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D-Glucose Transport System of *Zymomonas mobilis*

ANTHONY A. DiMARCO† AND ANTONIO H. ROMANO*
Microbiology Section, The University of Connecticut, Storrs, Connecticut 06268

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The properties of the D-glucose transport system of *Zymomonas mobilis* were determined by measuring the uptake of nonmetabolizable analogs (2-deoxy-D-glucose and D-xylose) by wild-type cells and the uptake of D-glucose itself by a mutant lacking glucokinase. D-Glucose was transported by a constitutive, stereospecific, carrier-mediated facilitated diffusion system, whereby its intracellular concentration quickly reached a plateau close to but not above the external concentration. D-Xylose was transported by the D-glucose system, as evidenced by inhibition of its uptake by D-glucose. D-Fructose was not an efficient competitive inhibitor of D-glucose uptake, indicating that it has a low affinity for the D-glucose transport system. The apparent K_m of D-glucose transport was in the range of 5 to 15 mM, with a V_{max} of 200 to 300 nmol min^{-1} mg of $protein^{-1}$. The K_m of *Z. mobilis* glucokinase (0.25 to 0.4 mM) was 1 order of magnitude lower than the K_m for D-glucose transport, although the V_{max} values for transport and phosphorylation were similar. Thus, glucose transport cannot be expected to be rate limiting at concentrations of extracellular glucose normally used in fermentation processes, which greatly exceed the K_m for the transport system. The low-affinity, high-velocity, nonconcentrative system for D-glucose transport described here is consistent with the natural occurrence of *Z. mobilis* in high-sugar environments and with the capacity of *Z. mobilis* for rapid conversion of glucose to metabolic products with low energetic yield.

There has been increasing interest during recent years in the bacterium *Zymomonas mobilis* because of the potential for developing processes to convert sugars to ethanol. Although yeasts have traditionally been used for alcohol production, *Z. mobilis* has a number of advantageous features: (i) it carries out efficient conversion of glucose to ethanol rapidly and in high yield (2, 16, 20, 22, 26); (ii) it is more tolerant to elevated temperature, ethanol concentration, and sugar concentration than *Saccharomyces cerevisiae* (20, 22, 26, 27, 34); (iii) it shows uncoupled growth and fermentation; that is, ethanol production proceeds in the absence of growth (4, 14, 21). However, *Z. mobilis* shows one major disadvantage: the range of sugars fermented is very limited—only glucose, fructose, and sucrose are utilized, and the latter are not utilized in all strains. There is no capacity for utilization of polysaccharides or many other monosaccharides, such as mannose, galactose, or pentoses (34). A number of laboratories are now engaged in efforts to extend the range of carbohydrate utilization by recombinant DNA techniques (9, 30, 32, 33, 35).

It seems clear that knowledge of sugar transport systems is fundamental to the development of sugar fermentation processes, and this knowledge is of significance in planning strategies to introduce new sugar utilization capacities by genetic manipulation. It is important to know whether transport is a rate-controlling step and how transport is energized and regulated. This paper represents an initial approach to these questions.

The biology and physiology of *Z. mobilis* have been reviewed comprehensively by Swings and DeLey (34). *Z. mobilis* is a strictly fermentative bacterium, with no capacity for oxidative phosphorylation. The salient feature of its physiology is that it ferments sugars not by the classical Embden-Meyerhof-Parnas pathway, but by the Entner-Doudoroff pathway (15). An important consequence of this pathway is that the energetic yield is very low: only one molecule of phosphoenolpyruvate (PEP) or ATP is generated per molecule of hexose utilized. Thus, the expenditure of PEP via a PEP-hexose phosphotransferase system to transport sugars, or the expenditure of ATP to power an active transport system for sugars, would be energetically uneconomical. Accordingly, in an earlier study (28) of glucose transport systems of fermentative bacteria, we found that *Z. mobilis* did not have a PEP-glucose phosphotransferase system, and active transport of the nonmetabolizable analog 2-deoxy-D-glucose could not be demonstrated. A facilitated diffusion system for glucose was suggested, but no direct evidence was advanced. This study extends those findings, and describes the characteristics of a stereospecific carrier-mediated, D-glucose facilitated diffusion system.

MATERIALS AND METHODS

Organisms and media. *Z. mobilis* 10988 and CP4 were maintained on slants of YEP medium (peptone [10 g], yeast extract [10 g], glucose, [20 g], agar [15 g], distilled H_2O [1 liter]; pH adjusted to 6.8). Cells for sugar uptake experiments were grown in liquid YEP medium, with 0.1 M glucose or fructose, or on the basal medium of Kluyver and Hoppenbrouwers (17) supplemented with yeast extract, denoted BMY medium (K_2HPO_4 [1 g], $(NH_4)_2SO_4$ and $MgSO_4 \cdot 7H_2O$, [0.5 g], yeast extract [1 g], tap water [1 liter], pH adjusted to 6.8; glucose or fructose was sterilized separately and added to a final concentration of 0.1 M). All incubations were at 30°C in a water bath reciprocal shaker at 100 strokes per min. No precautions were taken to exclude oxygen.

Selection of a glucokinaseless mutant. A glucokinase-deficient mutant, *Z. mobilis* CP4-M2, that could grow on fructose but not glucose as an energy source was selected as follows. *Z. mobilis* CP4 was grown overnight in BMY medium with 50 mM glucose and 50 mM fructose, harvested, washed, and suspended in sterile Na-K phosphate buffer (pH 6.8) containing 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter (suspension buffer). After incubation of the cell suspension at 30°C

* Corresponding author.
† Present address: Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

for 1 h to establish a resting state, ethylmethane sulfonate was added (0.03 ml of ethylmethane sulfonate per 2 ml of cell suspension) and incubation continued for 2 h. Cells were harvested by centrifugation, washed with suspension buffer to remove the ethylmethane sulfonate, and inoculated into 50 ml of YEP medium with 0.1 M fructose. After 18 h of incubation, 5 ml of the culture was transferred to BMY medium containing 0.1 M glucose and 1,000 U of penicillin G per ml and incubated for 5 h to obtain penicillin counterselection. Survivors were harvested, washed with suspension buffer, propagated overnight on BMY medium with 0.1 M fructose, and then plated on YEP medium with 0.1 M fructose. Colonies were replicated on plates with YEP medium with 0.1 M glucose, and colonies which appeared on YEP-fructose plates but showed no growth on YEP-glucose were picked as presumptive glucokinaseless mutants. Four such colonies were picked from ca. 5,000 colonies screened. One of these, designated strain CP4-M2, was selected for further study on the basis of the typical growth rate and colony morphology on fructose-containing media and lack of growth on complex media such as YEP or nutrient agar containing glucose or no sugar.

Glucokinase assay. Lack of glucokinase activity in cell extracts of *Z. mobilis* CP4-M2 was confirmed by growing this strain and the wild-type parent strain CP4 on YEP plus 0.1 M fructose and harvesting, washing, and suspending cells in 0.2 M glycylglycine buffer (pH 7.2). Cell extracts of each were then prepared by sonication (4- to 30-s sonication periods in an ice bath, with intermittent 1-min cooling periods). The cells were assayed for glucokinase by the spectrophotometric method of Anderson and Kamel (1), whereby the formation of glucose-6-phosphate was coupled to $NADP^+$ reduction with glucose-6-phosphate dehydrogenase (from yeast; Sigma Chemical Co.). Extracts of strain CP4-M2 showed no detectable activity, whereas extracts of parent strain CP4 showed typical activity (K_m = 0.25 mM, 0.25 μmol/min per mg of protein); addition of extract from strain CP4 to an extract of strain CP4-M2 restored full activity.

Uptake of labeled sugars. Two procedures were used. The first, the long-term uptake procedure, involved the timed sampling at 1- to 2-min intervals over a period of 12 min of a batch cell suspension to which isotopic sugars had been added. The second, the short-term uptake procedure, involved mixing of individual small volumes of cell suspension and isotopic sugar solutions for periods of 2 s to 1 min and stopping uptake by addition of ice-cold buffer, followed by rapid filtration. Details were as follows.

(i) Long-term uptake. Cells in the late logarithmic phase were harvested by centrifugation and suspended in a 0.05 M sodium or potassium phosphate buffer, pH 6.8, containing 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter (suspension buffer) at an optical density of 1.0 at 540 nm (0.2 mg of cell protein per ml). Cell suspensions (5 ml) were incubated in a water bath shaker at 30°C for 15 min to allow temperature equilibration. Uptake was initiated by adding 0.5 ml of isotopic sugar solutions at the concentrations and specific radioactivities specified. Samples (0.5 ml) were removed at 1- to 2-min intervals, filtered through membrane filters (0.8 μm pore size; Millipore Corp., Bedford, Mass.), and washed with 3 ml of suspension buffer. Filters with cells were transferred to 10 ml of Bray scintillation fluid (7) for radioassay in a Packard liquid scintillation spectrometer.

(ii) Short-term uptake. Cells in the late logarithmic phase were harvested by centrifugation, washed three times with suspension buffer by rapid agitation on a vortex mixer for 1 min, and suspended to a density of ca. 1 mg of cell protein per ml (this thorough washing procedure was found to be helpful in reducing filtration time, probably because surface capsular material was removed; in practice, cell density was adjusted before each experiment so that the dilution, filtration, and washing procedures described below could be completed in 5 to 7 s). Uptake was initiated by forcefully ejecting 0.1 ml of cell suspension from an automatic pipette into 0.1 ml of isotopic sugar solution, at concentrations and specific activities specified in a test tube (9 by 1.7 cm), gently stirred on a vortex mixer at room temperature. Uptake was stopped at appropriate times by rapid addition of 5 ml of ice-cold suspension buffer from an automatic syringe, filtration on a membrane filter (0.8 μm pore size), and washing with an additional 5 ml of cold buffer. Filters were immediately transferred to 10 ml of Bray fluid for radioassay as above.

In both of the above procedures, protein content of the cell suspension used in each experiment was determined by the method of Lowry et al. (23) after solubilizing the cell protein by extraction with 1 N NaOH at 100°C for 15 min. Results are expressed as nanomoles of sugar taken up per milligram of protein.

Inhibitors. When carbonylcyanide-*m*-chlorphenylhydrazone (CCCP) and *N*,*N*′-dicyclohexylcarbodiimide (DCCD) (both from Sigma Chemical Co.) were used as inhibitors in uptake determinations, they were added as ethanolic solutions (0.05 ml of 5 mM CCCP or 0.05 ml of 0.1 M DCCD per 5 ml of cell suspension). An equal volume of 95% ethanol was added to the control suspension.

Source of isotopes. D-[*U*-^{14}C]glucose, L-[1-^{3}H]glucose, L-[*U*-^{14}C]proline, and D-[*U*-^{14}C]fructose were from New England Nuclear Corp., Boston, Mass.; 2-deoxy-D-[*U*-^{14}C]glucose was from ICN, Irvine, Calif.; and D-[*U*-^{14}C]xylose was from Amersham-Searle Corp., Arlington Heights, Ill.

RESULTS AND DISCUSSION

Uptake of metabolizable sugars by wild-type cells. In initial experiments, the uptake of isotopic D-glucose or D-fructose by *Z. mobilis* 10988 grown on glucose or fructose was measured. Such experiments involving metabolizable sugars reveal little of the nature of the transport process itself, since the incorporation of isotopic sugar that is measured is the resultant of transport, metabolism, and incorporation into cellular materials. Nevertheless, they are useful in indicating characteristics of constitutivity versus inducibility, relative rates of overall uptake and incorporation, and patterns of preferential utilization or inhibition. Results shown in Fig. 1 indicate the following. (i) Overall uptake of D-glucose or D-fructose is constitutive. Although the rate of D-glucose uptake was always three- to fourfold higher than the rate of D-fructose uptake (note difference in scale in Fig. 1A versus Fig. 1B and C), the individual rates of uptake of D-glucose or D-fructose, respectively, were similar in glucose- or fructose-grown cells (to compare rates of D-glucose uptake in fructose- versus glucose-grown cells, compare Fig. 1A with Fig. 2). Rates of D-glucose or D-fructose uptake by sucrose-grown cells (data not shown) were similar to those shown for glucose- or fructose-grown cells. (ii) A 20-fold concentration excess of glucose strongly inhibited the overall uptake of D-fructose, whereas a similar concentration excess of D-fructose had only a small effect on D-glucose uptake. This may indicate that D-glucose and D-fructose share a common uptake system with a much greater affinity for D-glucose, but other explanations are possible, such as competition for

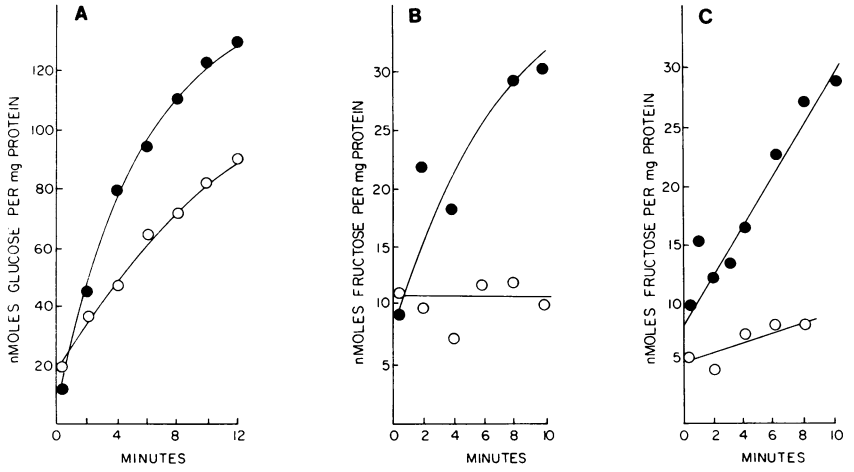

FIG. 1. Uptake of D-glucose and D-fructose by *Z. mobilis* ATCC 10988. Cells grown on YEP medium plus 0.1 M glucose (C) or fructose (A and B) were incubated with D-[^{14}C]glucose (A) or D-[^{14}C]fructose (B and C) (each 1 mM; 1 μCi μmol^{-1}) in the absence (●) or presence (○) of 20 mM nonradioactive D-fructose (A) or D-glucose (B and C) as indicated, added at −5 min.

available ATP for phosphorylation by intracellular kinases, noncompetitive inhibition of fructose uptake by glucose (catabolite inhibition) of the type shown in many other organisms (11, 18, 25), or inhibition of fructokinase by glucose or glucose-6-phosphate (12).

Uptake of nonmetabolizable analogs. Our initial approach to focus on the D-glucose transport system in the absence of metabolism was through the use of the nonmetabolizable analogs 2-deoxy-D-glucose and D-xylose, the latter being a D-glucose homomorph lacking the 5-CH_2OH group. Results of a long-term uptake experiment with *Z. mobilis* 10988 are shown in Fig. 2. As expected, D-glucose was extensively taken up and incorporated. However, uptake of 2-deoxy-D-glucose and D-xylose was minimal. Nevertheless, calculation of intracellular concentration of these analogs, based on intracellular water space of 3.3 μl per mg (dry weight) or 5.5 μl per mg of protein, as determined by the method of Maloney et al. (24), revealed an intracellular concentration near that of the external solution, with equilibration occurring within the first sampling interval.

The sampling procedure used in these initial long-term experiments proved inadequate for measuring uptake events during the first minute, since the sampling, filtration, and washing steps took ca. 30 s. We therefore developed the short-term procedure described above. We used *Z. mobilis* CP4 in the remainder of these experiments, since this strain more consistently yielded washed suspensions that could be filtered and washed within 5 s. (Strains CP4 and 10988 behaved similarly in long-term determinations of 2-deoxy-D-glucose uptake; data not shown). Results of a short-term uptake experiment with strain CP4 with 2-deoxy-D-glucose and D-xylose are shown in Fig. 3. Uptake of both 2-deoxy-D-glucose and D-xylose was very rapid, reaching plateaus within 10 s that were ca. 40 and 67%, respectively, of the external concentration. A facilitated diffusion system is clearly indicated, although a concentrative active transport system cannot be ruled out on the basis of these data, since the washed cells in the absence of a utilizable sugar may be in an energy-depleted state. This question is addressed later.

The uptake of both these analogs was strongly inhibited by D-glucose, indicating that they are transported by the D-glucose system.

Uptake of D-glucose by glucokinase-deficient mutant. A mutant deficient in the capacity to phosphorylate glucose offers the optimum system to study D-glucose transport, since the natural substrate could be used. The finding by Doelle (13) that *Z. mobilis* does not have a broad-specificity hexokinase, but has a distinct glucokinase and fructokinase offered a straightforward strategy for the selection of such a mutant. Thus, we derived a glucokinaseless mutant from *Z. mobilis* CP4 by mutagenesis and selection for the capacity to grow on fructose but not on glucose (see above). The long-term uptake of glucose by the glucokinaseless mutant strain CP4-M2 and the parent wild-type strain CP4 is compared in Fig. 4. Although the wild-type strain showed extensive incorporation of glucose carbon, the mutant strain CP4-M2 showed only a low level of D-glucose uptake which

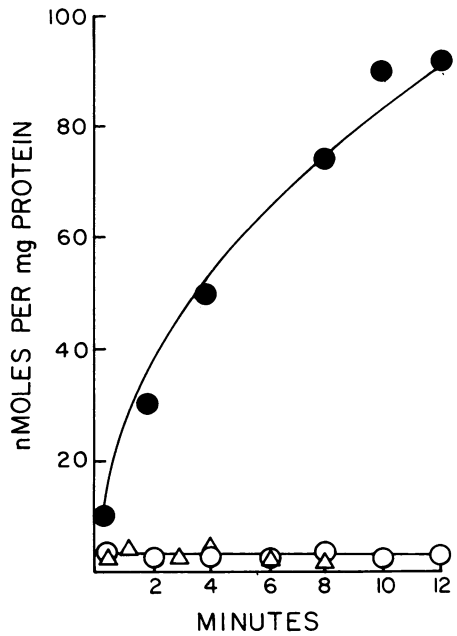

FIG. 2. Uptake of D-glucose, 2-deoxy-D-glucose, and D-xylose by *Z. mobilis* ATCC 10988. Cells grown on YEP medium with 0.1 M glucose were incubated with D-[^{14}C]glucose (1 mM; 1 μCi μmol^{-1} [●]), 2-deoxy-D-[^{14}C]glucose (0.5 mM; 0.2 μCi μmol^{-1} [△]), or D-[^{14}C]xylose (0.5 mM; 1 μCi μmol^{-1} [○]).

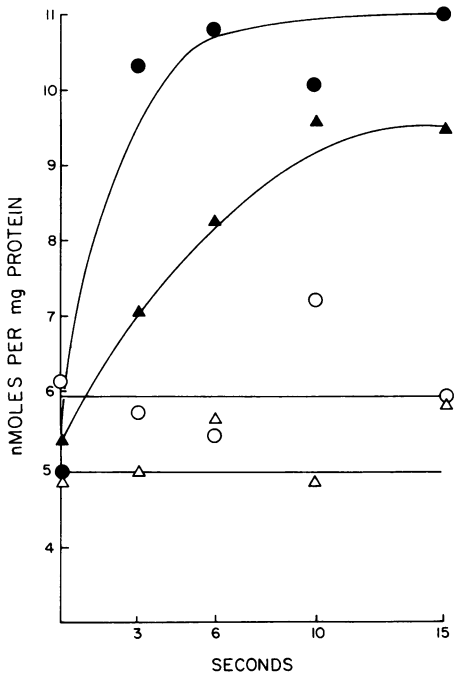

FIG. 3. Short-term uptake of 2-deoxy-D-glucose and D-xylose by *Z. mobilis* CP4. Cells grown on BMY medium plus 0.1 M glucose were incubated with 2-deoxy-D-[^{14}C]glucose (5 mM; 1 μCi μmol^{-1} [●, ○]) or D-[^{14}C-]xylose (2.5 mM; 1 μCi μmol [▲, △]) in the absence (solid symbols) or presence (open symbols) of 50 mM D-glucose added at −30 s.

reached a plateau within 30 s at a concentration near that of the external medium.

Figure 5 shows the short-term uptake at two concentrations of D-glucose by the glucokinaseless mutant strain CP4-M2. Uptake was rapid, reaching a steady-state concentration at 10 s that represented ca. 50% of the external concentration (it is probable that these measurements underestimate the maximum intracellular concentration because of leakage of substrate from the cell during filtration and washing). The presence of a 2.5- or 5-fold excess of D-fructose did not have a great effect on D-glucose uptake. These data do not allow a distinction to be made as to whether D-fructose is transported by a different system or whether it is transported by the same system with much lower affinity. However, these data do establish that the lack of concentration of D-glucose against a gradient was not due to lack of energy: the cells used in this experiment were grown on fructose and therefore would be expected to have been fully induced for the capacity to metabolize D-fructose and generate metabolic energy from it. A facilitated diffusion system for D-glucose is clearly indicated, therefore.

One of the characteristics of facilitated diffusion systems which is considered to provide strong evidence for the operation of a mobile carrier in such systems is the phenomenon of influx counterflow (or countertransport). When cells are preloaded with a nonradioactive transport substrate and then transferred to a solution containing a lower concentration of the radioactive substrate, there is an enhanced rate of inward flux of the radioactive species and an apparent transient accumulation against a concentration gradient, because the outward flux of the radioactive species is decreased by its displacement from the carrier by the nonradioactive species present in higher concentration on the internal side of the cytoplasmic membrane (31). Figure 6 demonstrates influx counterflow of D-glucose in *Z. mobilis* CP4-M2. It is clear that there was an enhanced uptake and a transient apparent concentration of D-[^{14}C]glucose in cells that had been preloaded with D-glucose.

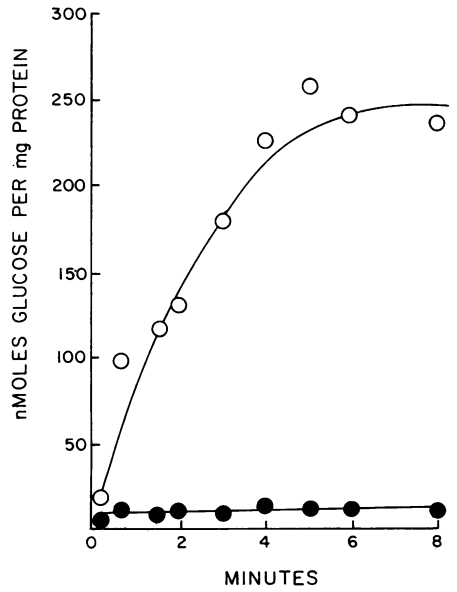

FIG. 4. Uptake of D-glucose by *Z. mobilis* CP4 (○) and glucokinaseless mutant CP4-M2 (●). Cells grown on BMY medium plus 0.1 M fructose were incubated with D-[^{14}C]glucose (1 mM; 0.5 μCi μmol^{-1}).

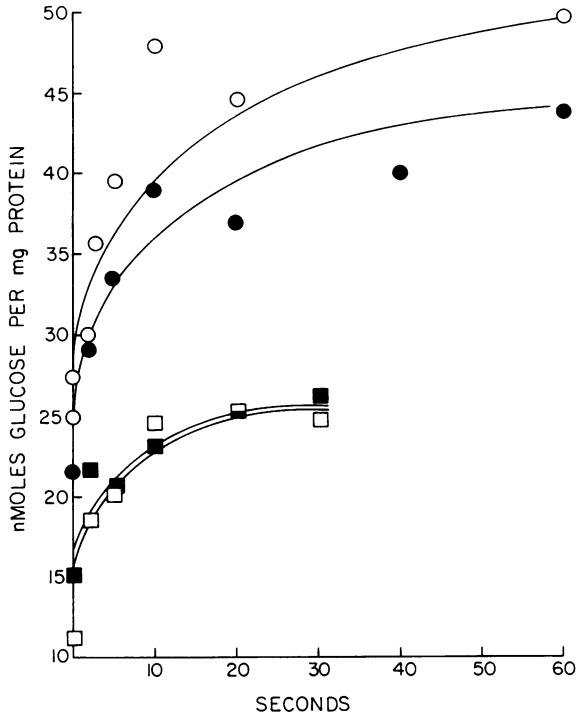

FIG. 5. Short-term transport of D-glucose by *Z. mobilis* CP4-M2; effect of D-fructose. Cells grown on BMY medium plus 0.1 M fructose were incubated with 10 mM (■, □) or 20 mM (●, ○) D-[^{14}C]glucose (0.2 μCi μmol^{-1}) in the absence (open symbols) or presence (closed symbols) of 50 mM D-fructose added at −30 s.

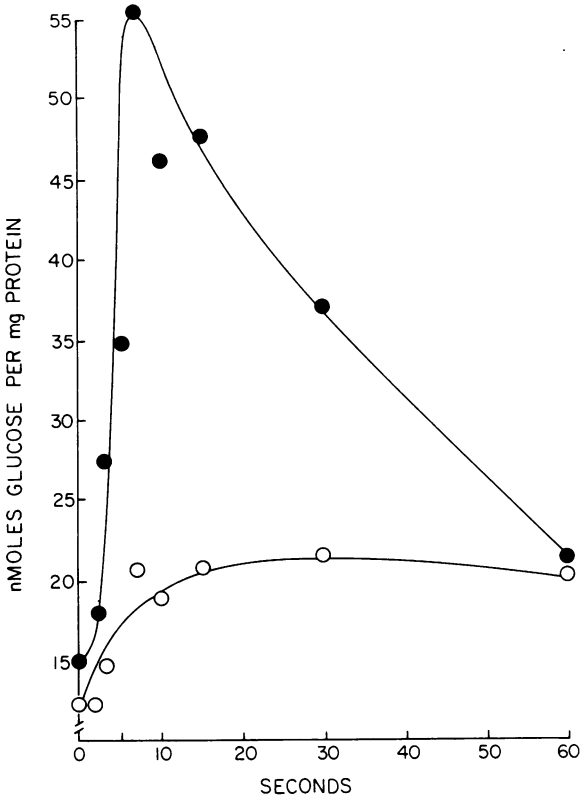

FIG. 6. D-Glucose influx counterflow. *Z. mobilis* CP4-M2 grown on BMY medium plus 0.1 M fructose was preloaded with 200 mM nonradioactive D-glucose for 10 min; at zero time, cells were diluted to 10 mM D-[^{14}C]glucose (final specific activity, 2 μCi μmol^{-1}) by adding 10 μl of cell suspension in 200 mM D-glucose to 0.4 μCi of carrier-free D-[^{14}C]glucose in 190 μl of buffer (●). Control cells were not preloaded but incubated with 10 mM D-[^{14}C]glucose, 2 μCi μmol^{-1} (○).

Figure 7 shows the result of a double-label uptake experiment with D-[^{14}C]glucose and L-[^{3}H]glucose. The uptake of glucose is clearly stereospecific, with D-glucose being taken up rapidly and reaching a plateau at 10 s. The measured L-glucose uptake represented a correction for rapid nonspecific adsorption to cells and filters, followed by a slower non-carrier-mediated simple diffusion component. At the substrate concentration used (10 mM for each isomer), this correction was significant, accounting for nearly one-third of the total measured uptake (D-glucose plus L-glucose) at 10 s.

Active transport of amino acids. All of the data presented above appear to establish clearly that *Z. mobilis* does not expend energy to take up glucose, its principal source of energy. The question arose as to whether this organism has any means of energizing its cytoplasmic membrane to drive other active transport systems, such as those for amino acids. This may be an important consideration for future efforts in genetic engineering to introduce capacities to transport and concentrate other sugars. Results of an investigation of L-proline are shown in Fig. 8. There was very little uptake of L-proline in the absence of an added energy source. Addition of glucose provoked a concentrative uptake of proline of approximately fivefold with respect to the external solution (similar results, not shown, were obtained in the presence of 100 μg of chloramphenicol per ml, indicating that this increase in proline uptake was not due to incorporation into protein). Uptake of L-proline in the presence of energy source was inhibited by the proton-conducting ionophore CCCP and the ATPase inhibitor DCCD. The most reasonable interpretation of these data is that the active uptake of L-proline is powered by a proton-motive force, which is dissipated by CCCP, or is not established in the presence of DCCD, an inhibitor of proton-translocating ATPase. Similar results were obtained with L-alanine and alpha aminoisobutyric acid as transport substrates (data not shown).

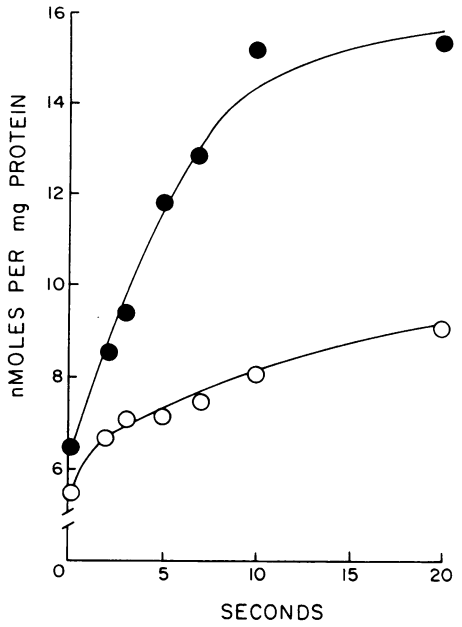

FIG. 7. Stereospecificity of D-glucose transport by *Z. mobilis* CP4-M2. Cells grown on BMY plus 0.1 M fructose were incubated in the presence of 10 mM D-[^{14}C]glucose (●) and 10 mM L-[^{3}H]glucose (○), each at 10 μCi μmol^{-1}.

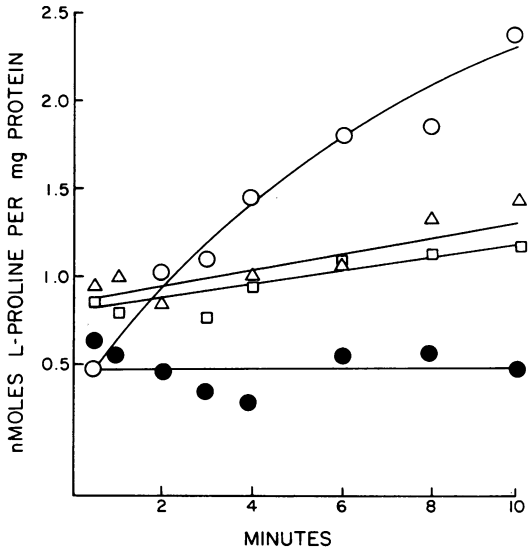

FIG. 8. Effect of energy source and inhibitors on the uptake of L-proline. *Z. mobilis* CP4 grown on BMY medium plus 0.1 M glucose was incubated with 0.1 mM L-[^{14}C]proline (0.5 μCi μmol^{-1}) in the absence of added energy source (●), in the presence of 20 mM glucose without further addition (○), or in the presence of 20 mM glucose and 50 μM CCCP (△) or 1 mM DCCD (□).

TABLE 1. Kinetics of D-glucose uptake and phosphorylation

D-Glucose uptake and phosphorylation	K_m (mM)	V_{max} (nmol min^{-1} mg of $protein^{-1}$)
D-glucose uptake		
Z. mobilis CP4	5	330
Z. mobilis CP4-M2	10–15[a]	200–300[a]
Glucokinase[b] (*Z. mobilis* CP4)	0.25	250

[a] Range of four separate determinations.
[b] Determined with crude cell extract.

Kinetic parameters of D-glucose uptake and phosphorylation. Results of K_m and V_{max} determinations for D-glucose uptake (calculated from Lineweaver-Burk plots of reciprocal initial velocities determined over 4 s [nanomoles of D-glucose at 4 s minus nanomoles of D-glucose at 0 time] versus reciprocal concentration) and for glucokinase in cell extracts are shown in Table 1. The values for the K_m for D-glucose uptake (5 to 15 mM) are in reasonable agreement with a determination by Belaich et al. (5) of the K_m for glucose permeation in *Z. mobilis* (3.2 mM) by a microcalorimetric method. These values are also of the same order of magnitude as the K_m for glucose uptake in bakers' yeast (1 to 6 mM), which also transports glucose via facilitated diffusion (6, 8, 19, 29). However, they are ca. 2 orders of magnitude higher than most bacteria, which carry out glucose uptake by active transport or by the PEP phosphotransferase system. Thus, the low-affinity glucose transport systems of *Z. mobilis* and *Saccharomyces cerevisiae* reflect their dependence upon higher sugar concentrations for optimum growth and their natural habitats in saccharine environments. The V_{max} values for glucose uptake by *Z. mobilis* are somewhat higher than those reported for bakers' yeast (70 to 150 nmol min^{-1} mg [dry weight]$^{-1}$) (6, 19, 29). The microcalorimetric values of Belaich et al. (5) for the V_{max} of glucose utilization are significantly higher (816 nmol mg of $cells^{-1}$ min^{-1} for *Z. mobilis* and 597 nmol mg of $cells^{-1}$ min^{-1} for *S. cerevisiae*). These differences are doubtlessly due to differences in the methods of measurement, and it is possible that our values are underestimates as a result of leakage of glucose from cells during filtration and washing.

Our value for the K_m of the glucokinase of *Z. mobilis* CP4 is in good agreement with that reported by Doelle (12) for *Z. mobilis* Z-10 (0.4 mM). Note that the K_m for glucokinase is an order of magnitude lower than the K_m for glucose uptake. Thus, the phosphorylating system for glucose would be fully saturated at external glucose concentrations near or above the K_m concentration for uptake. On the basis of microcalorimetric measurements of glucose utilization by *Z. mobilis*, Belaich et al. (5) concluded that in the range of glucose concentrations at which the organisms grew exponentially (above 2.2 mM), cell activity was limited by the maximal rate of the catabolic enzymes; at lower concentrations, glucose transport was the rate-limiting step. Our kinetic measurements of glucose uptake and subsequent phosphorylation are in accordance with this conclusion.

Thus, *Z. mobilis* has a low-affinity, high-velocity, but low-efficiency system for the uptake and utilization of glucose. Values for molar growth yield for *Z. mobilis* are among the lowest known for bacteria: 6.5 to 9.3 g (dry weight) per mol of glucose (3, 5, 10). It is this combination of properties that makes this organism efficient in the rapid conversion of sugars at high concentrations to metabolic products rather than to cellular material.

ACKNOWLEDGMENT

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