A route for fructose utilization by *Escherichia coli* involving the fucose regulon

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Fructose can be taken up by *Escherichia coli* via a variety of membrane-spanning proteins that recognize sugars with the 3,4,5-D-arabino-hexose configuration. Here, we describe a mutant that is devoid of those proteins but takes up fructose via the FucP carrier normally used for the transport of α -L-fucose; this implies that the fructose is taken up in the α - or β -fructopyranose form. For growth, the assimilated fructose is sequentially phosphorylated by ATP and (i) manno(fructo)kinase, to form fructose 6-phosphate, and (ii) phosphofructokinase, to form fructose 1,6-bisphosphate, which is a member of central routes of glycolysis and gluconeogenesis. The mutation that confers on the organism the ability to take up fructose via the fucose regulon was located as a deletion of the *fucA* gene with consequent induction of the proton-linked fucose transporter, FucP.

fructose metabolism | fructose uptake

We have previously (1) described the properties of a mutant of *Escherichia coli* designated HK 2148 that is able to grow on fructose as a sole source of carbon despite inactivation of all known routes involving the phosphoenolpyruvate/glycose phosphotransferase (PT) system for the uptake and utilization of this ketose (2). The entry of fructose into this strain is achieved by its facilitated diffusion through an isoform of the normally PT-linked glucose transporter, PtsG, which therefore requires that relatively high concentrations of fructose be provided in the medium (K_m for growth = ≈ 8 mM). As would be expected from this mode of fructose uptake, growth on this substrate is powerfully inhibited by analogs of glucose, such as methyl- α -D-glucoside (α MG). However, cultures of HK 2148 exposed for several days at 37° to 20 mM fructose plus 1 mM α MG gave rise to further mutants, some of which were not only impervious to the presence of α MG when growing on fructose but grew on this ketose at concentrations much lower (K_m for growth < 1 mM) than those observed with the parent HK 2148.

In the present article, we report the properties of one such mutant, designated HK 2298, and its derivatives, which provide evidence for the operation of a pathway for the uptake and utilization of fructose that has not been previously described.

Results

Uptake of [14 C]Fructose by HK 2298. Whereas suspensions of LB-grown HK 2298 readily took up and incorporated 14 C from [14 C]fructose at concentrations as low as 0.1 mM, similar suspensions of HK 2148 did so to only a negligible extent (data not shown). Neither PtsG nor the proteins specified by the PT-related fructose operon were involved in this uptake: A derivative of HK 2298 that also carried *ptsG::kanR* (strain HK 2385) grew on fructose and took up this ketose at rates indistinguishable from HK 2298, as did other derivatives of HK 2298 carrying either *fruA::kanR* (strain HK 2578) or a deletion covering the promoter region of the fructose operon as well as *fruB* and *fruK* (strain HK 2544).

Location of the Gene Responsible for Fructose Uptake. To avoid possible errors due to introduction of the PT-linked fructose

operon, a number of Hfr strains of *E. coli* transferring DNA from different points of origin and in different directions and carrying the Tn10 transposon at known sites (3) were crossed with strain HK 2578, a derivative of HK 2298 in which FruA activity had been deleted by the introduction of *fruA::kanR*; all exconjugants were tested for retention of the kanR marker. These tetracycline-resistant colonies were screened for loss of their ability to grow on 2.5 mM fructose. We found that the Hfr strain BW6169, which carries *argA::Tn10* located at minute 63.5 on the *E. coli* linkage map (4) and transfers its DNA counterclockwise from approximately minute 84.8, gave rise to tetracycline-resistant colonies, of which 40% failed to grow on fructose and which also took up 0.5 mM [14 C]fructose at only a negligible rate. This result placed the presumptive fructose uptake system at a site close to *argA*. This location (5) was more precisely defined by transduction of HK 2578 with phage carrying *fucP::Tn10*, a deletion of the gene specifying the proton-linked transport of fucose, which is sited at min 63.2. None of the 116 transductants obtained grew upon, or took up, fructose.

The identity of the fructose uptake system with a component of the fucose regulon (6) was investigated with two *argA::Tn10* transductants from the cross [P1 phage grown on BW6169 $\times$ HK 2578], one (HK 2621) having retained the ability to grow upon fructose and the other (HK 2622) being among the 40% that had lost it. We observed that the uptake of 0.5 mM [14 C]fructose by LB-grown HK 2621 was powerfully inhibited by 0.1 mM- α -L-(–)-fucose but not by D-(+)-fucose (Fig. 1A). HK 2622 took up the labeled fructose at <20% of the rate observed with HK 2621, which was virtually unaffected by the presence of L-(–)-fucose (Fig. 1B).

Furthermore, suspensions of LB-grown HK 2621 took up α -L-[3 H]fucose to a significant extent, whereas similar suspensions of HK 2622 did not (Fig. 2).

This uptake by HK 2621 (and by its parent, HK 2578) occurred rapidly during only the first few minutes of exposure to the isotope and thereafter increased to only a minor extent, which indicated that the fucose taken up was not further metabolized. In agreement with this interpretation, we observed that neither strain, HK 2578 nor HK 2621, grew on α -L-fucose, whereas their fructose-negative parents HK 2148 and HK 2632 did. Furthermore, addition of 1 mM- α -L-fucose to cultures of HK 2578 growing on 1–10 mM fructose greatly impeded growth, with kinetics indicating competitive inhibition (Fig. 3); whereas addition of α -L-fucose to cultures of HK 2632 growing on 5–20 mM fructose had no such effect (data not shown).

These observations suggested that the ability to take up and grow upon fructose via the fucose regulon was associated with a mutation in some member of that regulon. This was con-

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In the reverse sequence, phage grown on the Mak⁺ kanR strain 2193 were crossed with HK 2140: Again, none of the kanamycin-resistant transductants grew on 2.5 mM-fructose, but when a colony that grew on 20 mM-fructose (and had therefore

Scheme 1.

Discussion

Our present demonstration that both Mak⁺ and PfkA⁺ are required for the growth of HK 2298 and its derivatives supports the conclusion that the fructose taken up by the Fuc-F⁺ system subsequently follows the path

Table 1. Bacterial strains used in this study

Strain	Relevant genotype	Growth on 2.5 mM fructose	Source
HK 1796	<i>srlAΔ fruB fruKΔ pfkA rpsL (argHBCE⁺)</i>	—	Stock
HK 2140	<i>srlAΔ fruB fruA manXYZ::CmR rpsL</i>	—	Stock
HK 2148	<i>srlAΔ fruB manXYZ::CmR argHBCE rpsL</i>	—	Ref. 1
HK 2193	As 2148 but also <i>mak⁺::kanR</i>	—	Stock
HK 2198	<i>srlAΔ fruB fruKΔ manXYZ::CmR mak-o::Tn10</i>	—	Stock
HK 2385	As 2298 but also <i>ptsG::kanR</i>	—	This work
HK 2298	As 2148 but also resistant to αMG on 20 mM-fructose	+	This work
HK 2544	As 2298 but also (<i>promoter, fruB, fruK</i>)Δ	+	This work
HK 2578	As 2298 but also <i>fruA::kanR</i>	+	This work
HK 2621	[P1(BW 5659) × 2578] <i>argA::Tn10</i> (Fru ⁺)	+	This work
HK 2622	[P1(BW 5659) × 2578] <i>argA::Tn10</i> (Fru [−])	—	This work
HK 2632	As 2148 but also <i>fruA::kanR</i>	—	This work
BW 6169	Hfr (PO min 84.8, counterclockwise) <i>argA::Tn10</i>	+	Ref. 3

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Materials and Methods

Strains and Growth Conditions. The bacterial strains used are listed in Table 1. Bacteria were grown at 37°C either in a liquid culture consisting of LB broth (25 g of LB base per liter of water) or in defined media (10), or on such media solidified with 2% agar. Cell density was measured in 1-ml cuvettes (1-cm light path) with absorbance at 600 nm (A_{600} of 1.0 being equivalent to 0.26 mg of dry mass per milliliter). Procedures used for high-frequency recombination-mediated conjugations and for phage P1-mediated transductions were those described by Miller (11).

Uptake of Labeled Substrates. The uptake of 0.5 mM [¹⁴C]fructose or 0.5 mM [³H]fucose was measured as the quantities of radioactive material retained by cells at 0.2 mg of dry mass per milliliter, shaken in shallow vessels in a water bath at 30°C. Samples (0.1 ml) were taken at known times, filtered rapidly through Millipore (Billerica, MA) filters (pore size, 0.45 nm) and washed with 2 × 5 ml of 50 mM phosphate buffer, pH 7. The filters were plunged immediately into plastic cuvettes containing 5 ml of Ecoscint H (National Diagnostics, Atlanta, GA), and their radioactivity was assayed with an LS 6500 MultiPurpose scintillation counter (Beckman Coulter, Fullerton, CA). [¹⁴C]Fructose and [³H]fucose were obtained from American Radiolabeled Chemicals, (St. Louis, MO).

Analysis of Genomic DNA. Procedures for amplification of genomic DNA and manipulation and sequencing of DNA fragments were those previously described (1).

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