buffer (pH 6.5) containing 3×10^6 U of lysozyme and 2×10^3 U of mutanolysin, and incubated for 1.5 h at 37°C. Protoplasts were harvested by centrifugation (27,000 × *g* for 20 min) and resuspended in 1 liter of 50 mM potassium phosphate buffer (pH 7) containing 10 mM MgCl₂, DNase, and RNase. After 20 min at 37°C, 15 mM EDTA was added and the mixture was incubated for 10 min. The membranes and debris were harvested by centrifugation at 23,000 × *g* and then resuspended in 50 mM phosphate buffer (pH 7) and recentrifuged at 750 × *g* to remove cell debris. The supernatant was centrifuged at 48,000 × *g* for 30 min, and the pelleted membrane vesicles were resuspended in buffer, frozen in liquid nitrogen, and stored at -70°C. Vesicles, protoplasts, and whole-cell preparations were fixed in 2% glutaraldehyde (0.1% sodium cacodylate, pH 7.4) for 1 h at 4°C. They were then minced into small fragments and returned to the fixative for an additional hour. After being rinsed in buffer, the specimens were fixed in 1% osmium tetroxide for 1 h at 4°C, then rinsed in buffer, dehydrated in a graded ethanol series, and embedded in an Epon-aradite mixture. The sections were stained with uranyl acetate and lead citrate and observed with a Philips 201c transmission electron microscope.

For sugar uptake experiments, membrane vesicles were thawed, centrifuged at 30,000 × *g*, and resuspended in 0.5 M potassium phosphate–10 mM MgCl₂ buffer, pH 7. After 10 min at 48°C, the vesicles were centrifuged as described above and resuspended in 600 μl of 11.4 mM sodium maleate buffer (pH 6.5) containing 11.4 mM MgCl₂ and 400 mM lactose to give a vesicle concentration of approximately 1.2 mg of protein/ml. To start the reaction, 15 mM [¹⁴C]glucose (5 μCi/μmol) was added, and 50-μl samples were removed and filtered through 0.22-μm-pore-size filters. The filters were washed with cold quench buffer and dried, and radioactivity was determined as described above. Intravesicle volumes (4.3 μg of vesicular water/mg of protein) and membrane potentials were determined as described previously for whole cells (1, 7).

Other procedures. Protein concentrations in cell and vesicle preparations were determined by the method of Lowry et al. (8). All chemicals, except where noted otherwise, were obtained from Sigma.

RESULTS AND DISCUSSION

Kinetics of glucose transport. It was previously shown that when the PMF in *L. monocytogenes* Scott A was reduced by the addition of ionophores or uncouplers, uptake of glucose was

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‡ This paper is dedicated to the memory of Corrine Parker.

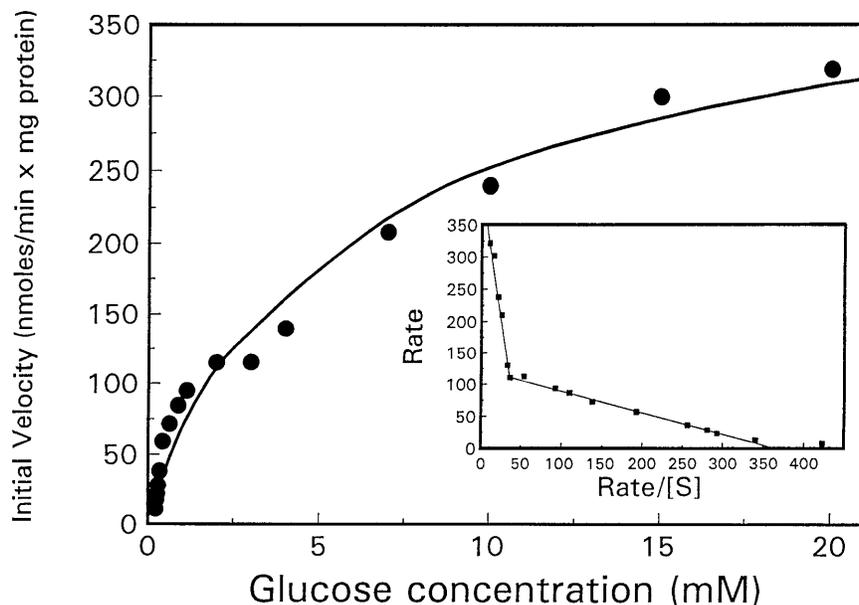


FIG. 1. Kinetics of glucose uptake by *L. monocytogenes* Scott A. Cells were incubated for 5 s with various concentrations of [^{14}C]glucose, filtered (in triplicate), and washed with ice-cold buffer containing 500 mM unlabeled glucose. An Eadie-Hofstee plot (inset) showed biphasic kinetics.

also inhibited but not eliminated entirely (2). That some glucose uptake still occurred, even when the PMF was completely dissipated, however, suggested that an alternative glucose transport system was functional. Furthermore, glucose was phosphorylated by *L. monocytogenes* Scott A cell extracts when PEP was added to the reaction mixture, indicating that this second transport system was a PEP-dependent PTS. We speculated that if *L. monocytogenes* uses two different systems for transporting glucose, these systems might be kinetically distinct. Indeed, when uptake rates at various glucose concentrations were determined, biphasic kinetics were observed, supporting the hypothesis that two systems were present (Fig. 1). One system was a high-affinity system, having an apparent K_m of 0.11 ± 0.09 mM (mean \pm standard deviation; $n = 3$), and the other system was a low-affinity system, having a K_m of 2.9 ± 1.05 mM ($n = 3$). The maximum velocities of the high- and low-affinity systems (means \pm standard deviations) were 50.1 ± 4 and 368.12 ± 15 nmol of glucose per min per mg of cell protein, respectively. The kinetics of these transport systems were not affected by the amount of glucose in the original growth medium (limiting versus excess glucose) or by the growth phase at which cells were harvested (data not shown).

Competition assays. Substrate competition experiments revealed other differences between the low- and high-affinity systems (Table 1). Uptake by the high- and low-affinity systems was determined by using 0.5 or 15 mM glucose, respectively. Although uptake by both systems would be expected to occur when glucose was present at the higher concentration (15 mM), some competing sugars did interfere with glucose uptake at either or both concentrations. Sugars such as galactose and arabinose did not interfere at all with glucose uptake when the latter was present at either the low (0.5 mM) or the high (15.0 mM) substrate concentration, whereas 2-deoxyglucose inhibited both systems. Of particular interest was the observation that 6-deoxyglucose inhibited the low-affinity system but had no effect on the high-affinity system, even when present at 100 times the glucose concentration. Because 6-deoxyglucose cannot be phosphorylated at the carbon-6 position, it cannot be

transported by the PTS. Thus, the inability of 6-deoxyglucose to inhibit the high-affinity system in our assay supports the hypothesis that this system is a PTS. In contrast, 6-deoxyglucose inhibited glucose uptake by the low-affinity system by about 50%. Mannose, which is accumulated by a glucose or glucose-mannose PTS in other gram-positive bacteria, including *Lactococcus lactis* subsp. *lactis* (18) and *Clostridium perfringens* (6), also inhibited the glucose high-affinity system in *L. monocytogenes* Scott A.

Based on the above results and the previous observation that glucose transport was inhibited by uncouplers and other agents that dissipate the PMF (2), we now argue that the PMF mediates uptake by the low-affinity glucose transporter in *L. monocytogenes* Scott A. We confirmed this hypothesis by measuring glucose and 2-deoxyglucose uptake in artificially energized cells and membrane vesicles (Fig. 2). In these experiments, energy to drive uptake was provided by generation of an artificial membrane potential ($\Delta\psi$). A $\Delta\psi$ in whole cells was

TABLE 1. Effects of competing sugars on high- and low-affinity glucose uptake systems in *L. monocytogenes* Scott A

Sugar added at 20 times excess	% Inhibition ^a	
	0.5 mM glucose	15 mM glucose
None	0	0
Glucose	58	58
2-Deoxyglucose	52	50
6-Deoxyglucose	2	47
3-O-methylglucose	18	31
Fructose	39	42
Galactose	0	18
Mannose	50	23
Arabinose	0	0
Xylose	12	24

^a Control glucose uptake rates at 0.5 and 15 mM glucose were 30 and 270 nmol per min per mg of protein, respectively.

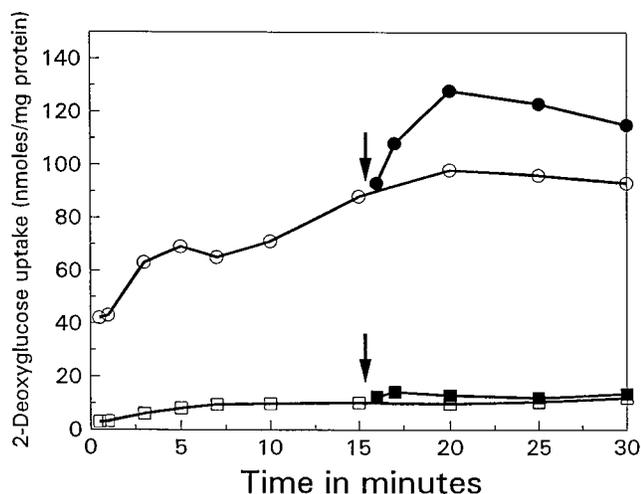


FIG. 2. 2-Deoxyglucose uptake by cells in response to valinomycin-induced potassium efflux. Cells were incubated in the presence of 100 mM potassium phosphate for 2 h, harvested by centrifugation, and resuspended in 50 mM sodium phosphate buffer containing either 0.5 (□) or 15 (○) mM [^3H]2-deoxyglucose. After 15 min, 15 μM valinomycin was added (closed symbols).

generated by treating de-energized, potassium-loaded cells with valinomycin, as described previously (11). Either 0.5 or 15 mM 2-deoxyglucose was added, and uptake was measured. 2-Deoxyglucose was used as the substrate rather than glucose to ensure that no energy could be derived via metabolism. When incubated in the presence of 0.5 mM 2-deoxyglucose, little uptake occurred in either de-energized or valinomycin-energized cells. Because the de-energized cells use the high-affinity PTS in the presence of 0.5 mM 2-deoxyglucose, energization by valinomycin treatment would not be expected to stimulate uptake under these conditions. Only when the valinomycin-treated cells were incubated in the presence of 15 mM 2-deoxyglucose did uptake occur, indicating that the low-affinity system was mediated by a PMF (or by the $\Delta\psi$). Furthermore, when cells were exposed to the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone following valinomycin treatment, transport by the low-affinity systems was abolished (data not shown).

Similar results were obtained with membrane vesicles (Fig. 3 and 4). Although the vesicles appeared to contain significant cytoplasmic material, no lactate dehydrogenase activity was detected in French press-lysed vesicle preparations (data not shown). The vesicles had a low membrane potential (mean \pm standard deviation, 39.9 ± 3.6 mV; $n = 4$), which increased to 49.6 ± 3.8 mV ($n = 4$) after valinomycin treatment. When glucose was added, transient uptake occurred. The intravesicular uptake of glucose was not maintained, however, as the free sugar apparently effluxed as the membrane potential dissipated.

Multiple glucose uptake systems in other gram-positive bacteria that have affinities within the range of those reported above have been described (4, 13, 16, 17). *Streptococcus mutans* transports glucose via a high-affinity PTS ($K_s = 6.8$ to 8.0 μM) and a low-affinity non-PTS ($K_s = 57$ to 125 μM); the latter is now thought to be an ATP-dependent, multiple-sugar metabolism transport system (3, 4). Two glucose transport systems are also present in *Bacillus licheniformis* NCIB 6349 (16). One uptake system, thought to be the dominant system, was driven by a PTS. The other, although not well-defined, has properties consistent with either an ATP- or a PMF-driven system. Re-

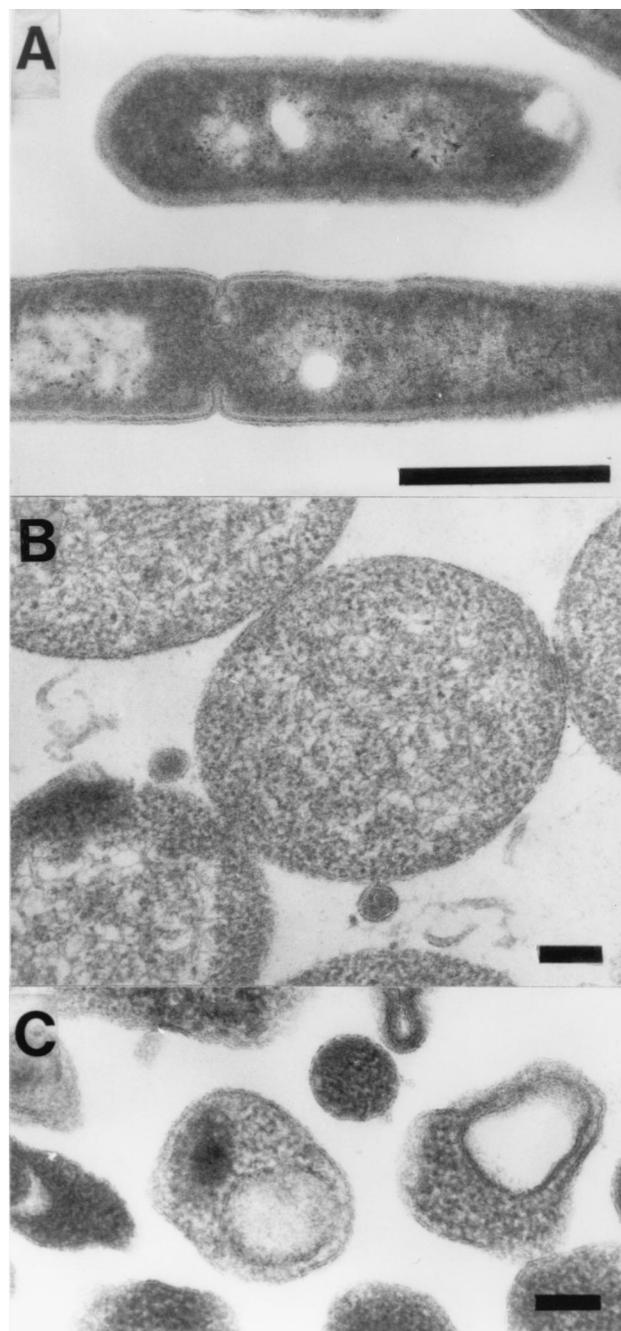


FIG. 3. Electron micrographs of a whole cell (A), protoplasts (B), and vesicles (C). Magnifications, $\times 60,000$ for whole cells and $\times 90,000$ for protoplasts and vesicles. Size markers were 0.5 (A) and 0.1 (B and C) μm .

cently, it was suggested that a non-PTS glucose uptake system in the related strain HWL10 may be regulated by the glucose PTS (17). In the ruminal bacterium *Streptococcus bovis*, a high-affinity glucose PTS and a low-affinity facilitated carrier are thought to confer optimal rates of carbon uptake in environments rich in glucose (e.g., the rumen), as well as under conditions in which sugar concentrations are low (9, 13). Similar advantages for the presence of high- and low-affinity glucose transport systems may also exist for *L. monocytogenes*. This organism is well adapted to growth in a variety of carbohy-

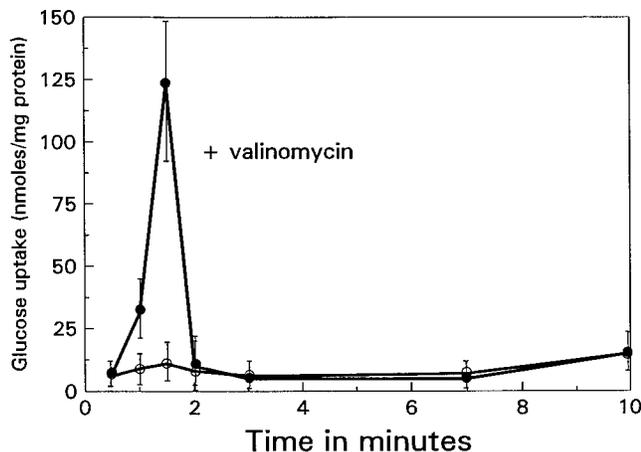


FIG. 4. Glucose uptake by membrane vesicles in response to valinomycin-induced potassium efflux. Vesicles were incubated in the presence of 500 mM potassium phosphate for 10 min, harvested by centrifugation, and resuspended in sodium maleate buffer containing 10 μ M valinomycin and 15 mM [2- 14 C]deoxyglucose (●). Valinomycin was not added to control cells (○). Values are means \pm standard deviations ($n = 3$).

drate-rich foods, as well as in foods that contain relatively little carbohydrate, such as meat, fish, cheese, and vegetables (5). As an intracellular parasite, *L. monocytogenes* also grows well within the cytoplasm of host tissue cells, where varying glucose concentrations might also be expected (19). Thus, when glucose concentrations are high, both the PMF-mediated system and the PTS are functional, but when the glucose levels are low, only the latter system would be active. In separate experiments, we have observed that the mRNAs coding for HPr and enzyme I genes of the PTS were transcribed when glucose concentrations were high (2a), suggesting that expression of these nonspecific PTS proteins by *L. monocytogenes* Scott A is constitutive.

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