ALTERNATE PATHWAYS OF D-FRUCTOSE TRANSPORT AND METABOLISM IN ARTHROBACTER PYRIDINOLIS

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SUMMARY

Phosphoenolpyruvate:fructose phosphotransferase negative mutants of Arthrobacter pyridinolis used fructose in the presence of malate. In isolated membrane vesicles from fructose-grown wild type cells, fructose uptake was stimulated by malate, but not by a variety of other compounds, to almost the same extent as by phosphoenolpyruvate. The stimulation by malate but not that by phosphoenolpyruvate was prevented by 2,4-dinitrophenol or potassium cyanide. D-Fructokinase activity was elevated in cells grown in malate plus fructose or sucrose.

During growth on fructose as sole carbon source, Arthrobacter pyridinolis first transports and phosphorylates the sugar using a phosphoenolpyruvate (PEP):fructose phosphotransferase (1). This activity has been characterized as a four-component enzyme system, and has been shown to catalyze translocation of fructose in isolated membrane vesicles (2). Glucose and α-glucosides are transported in A. pyridinolis by an inducible respiration-coupled system in which the sugar is not phosphorylated (3). Malate appears to be the substrate for the transport-linked respiration; the malate must be supplied exogenously because A. pyridinolis accumulates little intracellular malate unless malate or a precursor thereof is present in the medium (4).

While studying phosphotransferase-deficient mutants of A. pyridinolis, we observed that such mutants, which cannot grow on fructose alone, grew to a much greater density in medium containing malate plus fructose than could be accounted for on

the basis of the malate content of the medium. We now report that A. pyridinolis can transport fructose either by the PEP: fructose phosphotransferase or using a respiration-coupled transport system in which hexose is accumulated in the presence of exogenous malate. The latter transport system is also used for glucose transport in A. pyridinolis and may be similar to the transport system for glucose in Azotobacter vinelandii (5).

METHODS

A. pyridinolis, wild type and strain AP243, were grown in the media previously described (1,4), at 30°C, with shaking. Strain AP243 was previously found to be deficient in one of the constitutive components of the PEP:hexose phosphotransferase system (2). Mutants of strain AP243 were prepared as described previously (4). Growth studies were conducted using 300 ml sidearm flasks (5). Isolated membrane vesicles were prepared from fructose-grown wild type cells essentially by the method of Kaback (6) with a few modifications (2). Uptake of ^{14}C-[U]-fructose by these vesicles was assayed using a filtration assay (2). Cell extracts were prepared by sonication (4), dialyzed against assay buffer, and assayed for D-fructokinase (E C 2.7. 1.4) by the procedure of Sapico et al.(7) using a Gilford model 240 at 26°C.

RESULTS AND DISCUSSION

As shown in Fig. 1, phosphotransferase-negative A. pyridinolis strain AP243 grew to a much greater cell density on 0.015 M malate plus 0.05 M fructose than on 0.015 M malate alone. Sub-culturing of cells after 20 hours, indicated that the apparent utilization of fructose by this mutant was not due to reversion.

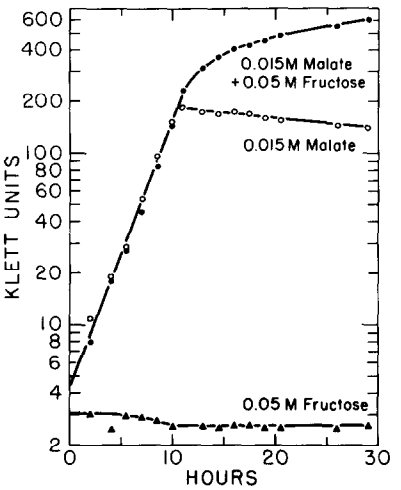

Figure 1. Growth of phosphotransferase-negative strain AP243 on fructose, malate, and malate plus fructose.

Fructose uptake was then studied using isolated membrane vesicles from fructose-grown wild type cells. The effects of a variety of compounds, summarized in Table I, indicated that: (1) malate caused an almost three-fold stimulation of fructose accumulation over that observed in the absence of additions to a level representing 65% of that seen in the presence of PEP; (2) all the other compounds tested caused much less stimulation, caused no stimulation, or even appeared to depress accumulation below that seen in the absence of additions; and (3) 2,4-dinitrophenol or KCN completely abolished malate-stimulated accumulation of fructose while causing no inhibition of PEP-stimulated accumulation.

In order to obtain mutants that were deficient in the respiration-coupled transport system, phosphotransferase-negative strain AP243 was mutagenized and plated on medium containing 0.005 M malate plus 0.05 M fructose. Strains which had formed small colonies and showed essentially normal growth on asparagine were further characterized. Two types of strains

TABLE I. The Effect of Various Compounds on Fructose Uptake by Isolated Membrane Vesicles

ADDITION	RELATIVE FRUCTOSE UPTAKE	ADDITION	RELATIVE FRUCTOSE UPTAKE
None	0.22	L-Malate	0.65
PEP	1.00	D-Lactate	0.34
ATP	0.10	Pyruvate	0.22
CTP	0.10	α-Glycerolphosphate	0.31
GTP	0.16	α-Hydroxybutyrate	0.28
UTP	0.11	Oxaloacetate	0.28
NAD	0.15	2-Phosphoglycerate	0.22
NADH	0.33	3-Phosphoglycerate	0.33
NADP	0.12	Malate + PEP	1.01
NADPH	0.33	Malate + 2,4-Dinitrophenol (10^{-3}M)	0.09
FAD	0.15	Malate + KCN (10^{-2}M)	0.14
Citrate	0.25	PEP + 2,4-Dinitrophenol (10^{-3}M)	1.08
Fumarate	0.25	PEP + KCN (10^{-2}M)	1.06
Succinate	0.36		

^{14}C-Fructose uptake by isolated membrane vesicles from fructose-grown A. pyridinolis was determined using 10 minute incubations at 35°C as previously described (2), in the presence of the additions shown. Unless indicated, the additions were present at 0.1 M. The values are expressed relative to the uptake of fructose in the presence of PEP (0.2 nmoles/min./mg vesicle protein).

were found: (1) those that grew poorly on malate relative to the parent strain, and showed little or no increase in growth when fructose or glucose was added; and (2) strains which grew normally on malate and used glucose, but not fructose, when malate was present. The first type of mutant might be deficient in the malate dehydrogenase that was involved in transport. The

second type of mutant was presumably deficient in a sugar-specific component of the transport system.

The apparent existence of a transport system that did not involve a phosphate donor, raised the question of how the

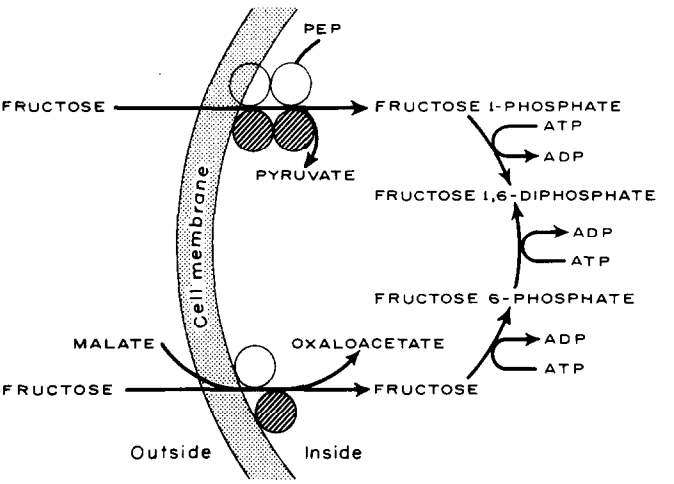

Figure 2. Schematic representation of the routes of fructose uptake and metabolism in A. pyridinolis. Open circles represent inducible proteins and hatched circles represent constitutive proteins involved in transport.

fructose thus transported was phosphorylated for metabolism. We had previously failed to find fructokinase in extracts of fructose-grown cells (1). Upon reexamination, using concentrated extracts, fructokinase activity was detected in malate- or fructose-grown wild type A. pyridinolis (4.0 nmoles of fructose 6-phosphate formed/min./mg protein). No activity was detected if fructose 1-phosphate was substituted for ATP in the assay. The fructokinase activity was elevated approximately 2.5 fold, over that in fructose-grown cells, in wild type or AP243 grown on 0.015 M malate plus 0.05 M fructose or in wild type cells grown on 0.015 M malate plus 0.05 M sucrose. Kelker, Hanson, and Anderson (8) found that D-fructokinase was induced by sucrose in wild type Aerobacter aerogenes, and by fructose in a mutant of this species lacking fructose 1-phosphate kinase.

A schematic representation of the pathways for fructose transport and metabolism in A. pyridinolis is presented in Fig. 2. The inducible membrane-bound and soluble components (open circles) and the two constitutive components (hatched circles) are shown as part of the PEP-dependent phosphotransferase; such systems have been extensively characterized by Roseman and his co-workers (9). The respiration-coupled system is represented by two proteins proposed to be a constitutive malate dehydrogenase (hatched circle) and a sugar-specific (open circle) component. Malate must be supplied exogenously, but its oxidation may occur on the inside of the membrane. The requirement for exogenous oxidizable substrate will be useful in determining whether substrates other than malate can be utilized in malate dehydrogenase-deficient mutants in analogy with findings by Hong and Kaback (10) in other species. This requirement will also be useful in future assessments of other compounds (e.g., amino acids) whose transport may be respiration-coupled.

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