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Glucose Uptake by *Listeria monocytogenes* Scott A and Inhibition by Pediocin JD†

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Glucose uptake by *Listeria monocytogenes* Scott A was inhibited by the bacteriocin pediocin JD and by the protonophore carbonyl cyanide *m*-chlorophenyhydrazone. Experiments with monensin, nigericin, chlorhexidine diacetate, dinitrophenol, and gramicidin, however, showed that glucose uptake could occur in the absence of a proton motive force. *L. monocytogenes* cell extracts phosphorylated glucose when phosphoenolpyruvate (PEP) was present in the assay mixture, and whole cells incubated with 2-deoxyglucose accumulated 2-deoxyglucose-6-phosphate, indicating the presence of a PEP-dependent phosphotransferase system in this organism. Glucose phosphorylation also occurred when ATP was present, suggesting that a proton motive force-mediated glucose transport system may also be present. We conclude that *L. monocytogenes* Scott A accumulates glucose by phosphotransferase and proton motive force-mediated systems, both of which are sensitive to pediocin JD.

Listeria monocytogenes is a facultative anaerobic pathogen that has been implicated in numerous food poisoning outbreaks and food product recalls (8). Despite significant interest in developing processes to control this organism, relatively little is known about the basic physiology of *Listeria* spp. (20). Although resting cells of *L. monocytogenes* Scott A at near neutral pH maintain a proton motive force (Δp) of about −120 mV (6), and energized, growing cells maintain a somewhat higher Δp (about −160 mV [4]), there are only a few reports demonstrating Δp-coupled metabolic activities in this organism (16, 22). That the Δp in *L. monocytogenes* is dissipated by nisin (4, 5), pediocins (5–7), and other bacteriocins (16) suggests that bacteriocin-mediated inhibition of *Listeria* spp. might occur as a result of the inhibition of an essential Δp-coupled activity, such as glucose transport. Therefore, our objective was to identify the glucose transport system in *L. monocytogenes* and to determine whether the dissipation of the Δp, either by bacteriocins or other agents, leads to diminished rates of glucose uptake.

Effect of uncouplers on the proton motive force. *L. monocytogenes* Scott A was grown overnight at 37°C in tryptic soy broth containing 0.5% yeast extract (Difco Laboratories, Detroit, Mich.) to a density of 0.25 mg/ml (dry weight). The pH of the broth was then adjusted to 5.0 with 1 N NaOH. Individual cell suspensions were treated with 0.1 mM dicyclohexylcarbodimide (DCCD), 0.014 mM nigericin, 0.05 mM gramicidin, 0.05 mM 2,4 dinitrophenol (DNP), 0.2 mM carbonyl cyanide *m*-chlorophenyhydrazone (CCCP), 0.2 mM chlorhexidine diacetate, or 0.017 mM monensin or left untreated. In some experiments, cells were treated with pediocin JD, a bacteriocin produced by *Pediococcus acidilactici* JD1-23 (2). Pediocin JD concentration (expressed as arbitrary units) and activity assays were performed as described previously (7). All cell suspensions were incubated for 20 min at room temperature. Estimates of the pH gradient (calculated as $Z\delta pH$, where Z = 59 mV per pH unit) and the membrane potential ($\Delta\psi$) were determined as described previously (7, 12).

Stationary-phase, nonenergized (glucose-depleted) *L. monocytogenes* cells maintained a Δp of −98 mV. As expected, the addition of the ionophores nigericin and monensin to these cells caused the ΔpH to collapse but had little or no effect on the $\Delta\psi$ (Table 1). Chlorhexidine diacetate and DCCD had little effect on either the ΔpH or the $\Delta\psi$, but addition of the uncouplers, CCCP and gramicidin, and pediocin JD led to dissipation of both the ΔpH and the $\Delta\psi$. An intermediate effect was observed with DNP. In contrast, growth of *L. monocytogenes* was inhibited by all of the ionophores and by chlorhexidine diacetate and pediocin JD (data not shown). In the presence of DNP, cells had a longer lag phase and longer generation time; DCCD had no effect on cell growth. Monensin, CCCP, nigericin, and gramicidin caused the ΔpH to collapse, suggesting that growth inhibition of *L. monocytogenes* might be caused either by dissipation of the ΔpH or by low intracellular pH, as suggested previously (11, 23). Collapse of the Δp or one of its components would also interfere with energy metabolism and result in diminished capacity of the cells to accumulate Δp-driven solutes. The latter has been suggested by various investigators as being responsible for the growth inhibition observed for bacteriocin-treated cells (4, 9, 16, 21), although inhibition of active sugar transport by bacteriocins has not been demonstrated.

Glucose and 2-deoxyglucose uptake. Log-phase *L. monocytogenes* was harvested by centrifugation at 6,300 × *g* (model J2-21; Beckman Instruments), washed, and resuspended to a density of 0.25 mg (dry weight) per ml in 50 mM sodium phosphate buffer, pH 6.5. When indicated, cells were treated with either active pediocin JD (200 arbitrary units per ml), inactive pediocin JD (treated with trypsin, 0.2 mg/ml), gramicidin, nigericin, DCCD, DNP, or chlorhexidine diacetate, at concentrations listed above. Assays were started by addition of [^{14}C]glucose (1 mM final concentration; 3.7 mCi/mmol; Sigma Radiochemicals, St. Louis, Mo.). At various times, 1-ml samples were removed and centrifuged (12,000 × *g* for 1 min) through silicon oil, and the radioactivity was determined by liquid scintillation counting as described previously (7). Glucose uptake occurred in the presence of monensin and nigeri-

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TABLE 1. Effect of inhibitors on the proton motive force in *L. monocytogenes* Scott A

Treatment	Value (mV)[a]		
	59ΔpH	Δψ	Δp
Control	−53 ± 2	−45 ± 23	−98 ± 23
Nigericin	−12 ± 3	−68 ± 3	−80 ± 3
Gramicidin	−10 ± 1	−18 ± 9	−28 ± 8
Monensin	−13 ± 2	−44 ± 30	−57 ± 45
Chlohexidine diacetate	−27 ± 4	−72 ± 7	−99 ± 7
DCCD	−55 ± 1	−38 ± 26	−93 ± 23
DNP	−17 ± 4	−28 ± 13	−45 ± 18
CCCP	−11 ± 3	−27 ± 16	−38 ± 18
Pediocin JD	−12 ± 2	−11 ± 6	−23 ± 5
Trypsin-treated pediocin JD	−41 ± 1	−31 ± 6	−72 ± 7

[a] Values are given as the averages ± standard deviation from at least 4 duplications.

cin, even though these agents had inhibited growth and had depleted the ΔpH (but not the Δψ) indicating that a ΔpH was not required to drive transport (Fig. 1). In contrast, both the ΔpH and the Δψ were reduced by addition of CCCP and pediocin JD, and glucose uptake decreased markedly (Fig. 1 and 2), indicating that glucose transport may be driven by a Δp. The latter agent inhibited uptake in a dose-dependent manner and caused efflux of preaccumulated label (data not shown). However, chlorhexidine diacetate, an agent known to inhibit the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) (17), inhibited growth and glucose uptake, despite having had no effect on the magnitude of the Δp. Also, gramicidin and DNP reduced both the ΔpH and the Δψ but

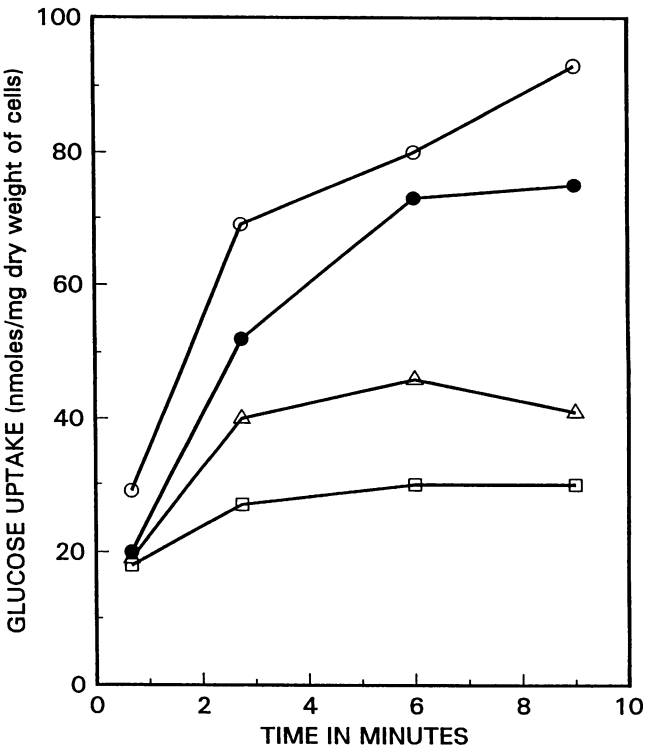

FIG. 2. Effect of pediocin JD on glucose uptake by *L. monocytogenes* Scott A. Cells were treated with 120 (●), 180 (△), or 240 (□) arbitrary units of pediocin JD per ml or with trypsin-treated pediocin (○).

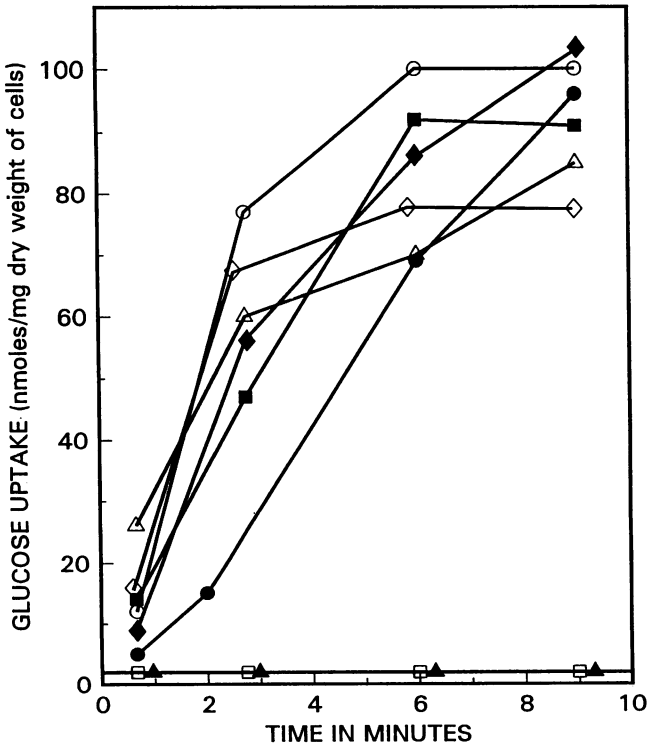

FIG. 1. Effect of gramicidin (◇), monensin (■), nigericin (●), DCCD (△), DNP (○), CCCP (□), chlorhexidine diacetate (▲), or no additions (◆) on glucose uptake by *L. monocytogenes* Scott A.

had no effect on glucose uptake. Therefore, we speculated that pediocin JD-mediated and chlorhexidine diacetate-mediated inhibition of *Listeria* spp. might be caused by inhibition of a glucose PTS transport system. However, the only PTS previously reported in *L. monocytogenes* is a fructose-specific PTS (18); there are no reports describing glucose uptake systems in this organism (13). That CCCP inhibited glucose uptake, as well as cell growth, suggests that this agent may have had a broader effect.

PTS activity. Log-phase *L. monocytogenes* cells were harvested, washed, and resuspended to a density of approximately 1.25 mg (dry weight) of cells per ml in 100 mM Tris buffer containing 5 mM $MgCl_2$, pH 7.0. A toluene:ethanol (1:9) mixture was added (30 μl/ml of cells) and the cells were vortexed for 1 min (17). PTS assays were performed by the method of Kornberg and Reeves (14). Reaction mixtures (1.0 ml) consisted of 50 mM Tris buffer (pH 7.0), 5 mM PEP or ATP, 5 mM $MgCl_2$, 0.11 mM NADH, 6 U of lactate dehydrogenase, and 50 μl of toluene-ethanol permeabilized cells. The reaction was started by addition of 5 mM sugar, and formation of NAD was measured by the decrease in A_{340}. In other experiments, PEP- or ATP-dependent formation of glucose-6-phosphate from glucose was determined directly, based on the rate of NAD reduction by glucose-6-phosphate dehydrogenase.

Phosphorylation of glucose by toluene-solubilized cell extracts occurred only when either PEP or ATP was available as the phosphoryl group donor (Table 2). However, PEP-dependent phosphorylation rates were about 35% greater than ATP-dependent rates (Table 2, trial 1). These results suggest the presence of a PTS as well as hexokinase or glucokinase.

TABLE 2. Phosphorylation of glucose by permeabilized *L. monocytogenes* Scott A

Treatment	% Activity[a]	
	Trial 1	Trial 2
Complete	100[b]	100[b]
− PEP, + ATP	73	0
− PEP, − ATP	18	0
+ PEP, + ATP	146	ND[c]
− glucose	0	0
+ pediocin JD	14	24
+ trypsin-treated pediocin JD	51	85

[a] Activities are expressed as a percentage of the activity of the complete assay mixtures (containing components listed in text). The trial 1 assay was glucose-6-phosphate dehydrogenase coupled, and trial 2 was coupled to lactate dehydrogenase.

[b] The mean ($n = 5$) rates of the complete assay mixtures for trials 1 and 2 were 76 ± 12 and 145 ± 25 nmol of glucose phosphorylated per mg of protein per min, respectively.

[c] ND, not done.

Pediocin JD caused an 85% decrease in PTS activity, an effect which could not be due to a pore-forming mechanism, since permeabilized cells were used in the PEP-supplemented PTS assays. The bacteriocin, however, had no effect on the activity of glucose-6-phosphate dehydrogenase, which was used as a coupling enzyme in this assay. When PTS activity was determined based on formation of pyruvate from PEP, similar results were obtained (Table 2, trial 2); however, no phosphorylation occurred when PEP was absent from the reaction mixture. Again, addition of pediocin JD caused a significant reduction in PTS activity, although part of this effect was due to inhibition of the coupling enzyme, lactate dehydrogenase, by pediocin JD.

To obtain more direct evidence that *L. monocytogenes* transported glucose via a PTS, [^{3}-H]2-deoxyglucose (2-DG; 1 mM final concentration; 5.1 mCi/mmol; Sigma Radiochemicals) was added to energized (fructose-treated) cell suspensions and the intracellular product was extracted with 5% butanol and spotted on silica gel thin-layer chromatography plates (Whatman, Hillsboro, Oreg.). Plates were developed in acetone:water (4:1) and air dried, and 1-cm sections were scraped with a razor blade and collected in vials for scintillation counting (10). Thin-layer chromatography of the intracellular product revealed two radioactive compounds, one (making up about 65% of the total intracellular radioactivity) which cochromatographed with the 2-DG standard ($R_f = 0.83$) and another (making up about 35% of the total intracellular radioactivity) which cochromatographed with the 2-DG-6-phosphate standard ($R_f = 0.45$). When the latter compound was eluted from the plate, treated with alkaline phosphatase, and rechromatographed, it comigrated with the 2-DG standard. The presence of free 2-DG indicates that a non-PTS permease may be present in *L. monocytogenes* Scott A.

There is now convincing evidence showing that pediocin JD, nisin, and other bacteriocins dissipate the Δp and interfere with membrane-associated functions in *L. monocytogenes* and other food-borne pathogens (3–7, 16, 19, 21). However, fewer data are available to demonstrate how depletion of the electrochemical or other ion gradients actually causes cell inhibition. Various investigators have suggested that bacteriocin-mediated deenergization of the cytoplasmic membrane was responsible for reduced rates of amino acids and potassium uptake by sensitive cells (22). Recently, Abee et al. (1) showed that lactacin F, a two-component bacteriocin produced by *Lactobacillus johnsonii*, caused phosphate efflux, resulting in hydrolysis of intracellular ATP and decreased growth rates in sensitive cells. Similarly, Chikindas et al. (6) reported that pediocin PA-1, a bacteriocin similar to, if not identical to, pediocin JD, forms aggregates within the membrane of sensitive cells that allow efflux of ions and other small molecules.

In this report, we show that pediocin JD and other agents that dissipate the $\Delta\psi$ component of the Δp inhibit glucose uptake, indicating the presence of an ion-coupled transport system in *L. monocytogenes*. We further suggest that this organism can alternatively transport glucose via a PTS. Pediocin JD inhibited the activity of this PTS, both in vivo and in a permeabilized extract. Although lactic acid bacterium-produced bacteriocins and colicins of the E1 group inhibit a variety of Δp-driven solute transport systems (15), inhibition of PTS-mediated processes by bacteriocins or colicins has not previously been reported. We also noted that the trypsin-inactivated bacteriocin had residual PTS-inhibitory activity (Table 2), suggesting that PTS inhibition by the pediocin may occur via interference of the bacteriocin or a trypsin-treated bacteriocin hydrolysis product with a PTS component rather than by leakage of PEP or an essential ion. We conclude that *L. monocytogenes* Scott A accumulates glucose by PTS and Δp-mediated systems, both of which are sensitive to pediocin JD.

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