Uptake of D-Xylose and D-Glucose by *Spirochaeta aurantia*

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Uptake of D-[14C]glucose and D-[14C]xylose by *Spirochaeta aurantia* was demonstrated to be osmotic shock sensitive and to require a high-energy phosphorylated compound rather than a proton motive force. These features are similar to those of binding protein-mediated transport systems in other gram-negative bacteria.

Spirochaeta aurantia is a strictly saccharolytic, facultatively anaerobic spirochete capable of utilizing a variety of sugars as energy sources for growth (3). One of these sugars, mannitol, enters cells via a phosphoenolpyruvate-dependent pathway similar in most ways to phosphotransferase systems of other bacteria (9, 10). The only available information regarding uptake of other sugars by *S. aurantia* is that a phosphotransferase system is not involved (10), and virtually nothing else is known about substrate transport in other spirochetes. To better understand catabolism in *S. aurantia*, it is necessary to characterize uptake of sugars other than mannitol. Furthermore, studies of sugar uptake in *S. aurantia* may lead to a better understanding of chemotaxis in this organism. In other gram-negative bacteria certain components of transport systems are also components of the chemotaxis system, even though transport of a chemoeffector is not required for a tactic response (1, 6, 7). For example, it has been demonstrated in several cases that periplasmic binding proteins are shared by chemotaxis and active transport systems (6, 7). Although a variety of sugars are effective attractants for *S. aurantia*, mannitol is not (5). To address the question of whether chemotaxis and transport in *S. aurantia* share any components, it was imperative to study transport of compounds that serve as attractants for this organism. Thus, in the present communication we present results of studies on uptake of two effective attractants, D-glucose and D-xylose (5) by *S. aurantia*.

The organism used with *S. aurantia* M1 (3). The growth medium contained 0.2 g of D-xylose, 0.5 g of Trypticase (BBL Microbiology Systems), and 0.2 g of yeast extract (Difco Laboratories) per 100 ml of deionized water. The medium pH was adjusted to 7.5 with KOH before autoclaving. Cells were grown in 250-ml Erlenmeyer flasks without shaking (100 ml of growth medium per flask). After incubation for ca. 40 h at 30°C, cells were harvested by centrifugation (27,000 × g for 10 min at 10°C). Inocula (2% [vol/vol]) were from 24-h cultures as described elsewhere (5).

Cells harvested as described above were washed and resuspended in either potassium phosphate buffer (10 mM, pH 7.0) or potassium arsenate buffer (10 mM, pH 7.0) to a density of ca. 1.7×10^9/ml. For osmotic-shock experiments, cell suspensions were prepared by a procedure based on that of Neu and Heppel (8). Cells were suspended in phosphate buffer plus sucrose (20% [wt/vol]), and after centrifugation these cells were resuspended in ice-cold phosphate buffer without sucrose. The cells were then separated from the shock fluid by centrifugation and were finally suspended in phosphate buffer (final density, ca. 1.7×10^9 cells per ml). As controls for the osmotic-shock experiments, a portion of

the culture was carried through the same regime, except it was not suspended in the presence of sucrose. In all cases centrifugation was 27,000 × g for 10 min at 10°C.

Generally, for uptake assays 0.1 ml of cell suspension was added to 10 mM phosphate buffer (pH 7.0). Uptake was initiated by addition of radiolabeled substrate (0.1 ml). Final volume was 1.0 ml. Unless otherwise specified, the radiolabeled substrate was either D-[U-14C]glucose or D-[U-14C]xylose at a final concentration of 100 μM (0.5 μCi) in phosphate buffer. For some experiments on the energetics of uptake, potassium arsenate was used in place of potassium phosphate. In such cases 1 μM phenazine methosulfate (PMS) and 10 mM sodium ascorbate were provided for maintenance of proton motive force (4).

After initiation of uptake, 0.1-ml samples were removed from reaction mixtures, immediately diluted in 5 ml of phosphate buffer to stop further uptake, and filtered through 25-mm-diameter membrane filters (pore size, 0.2 μm), and cells retained on the filter were washed with 5 ml of phosphate buffer. The amount of radiolabel retained on each filter was determined by standard scintillation counting procedures and is expressed in terms of nmoles of D-glucose or D-xylose. All uptake assays were at ambient temperature (ca. 25°C).

In some experiments, 2 μM cyanide-*m*-chlorophenyl hydrazone (CCCP), or PMS and ascorbate were present in reaction mixtures as indicated. In such cases these compounds were added 2 to 3 min before addition of the radiolabeled sugar.

Potassium arsenate, sodium ascorbate, PMS, CCCP, and unlabeled sugars were purchased from Sigma Chemical Co. D-[U-14C]glucose and D-[U-14C]xylose were purchased from Amersham Corp. The CCCP was dissolved in methanol. In cell suspensions containing CCCP the methanol concentration did not exceed 0.1%. This concentration of methanol did not inhibit glucose or xylose uptake.

Apparent K_m and V_{max} values for glucose and xylose uptake were determined by Lineweaver-Burk analyses of initial velocities of uptake at sugar concentrations between 5 and 200 μM. For D-glucose the K_m was calculated to be 1 μM, and the V_{max} was 0.45 nmol/min per 10^8 cells. Linear regression analysis gave a coefficient of correlation greater than 0.94. For D-xylose the K_m was 9 μM and the V_{max} was 0.3 nmol/min per 10^8 cells. A linear regression analysis yielded a coefficient of correlation greater than 0.92.

The metabolic inhibitors, arsenate and CCCP, were employed to study the energetic requirements for glucose and xylose uptake. It has been demonstrated previously that when *S. aurantia* cells are suspended in arsenate buffer containing PMS and ascorbate as the electron donor, ATP levels are extremely low (less than 2% of the ATP concentra-
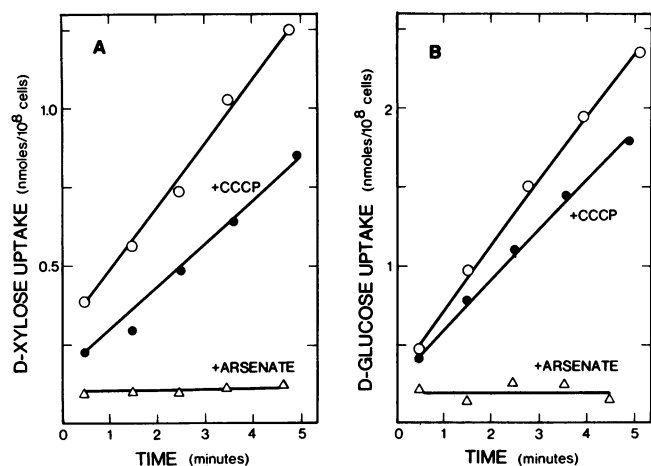
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FIG. 1. Effects of CCCP and arsenate on sugar uptake in *S. aurantia*. (A) D-[^{14}C]xylose uptake; (B) D-[^{14}C]glucose uptake. Symbols: ○, cells suspended in potassium phosphate; ●, cells suspended in potassium phosphate plus CCCP (2 μM); △, cells suspended in potassium arsenate plus PMS and ascorbate.

tion in the absence of arsenate), but the proton motive force is maintained. Addition of CCCP to cells in phosphate buffer results in a collapse of proton motive force, but the intracellular ATP concentration remains relatively high (4). Both D-glucose and D-xylose uptake remained relatively high when cells were suspended in phosphate buffer plus CCCP, and uptake of both sugars was completely inhibited in arsenate buffer plus PMS and ascorbate (Fig. 1). Motility of *S. aurantia* which is driven by a proton motive force (4) was monitored, and as expected, cells treated with CCCP were immotile and those suspended in arsenate plus PMS and ascorbate were motile. Apparently, the energy for uptake of both sugars is provided by a high-energy phosphorylated compound. A proton motive force is not sufficient to energize D-glucose or D-xylose uptake.

In those gram-negative bacteria that have been studied, high-affinity transport systems which require a high-energy phosphorylated compound typically involve periplasmic binding proteins (12). An osmotic shock causes the release of proteins residing in the periplasm and thus reduces uptake of substances transported by one of these systems (11). Osmotic shock severely inhibited glucose and xylose uptake (Table 1) without inhibiting motility. The fact that osmotically shocked cells retained their motility indicated that this treatment did not result in a loss of membrane integrity. Rather, these experiments indicate that periplasmic binding proteins are a component of the xylose and glucose uptake systems of *S. aurantia*.

TABLE 1. Effect of cold osmotic shock on sugar uptake in *S. aurantia*

Sugar (100 μM)	Rate of uptake (nmol/min per 10^8 cells)	
	Without osmotic shock	With osmotic shock
D-Glucose	0.43	0.09
D-Xylose	0.24	0.05

In conclusion, these experiments demonstrate that both D-glucose and D-xylose enter *S. aurantia* cells by a system that requires a high-energy phosphorylated compound (Fig. 1) and possesses an osmotic shock-sensitive component (Table 1). Furthermore, it has been previously demonstrated that in *S. aurantia* these sugars are phosphorylated by cellular kinases rather than a phosphotransferase system (10). All of these data are consistent with the notion that D-glucose and D-xylose enter *S. aurantia* cells via a periplasmic binding protein-mediated system that is similar in nature to binding protein-mediated transport systems of other gram-negative bacteria (2, 11), for example, the high-affinity D-xylose transport system of *Escherichia coli* (2). *S. aurantia* D-glucose and D-xylose binding proteins have not yet been identified, and the possibility that components of the transport and chemotaxis systems are shared remains to be addressed.

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