Identification of a Pathway for the Utilization of the Amadori Product Fructoselysine in Escherichia coli*

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Escherichia coli was found to grow on fructoselysine as an energetic substrate at a rate of about one-third of that observed with glucose. Extracts of cells grown on fructoselysine catalyzed in the presence of ATP the phosphorylation of fructoselysine and a delayed formation of glucose 6-phosphate from this substrate. Database searches allowed us to identify an operon containing a putative kinase (YhfQ) belonging to the PKB/ribokinase family, a putative deglycase (YhfN), homologous to the isomerase domain of glucosamine-6-phosphate synthase, and a putative cationic amino acid transporter (YhfM). The proteins encoded by YhfQ and YhfN were overexpressed in E. coli, purified, and shown to catalyze the ATP-dependent phosphorylation of fructoselysine to a product identified as fructoselysine 6-phosphate by 31P NMR (YhfQ), and the reversible conversion of fructoselysine 6-phosphate and water to lysine and glucose 6-phosphate (YhfN). The \( K_m \) of the kinase for fructoselysine amounted to 18 \( \mu \text{M} \), and the \( K_m \) of the deglycase for fructoselysine 6-phosphate, to 0.4 \( \mu \text{M} \). A value of 0.15 \( \text{M} \) was found for the equilibrium constant of the deglycase reaction. The kinase and the deglycase were both induced when E. coli was grown on fructoselysine and then reached activities sufficient to account for the rate of fructoselysine utilization.

Fructosamines are the products of a non-enzymatic reaction of glucose with primary amines followed by an Amadori rearrangement. These reactions, known as glycosylation (to be distinguished from glycosylation, which is enzymatically catalyzed), typically modify the amino terminus and the lysine side-chains of proteins (reviewed in Refs. 1–3), as well as a variety of low molecular weight compounds including aminophospholipids (4). Their interest for human physiopathology is 2-fold. A first aspect is that fructosamines form spontaneously and slowly in the body in proportion to the blood glucose concentration. The concentration of protein-bound fructosamine in the serum and the level of HbA1c (a form of hemoglobin with a fructosamine residue) are used to estimate the mean blood glucose concentration in the preceding weeks or months (5–7). Furthermore, fructosamines may participate in the development of diabetes complications (8). A second aspect is that low amounts of fructosamines are present in various foods (8). Fructoselysine, which is presumably released from glycated proteins in the course of digestion, is apparently partly metabolized by bacteria in the hind gut (8) (see also “Discussion”).

The understanding of the metabolism of fructosamines has significantly progressed during the last few years. Various microorganisms (Pseudomonas sp., Corynebacterium sp., Aspergillus sp.) have been shown to produce amadoriases, which catalyze the oxidative cleavage of low molecular weight fructosamines (9–13). In addition, a mammalian fructosamine 3-kinase acting on low molecular weight and protein-bound fructosamines has recently been identified, purified, and cloned. The role of this enzyme is most likely to initiate an intracellular protein “deglycation” process (14–17).

While overexpressing mammalian fructosamine 3-kinase in Escherichia coli, we noted that control bacterial extracts contained low activities of an enzyme capable of phosphorylating fructoselysine. This stimulated us to study the metabolism of this compound in E. coli. In this paper, we show that fructoselysine can sustain growth of E. coli and that it is metabolized by a pathway involving a fructoselysine 6-kinase and an enzyme converting fructoselysine 6-phosphate to glucose 6-phosphate and lysine.

EXPERIMENTAL PROCEDURES

Materials—Reagents, of analytical grade whenever possible, were from Acros, Biowhitaker Europe, Merck, or Sigma. Radiochemicals, Sephacryl S-200, Sephacryl S-300, and DEAE-Sephacel were from Amersham Biosciences. AG 1-X8 (200–400 mesh) and AG 50W-X4 (100–200 mesh) were purchased from BioRad. Auxiliary enzymes, from Roche Molecular Biochemicals, were desalted by centrifugation before use.

Non-radioactive fructoselysine and [14C]fructoselysine (labeled on its deoxyfructose moiety) were synthesized as described elsewhere (17). For the synthesis of fructose-3-phosphate, [14C]fructoselysine (7.10⁶ cpm) was incubated for 30 min at 30 °C with 240 \( \mu \text{g} \) of purified recombinant mouse fructosamine 3-kinase (15) in the presence of 25 mM Tris-HCl, pH 7.8, 5 mM ATP-Mg, 1 mM EGTA, and 0.1 mg/ml bovine serum albumin in a final volume of 2.5 ml. The sample was diluted with one volume of water and loaded onto a 10-ml column of AG 50W-X4 (Na+) equilibrated with 10 mM Hepes, pH 7.1. The column was washed with water to elute fructose 3-phosphate, whereas fructoselysine remained bound to the column.

Non-radioactive fructoselysine was incubated for 30 min at 30 °C in a mixture (15 ml) containing 10 mM ATP-Mg, 50 mM Hepes, pH 7.1, and 5 units/ml of purified fructoselysine 6-kinase (YhfQ), which led to its complete phosphorylation. The reaction was stopped by the addition of 7.5 ml of ice-cold 10% (v/v) HClO₄. After centrifugation, the supernatant was neutralized with 3 M KHCO₃ and diluted 5-fold with 20 mM sodium acetate, pH 5.0. The

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† The amino acid sequences described in this paper are registered in the Swiss Protein Database under accession numbers P45539, P45540, P45541, P45542, P45543, P45544, O29152, O29153, and P17169, in the GenBank database with accession number AAAG58480, or in NCBI Protein Database with accession numbers CAB15251, T44930, and T44931.

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Pathway for Utilization of Fructoselysine in *E. coli*

sample was applied onto a 25-ml AG 1-X8 (Cl\(^-\)) column equilibrated with 20 mM sodium acetate, pH 5.0. The column was washed with 40\,μl of 0.2 M Tris-HCl, pH 8.0. The retained radioactivity was eluted by applying 8\,ml of 500 mM NaCl. The fractions were mixed with Optima Gold (Packard) scintillation fluid and counted for radioactivity.

Formation of Glucose-6-phosphate from Fructoselysine and ATP Catalyzed by *E. coli* Extracts—Reactions were carried out in glass cuvettes in a mixture (1 ml) containing 25\,mM Hepes, pH 7.1, 0.25\,mM NADP, 5\,mM MgCl\(_2\), 1\,mM dithiothreitol, 1\,mM EGTA, 5\,mM ATP-Mg, 0.1\,mM fructoselysine, 5\,μg of yeast glucose-6-phosphate dehydrogenase (grade I), and a bacterial extract (35\,μg of protein). *A. salmonii* was monitored to follow the appearance of NADPH.

Fructoselysine-6-kinase Assays—All enzymatic assays were carried out at 30 °C. Fractions of the DEAE-Sepharose column were collected by spectrophotometrically with a pyruvate kinase/lactate dehydrogenase-coupled assay. The assay mixture (1 ml) contained 25\,mM Hepes, pH 7.1, 25\,mM KCl, 1\,mM MgCl\(_2\), 1\,mM dithiothreitol, 0.25\,mM phosphoenolpyruvate, 0.15\,mM NADH, 1\,mM ATP-Mg, 0.5\,mM fructoselysine, 1\,mM phenylalanine, 1\,mM l-lysine (as assessed spectrophotometrically) could be obtained in a final concentration of 0.4 mM. After a further incubation at 18 °C for 22–24 h, the cells were collected by centrifugation, resuspended in 50\,ml of buffer A (20\,mM Hepes, pH 7.4, 5\,mM EDTA, 1\,mM dithiothreitol, 0.5\,mM phenylmethylsulfonyl fluoride, 5\,μg/ml leupeptin, 5\,μg/ml antipain, 1\,mg/ml lysozyme) and submitted to three cycles of freezing and thawing. The bacterial extract was incubated on ice for 1 h with 5\,mg of DNase in the presence of 10\,mM MgSO\(_4\) and centrifuged for 30 min at 10,000 \(\times g\). The pellet was washed twice with 1\,ml of 20\,mM Hepes, pH 7.1, 20 mM polyethylene glycol 6000 and centrifuged for 15 min at 10,000 \(\times g\). The supernatant was diluted 2-fold with buffer B (25\,mM Hepes, pH 7.1, 1\,mM KCl, 1\,mM dithiothreitol, 5\,μg/ml leupeptin, 5\,μg/ml antipain) and loaded onto a DEAE-Sepharose column (30\,ml) equilibrated with 20\,mM Hepes, pH 7.1. The column was washed with 150\,ml of buffer B, and protein was eluted with a linear NaCl gradient (0–0.5 in 2\,× 25\,ml of buffer B). Fractions of 3.5\,ml were collected. Protein was assayed (19) using bovine γ globulin as a standard.

Preparation and NMR Analysis of Phosphorylated Fructoselysine—Twenty-five \(\mu\)moles of fructoselysine were incubated in the presence of 10\,mM ATP-Mg, 50\,mM Hepes, pH 7.1, 40\,μg of fructoselysine kinase durin 15 min at 30 °C. The incubation medium was mixed with 0.5\,vol of cold 10% (w/v) HClO\(_4\), and the supernatant was neutralized with 3\,M KHCO\(_3\). The sample was centrifuged for 10 min, and the resulting supernatant was diluted 3-fold with buffer C (20\,mM Hepes, pH 7.1, 1\,mM dithiothreitol, 1\,mM EGTA, 5\,μg/ml leupeptin, and 5\,μg/ml antipain) and applied onto a sodium cholate column (30\,ml) equilibrated with 20\,mM Hepes, pH 7.1. The column was washed with 150\,ml of buffer C, and protein was eluted with a linear NaCl gradient (0–0.5 in 2\,× 25\,ml of buffer C). Fractions of 3.5\,ml were collected.
curred in the absence of ATP, suggesting that the phosphorylation did not take place after cleavage of [14C]fructoselysine to lysine and a radioactive sugar.

Because fructoselysine contains a carbohydrate moiety and appeared to be phosphorylated, we wondered if it would be converted to glucose 6-phosphate in bacterial extracts. In the experiment shown in Fig. 1B, we monitored the formation of glucose 6-phosphate in extracts of E. coli incubated with NADP and glucose-6-phosphate dehydrogenase. A progressive increase in the formation of NADPH took place after a lag period in extracts of induced cells incubated with fructoselysine and ATP, but not if one of these two substrates was omitted, or if an extract of cells grown on glucose was used. If exogenous glucose-6-phosphate dehydrogenase was omitted, a smaller increase was observed, presumably due to the presence of endogenous glucose-6-phosphate dehydrogenase. These results indicated therefore that the product of phosphorylation of fructoselysine was converted to glucose 6-phosphate in cell-free extracts of E. coli that had grown on this fructosamine.

Because the E. coli genome contains a sequence showing about 30% identity with mammalian fructosamine 3-kinase (15), extracts of cells grown with fructoselysine were incubated with radiolabeled [14C]fructoselysine 3-phosphate with or without ATP. Analysis of the reaction mixtures by chromatography on anion-exchanger, under conditions allowing the separation of hexose-monophosphates from fructoselysine 3-phosphate did not indicate conversion of fructoselysine 3-phosphate in these extracts (not shown). Furthermore no evidence for the conversion of fructoselysine 3-phosphate (100 µM) to glucose 6-phosphate could be obtained in experiments similar to those presented in Fig. 1B (not shown).

Taken together, these results indicated therefore that the metabolism of fructoselysine in E. coli proceeded through the phosphorylation of its deoxyfructose moiety on a carbon distinct from C3. Because sugar kinases commonly phosphorylate a hydroxyl group on a terminal carbon, this carbon was most likely C6. There is indeed no hydroxyl group on C1 in fructoselysine.

Identification of an Operon Containing the Enzymes Responsible for the Metabolism of Fructoselysine—We next searched the E. coli genome for a potential operon (in the present paper, we use this term to mean a series of neighboring ORFs, all in the same orientation, without actual proof that they are transcribed as a single mRNA) responsible for the degradation of fructoselysine. Our hypothesis was that such an operon should contain sequences encoding: 1) a transporter for fructoselysine, possibly homologous to cationic amino acid transporters; 2) a kinase able to phosphorylate fructoselysine on C6 of its deoxyfructose moiety; this kinase would probably belong to the PfkB/ribokinase protein family (20–22), which comprises a series of enzymes phosphorylating a hydroxymethyl group bound to a furanose ring; and 3) a "deglycase", able to catalyze the conversion of fructoselysine 6-phosphate to lysine and glucose 6-phosphate; such a reaction is analogous to those catalyzed by glucosamine-6-phosphate synthase and glucosamine-6-phosphate isomerase (see "Discussion").

PSI-BLAST (23) searches were carried out to identify E. coli homologues of several of the proteins mentioned above (lysine and arginine transporters, PfkB, glucosamine-6-phosphate synthase, glucosamine-6-phosphate deaminase). For each hit, we tried to identify the function of the surrounding ORFs by performing additional BLASTp (24) or PSI-BLAST searches.

The abbreviations used are: ORF, open reading frame; HMQC, H-detected heteronuclear multiple-quantum coherence via direct coupling.

Metabolism of Fructoselysine in Bacterial Extracts—As shown in Fig. 1A, extracts of E. coli grown in the presence of fructoselysine catalyzed the ATP-dependent conversion of [14C]fructoselysine (labeled on its deoxyfructose moiety) to anionic products suggesting the existence of a fructoselysine-kinase activity. No such activity could be detected under these experimental conditions with an extract of cells that had been grown on glucose instead of fructoselysine. Analysis of the reaction products on cation-exchanger under acidic conditions (not shown) indicated that no conversion of fructoselysine oc-

![Fig. 1](http://www.jbc.org/)

**Pathway for Utilization of Fructoselysine in E. coli**

42525

**RESULTS**

**Metabolism of Fructoselysine in Bacterial Extracts**—As shown in Fig. 1A, extracts of E. coli grown in the presence of fructoselysine catalyzed the ATP-dependent conversion of [14C]fructoselysine (labeled on its deoxyfructose moiety) to anionic products suggesting the existence of a fructoselysine-kinase activity. No such activity could be detected under these experimental conditions with an extract of cells that had been grown on glucose instead of fructoselysine. Analysis of the reaction products on cation-exchanger under acidic conditions (not shown) indicated that no conversion of fructoselysine oc-

**Control extract**

**ATP**

**G6PDH**

**Induced extract**

**FL**

**[NADPH (µM)]**

**Time (min)**

**Anionic products (µM)**

**Supernatant**

**% of total radioactivity**

**Fig. 1. ATP-dependent phosphorylation of fructoselysine (A) and conversion of fructoselysine to glucose 6-phosphate (B) in E. coli extracts.** A, extracts of cells grown for 25 h in the presence of 20 mM fructoselysine (induced extract, closed circles) or 20 mM glucose (control extract, closed squares) were incubated with 20,000 cpm [14C]fructoselysine and 5 mM ATP-Mg for the indicated times. Anionic radiolabeled compounds were isolated by anion-exchange chromatography. An incubation in which ATP was omitted was also performed in the case of extracts of the cells grown on fructoselysine (open triangles). One representative experiment of four is shown. B, the same extracts were incubated with 100 µM fructoselysine, 1 mM ATP-Mg, 0.25 mM NADP, and glucose-6-phosphate dehydrogenase (G6PDH), and the formation of NADPH was followed by measuring A260 for the indicated times. Controls with induced extract but without ATP (open triangles), fructoselysine (open squares), or glucose-6-phosphate dehydrogenase (open circles) were also run. Results shown are means ± S.E. for three independent experiments.

**0.22-µm membrane.** Samples (1 ml) were taken at different times to measure A260. They were then immediately centrifuged for 15 min at 5000 × g. Percoll extracts were prepared from the resulting supernatants to measure glucose and fructoselysine. The cell pellets were resuspended in 50 µl of buffer A and extracted as described for fructoselysine 6-kinase. Proteins, fructoselysine 6-kinase, and deglycase activities were measured in the supernatants obtained after a 30-min centrifugation at 10,000 × g.
One operon with the expected features was identified (Fig. 2). It contains ORFs registered as YhfM, YhfN, YhfO, YhfQ, YhfR, and Yhrf.

YhfM (P45539) is a putative transporter sharing \(-25\%\) identity with eukaryotic and prokaryotic cationic amino acid transporters (not shown). YhfN (P45540) belongs to the same family of proteins as the isomerase domain of glucosamine-6-phosphate synthase (P17169) (Fig. 3). One of its closest homologues is MocD (T44930) most probably catalyzes the phosphorylation of santhopine (fructoseglutamine) and the “deconjugation” of santhopine 6-phosphate, respectively.

Identification of the Phosphorylation Product as Fructoselysine 6-phosphate—The product of fructoselysine phosphorylation was purified. Mass spectrometry analysis indicated the presence of a negative ion with the expected mass of 28 kDa (Fig. 5), indicating that it is a monomer.

The kinetic properties were studied on the purified preparation. The \(K_m\) for fructoselysine was 18 mM in the presence of 5 mM ATP-Mg, and the \(K_m\) for ATP was 50 mM in the presence of 0.5 mM fructoselysine. Deoxymorpholinofructose (\(3\)JPOCH) showed triplet resonances, indicating that phosphate was esterified to a carbon bearing two hydrogen atoms, \(C_6\) of \(2\)-furanose form of fructoselysine 6-phosphate. The coupling constant \(J_C6_C5\) was 6.4 Hz. Three resonances were observed with chemical shifts of 1.32, 0.84, and 0.70 ppm and resonances that could be unambiguously assigned to the hydrogens bound to carbon \(C_6\) of the lysine moiety and to \(N_\text{e}\) of the amino group. These resonances were further confirmed in a two-dimensional spectrum correlating \(^3\)P and \(^1\)H resonances. The coupling between phosphorus and methylene protons was further confirmed in a two-dimensional spectrum correlating \(^3\)P and \(^1\)H (HMOC spectrum, not shown). \(^1\)H-NMR spectra (one dimensional and COSY) indicated the presence of several distinct resonances that could be unambiguously assigned to the hydrogens bound to carbon \(\alpha\) to the lysine moiety and to carbons 1 and 6 of the deoxyfructose moiety. Resonances

![Fig. 2. The fructoselysine operon in E. coli and related operons in B. subtilis and the Ti plasmid of A. tumefaciens. Sequences encoding homologous proteins are indicated by hatched, shaded or black arrows. YhfO (P45541) and YhfP (P45542) form probably one single ORF (see “Discussion”). Based on their homology with YhfQ and YhfN, MocE (T44929) and MocD (T44930) most probably catalyze the phosphorylation of santhopine (fructoseglutamine) and the “deconjugation” of santhopine 6-phosphate, respectively.](http://www.jbc.org/)

![Fig. 3. Alignment of E. coli YhfN (degeny case, P45540) with homologous sequences from the operons of B. subtilis (YurP, P45544) and A. tumefaciens (MocE, T44929) and with the isomerase domain of E. coli glucosamine-6-phosphate synthase (GlmEc, P17169). Residues conserved between two sequences of this alignment are shown in bold. Residues participating in catalysis (Glu-485, His-504, and Lys-603) are underlined in the GlnmEc sequence.](http://www.jbc.org/)
corresponding to hydrogens bound to C3 to C5 of the deoxyfructose moiety could not be assigned unambiguously due to strong overlap of resonances.

Expression and Characterization of the Putative Deglycase—We prepared two expression vectors containing either the first or the second AUG codon of YhfN (P45540) as initiation codon. The first of the two constructs led to the production of a soluble protein of the expected size (39 kDa; Fig. 4B), whereas the second construct yielded an insoluble protein (not shown).

Extracts of cells expressing the first construct catalyzed the conversion of 100 \( \mu \text{M} \) fructoselysine 6-phosphate to glucose 6-phosphate at a rate of 60 nmol/min/mg protein. No such activity was detected with extracts of control cells or of cells expressing the shorter form of YhfN. The deglycase was purified by chromatography on DEAE-Sepharose, from which it was eluted at a high salt concentration (400 mM NaCl, Fig. 7), in agreement with the calculated pI value (4.79) of the protein.

The enzyme was homogeneous after this purification step and displayed a specific activity of 0.14 mol/min/mg protein when measured with 100 \( \mu \text{M} \) substrate. About 270 mg purified protein were obtained from a 1-liter culture. The enzyme was free from phosphoglucose isomerase, which allowed us to conclude that it produced glucose 6-phosphate and not fructose 6-phosphate. Gel-filtration on Sephacryl S-300 indicated that the protein had an apparent molecular mass of 450 kDa, suggesting a dodecameric structure.

The enzyme was stimulated 2-fold by EGTA, which had a maximal effect at a concentration of 10 mM, and by EDTA, which had a maximal effect at higher concentrations (0.1 M). It was strongly inhibited by ZnCl\(_2\), which, at 10 mM, completely suppressed the activity; 0.1 mM EGTA was therefore included in all further assays. The \( K_m \) for fructoselysine 6-phosphate amounted to 0.4 mM.

The reaction catalyzed by the deglycase could also be measured in the reverse direction through the conversion of \( ^{14} \text{C} \)glucose 6-phosphate and lysine to a radioactive product that was retained on cation exchanger at acidic pH (Fig. 8). Prolonged incubation of the deglycase with 100 and 250 mM lysine led to the conversion of glucose 6-phosphate to extents of 40 and 62%, respectively. This allowed us to calculate an equilibrium constant ([lysine] x [glucose 6-phosphate]/[fructoselysine 6-phosphate]) of 0.15 mM.
Growth of E. coli on Fructoselysine—Fig. 9A illustrates that E. coli grows on fructoselysine at a rate of about one-third of that observed with glucose as a carbon source. Lysine itself did not support growth in the absence of other carbon source and did not affect the growth observed with glucose. Fig. 9B shows the rate of disappearance of glucose and fructoselysine (assayed using fructoselysine 6-kinase and the deglycase). The latter amounted to ~1.5 nmol/ml/hour at mid logarithmic phase corresponding to ~70 nmol/min/mg soluble protein (at 37 °C). The specific activity of fructoselysine 6-kinase (measured with a deglycase-coupled assay) in extracts of cells grown on fructoselysine amounted to ~30 nmol/min/mg protein (at 30 °C) and that of fructoselysine-6-phosphate deglycase, to ~6 nmol/min/mg of protein (at 100 μs fructoselysine 6-phosphate and 30 °C). No activity was detectable in cells grown on glucose with or without lysine with the spectrophotometric assay (not shown). Assays of the kinase with radiolabeled fructoselysine (10 μM) indicated an activity of ~0.02 nmol/min/mg protein.

DISCUSSION

Nature of the Catalyzed Reactions—We describe in this paper a novel pathway for the utilization of the Amadori product fructoselysine, involving a kinase and a deglycase. The first one phosphorylates fructoselysine on C6 of the deoxyfructose moiety, as indicated by NMR analysis. This conclusion is also consistent with the fact that the kinase belongs to the PfkB-ribokinase family (20–22), which comprises enzymes that phosphorylate primary alcohols (e.g. C1 of fructose 6-phosphate or fructose, C5 of ribose, C6 of fructose 1-phosphate). The deglycase catalyzes a reaction that is easily reversible, at least in vitro. The fact that lysine and glucose 6-phosphate are used in the reverse reaction confirms that they are the products of the forward reaction. The equilibrium constant of the reaction (0.15 M) suggests, however, that the enzyme serves in vivo to produce glucose 6-phosphate and lysine from fructoselysine 6-phosphate rather than for the opposite conversion.

When considered in the non-physiological direction, the reaction catalyzed by the deglycase most likely involves the formation of a Schiff base with C1 of glucose 6-phosphate, followed by an isomerization step. It is therefore similar to the reaction catalyzed by glucosamine-6-phosphate synthase. This enzyme contains a glutaminase domain and a sugar isomerase domain, both of which have been crystallized (26–28). Ammonia generated by the glutaminase domain serves to form a Schiff base with C2 of fructose 6-phosphate, and the Schiff base is then isomerized to glucosamine 6-phosphate. The role of C2 of glucose 6-phosphate in fructoselysine-6-phosphate deglycase is analogous to that played by C1 in glucosamine-6-phosphate synthase. Remarkably, fructoselysine-6-phosphate deglycase is homologous to the isomerase domain of glucosamine-6-phosphate synthase. Two of the residues thought to be involved in catalysis (His-504 and Glu-488, underlined in Fig. 3) in glucosamine-6-phosphate synthase are conserved in the fructose-6-phosphate deglycase. The first one is thought to participate in ring opening, and the second, in a proton transfer event catalyzed by glucosamine-6-phosphate synthase. This suggests that Schiff base formation in the reverse deglycase reaction occurs directly with the epsilon amine of the substrate.
single ORF in E. coli 0157H7 (AAG58480). Their existence as two separate ORFs in E. coli K12 (P45541, P45542) may be due to a mutation or to a sequencing error. When this putative mutation is “corrected,” ORF “OP” shows 21% sequence identity with tagatose 3-epimerase (30), a bacterial enzyme that interconverts D-tagatose and D-sorbose, as well as D-fructose and D-psicose (31). We speculate that Yhf OP catalyzes the reversible isomerization of psicoselysine to fructoselysine or of psicoselysine 6-phosphate to fructoselysine 6-phosphate.

tBLASTn searches with finished and unfinished bacterial genomes suggest that the occurrence of the fructoselysine operon is not common. One of the two closest protein homologues that we have found for the deglycase encodes an enzyme (MocD) involved in mannopine utilization. It has previously been suggested that MocD acts on santhopine (fructoseglutamine), the oxidation product of mannopine (25). The facts that the same operon contains a kinase homologous to YhfQ (MocE) and that MocD is similar to the isomerase domain of glucosamine-6-phosphate synthase suggest that santhopine (fructoseglutamine), the oxidation product of mannopine (25). The facts that the same operon contains a kinase homologous to YhfQ (MocE) and that MocD is similar to the isomerase domain of glucosamine-6-phosphate synthase suggest that santhopine is converted to glutamine and glucose 6-phosphate in a reaction analogous to that catalyzed by the fructoselysine-6-phosphate deglycase. The role of the deglycase homologue (YurP) found in the B. subtilis genome remains to be established.

Physiological Role—The two enzymes that we have identified and purified are most likely responsible for the metabolism of fructoselysine by E. coli. This is not only indicated by the observation that both enzymes are induced by fructoselysine, but also by the fact that their activities in extracts of cells grown in the presence of fructoselysine can account for the rate of fructoselysine metabolism, if one takes into account the effect of temperature and the fact that the deglycase activity was measured at a subsaturating (0.1 mM as compared with a K_m of 0.4 mM) concentration of substrate.

Balance studies have indicated that only a minor part (up to about 10%) of ingested fructoselysine (in the form of glycated casein) is excreted in the urine or in the feces in man, suggesting metabolism of this compound (8). Since fructoselysine is slowly taken up by the intestinal mucosa, by hepatocytes, and kidney cells (8), one possibility is that part of this metabolism takes place in tissues and involves phosphorylation by fructosamine 3-kinase and degradation of the latter to deoxyglucosone, lysine, and inorganic phosphate (15–17). Another possibility is that fructoselysine is utilized by bacteria present in the gut. The latter is consistent with the finding that fructoselysine (as glycated casein) is progressively consumed when incubated with feces in the absence but not in the presence of an antimicrobial agent (8). Our results indicate that E. coli may participate in this intraintestinal metabolism.

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Note added in proof—Following a suggestion of Dr. Rudd (University of Miami), we propose to designate the fructoselysine operon “frl.” ORFs yhfM, yhfN, yhfOP and hfQ become frlA, frlB, frlC and frlD, respectively. The putative regulator yhR is designated frR.

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