# Fructoselysine 3-epimerase, an enzyme involved in the metabolism of the unusual Amadori compound psicoselysine in *Escherichia coli*

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The frl (fructoselysine) operon encodes fructoselysine 6-kinase and fructoselysine 6-phosphate deglycase, allowing the conversion of fructoselysine into glucose 6-phosphate and lysine. We now show that a third enzyme encoded by this operon catalyses the metal-dependent reversible interconversion of fructoselysine with its C-3 epimer, psicoselysine. The enzyme can be easily assayed through the formation of tritiated water from [3-$^3$H]fructoselysine. Psicoselysine supports the growth of *Escherichia coli*, causing the induction of the three enzymes of the frl operon. No growth on fructoselysine or psicoselysine was observed with Tn5 mutants in which the putative transporter (FrlA) or fructoselysine 6-phosphate deglycase (FrlB) had been inactivated, indicating the importance of the frl operon for the metabolism of both substrates. The ability of *E. coli* to grow on psicoselysine suggests the occurrence of this unusual Amadori compound in Nature.

Key words: fructosamine, fructoselysine, glycation, osamine, psicosamine.

## INTRODUCTION

Fructosamines are formed through the condensation of glucose with amines, followed by an Amadori rearrangement (reviewed in [1,2]). Other sugars also react with amines to form other types of ketosamine, but the occurrence of these in Nature is much less known. Several enzymes that can metabolize fructosamines have recently been identified.

In mammalian cells, fructosamine 3-kinase phosphorylates protein-bound fructosamines, leading to the formation of unstable fructosamine 3-phosphates, which detach from proteins to regenerate the amine in its pristine state [3–5]. A related mammalian enzyme was recently shown to catalyse a similar reaction, though not on fructosamines, but on their C-3 epimers (psicosamines) and on ribulosamines [6].

In micro-organisms, low-molecular-mass fructosamines can be metabolized through an oxidative mechanism, involving 'amadoriases' that sever the bond of the amine either with the aglycone or with the sugar portion [7–11]. A non-oxidative metabolism has also been identified in *Escherichia coli*, which involves fructoselysine 6-kinase (YhfQ, now termed FrlD) and fructoselysine 6-phosphate deglycase (YhfN/FrlB), as well as a putative transporter (YhfM/FrlA) [12]. These three proteins are encoded by the frl (fructoselysine) operon.

In this paper we have studied the function of a fourth ORF (open reading frame), yhfO-P/frlC, in the same operon. The encoded protein is an epimerase that allows *E. coli* to utilize psicoselysine.

## EXPERIMENTAL

### Materials

D-[3-$^3$H]Glucose was from Moravek Biochemicals (Brea, CA, U.S.A.) or Amersham Biosciences (Amersham, Little Chalfont, Bucks., U.K.). *N*-$\alpha$-t-Boc-lysine (*N*-$\alpha$-t-butoxycarbonyl-lysine) was from Novabiochem (Laüfelfingen, Switzerland) and D-allose from Sigma (St. Louis, MO, U.S.A.) or ICN (Irvine, CA, U.S.A.). Amberlite IRN77 was from Sigma. AG 50W-X4 (100–200 mesh) and Biogel P2 fine were purchased from Bio-Rad (Hercules, CA, U.S.A.). Auxiliary enzymes, from Roche Applied Science Biochemicals (Mannheim, Germany), were desalted before use. Fructoselysine 6-kinase and fructoselysine-6-phosphate deglycase were prepared as described previously [12].

The *E. coli* Tn5 mutants *yhfM*/*frlA*$^-$ (FB21138), *yhfN*/*frlB*$^-$ (FB21140) and *yjcU*/*AlsE*$^-$ (FB21829) were obtained from the Blattner Laboratory (University of Wisconsin, Madison, WI, U.S.A.). Details of these Tn5 mutants can be found at http://asap.ahabs.wisc.edu/annotation/php/home.php.

For the synthesis of [3-$^3$H]fructoselysine, D-[3-$^3$H]glucose (100 $\mu$Ci; 5 nmol) and *N*-$\alpha$-t-Boc-lysine (16 $\mu$mol) were incubated for 16 h in 0.8 ml of methanol at 50 °C. The sample was freeze-dried, resuspended in 1 ml of water, brought to pH 1 with 1 M HCl and kept overnight at room temperature to deprotect the $\alpha$-amino group of lysine. It was then diluted with 4 vol. of 10 mM HCl and loaded on to a 1 ml cation-exchange column (AG 50W-X4, $H^+$ form). The column was washed with 5 ml of water, and the cationic radioactive material (i.e. Schiff base and Amadori compound) was eluted with 0.5 M NaCl and desalted on a Biogel P2 column. An additional chromatographic step on an AG 50W-X4 column (1 ml; $Na^+$ form) separated the labile Schiff base from radiolabelled fructoselysine, which was eluted with 0.5 M NaCl and desalted on Biogel P2. The yield with respect to radioactive glucose was approx. 35 %.

Psicoselysine was synthesized in a similar manner to fructoselysine [5,13]. Briefly, D-allose (5 mmol) and *N*-$\alpha$-t-Boc-lysine (5 mmol) were heated in methanol (225 ml) for 4 h under reflux and $N_2$. The sample was evaporated to dryness and resuspended in water (100 ml), brought to pH 1 with 7.5 M HCl, maintained for 40 h at this pH at room temperature and loaded on to a 25 ml cation-exchange column (Amberlite IRN77, $H^+$ form). The column was washed with 250 ml of water, and psicoselysine was eluted with 0.25 M $NH_4OH$. Fractions containing psicoselysine were pooled, concentrated 10-fold, decolourized with 2.5 % charcoal and neutralized with HCl. The purity of the final product was checked by paper chromatography [6]. The yield of psicoselysine was 2.5 mmol, as assessed by measuring the reducing power [14] or with the specific enzymic assay (see below).

Abbreviations used: ORF, open reading frame; *N*-$\alpha$-t-Boc-lysine, *N*-$\alpha$-t-butoxycarbonyl-lysine.

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### Overexpression and purification of fructoselysine 3-epimerase

A 5′ primer containing the putative ATG codon (C*CATATG*AAAACAGGTATGTTTACCTG) in an *Nde*I site (in italics) and a 3′ primer containing the putative stop codon (GG*GGATCC*TCACTCATCCTCCGGCAG) flanked by a *Bam*HI site (in italics) were used to PCR-amplify genomic DNA from *E. coli* BL21(DE3) with 2.5 units of *Pwo* polymerase. This PCR product was subcloned in pBlueScript and checked by sequencing. A *Nde*I–*Bam*HI fragment was removed from the pBlueScript plasmid and ligated in pET-3a. The resulting plasmid was used to transform *E. coli* BL21(DE3)pLysS [15]. Expression of the protein was carried out as described previously [12].

Bacteria derived from a 1 litre culture were collected by centrifugation, resuspended in 50 ml of buffer A, containing 20 mM Hepes, pH 7.1, 0.5 mM PMSF, 5 $\mu$g/ml leupeptin, 5 $\mu$g/ml antipain and 1 mg/ml lysozyme, and submitted to three cycles of freezing and thawing. The bacterial extract was incubated for 1 h on ice in the presence of 5 mg of DNase I and 10 mM $MgSO_4$, and then centrifuged for 30 min at 10 000 ***g***. The resulting supernatant (50 ml) was diluted 3-fold with buffer B (25 mM Hepes, pH 7.1, 5 $\mu$g/ml leupeptin, 5 $\mu$g/ml antipain and 0.1 mM $NiCl_2$) and loaded on to 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 the retained proteins were eluted with a linear NaCl gradient (0–0.5 M in 2 × 125 ml of buffer B). Fractions of 3.5 ml were collected, and protein concentration was determined using bovine $\gamma$-globulin as a standard [16].

### Fructoselysine 3-epimerase assay

Two different types of assay were used. Both were carried out at 30 °C in a medium containing 25 mM Hepes, pH 7.1, 1 mM $MgCl_2$ and 0.1 mM $NiCl_2$. If needed, the enzymic preparation to be assayed was extemporaneously diluted in a buffer containing 25 mM Hepes, pH 7.1, 0.1 mM $NiCl_2$ and 0.5 mg/ml BSA.

The first assay method is based on the fact that fructoselysine 3-epimerase catalyses the exchange of tritium from [3-$^3$H]fructoselysine with water. The assay mixture (0.2 ml) contained 0.1 mM fructoselysine and 20 000 c.p.m. of [3-$^3$H]fructoselysine. The assay was started by the addition of 10 $\mu$l of enzyme and stopped with 0.1 ml of ice-cold 10 % (w/v) perchloric acid. After centrifugation, 0.25 ml of the supernatant was diluted with 0.75 ml of water and applied to an AG 50W-X4 column ($H^+$ form, 1 ml). The column was washed twice with 2 ml of water. The resulting fractions were mixed with Optima Gold scintillation liquid and counted for radioactivity. One unit of enzyme is the amount that catalyses the conversion of 1 $\mu$mol of substrate/min under the indicated conditions.

The second method is a measure of the formation of fructoselysine from psicoselysine in a reaction mixture (2.5 ml) containing 0.5 mg/ml BSA and 1 mM psicoselysine. The reaction was stopped after various times (0–120 min) by mixing 0.2 ml samples of the incubation mixture with 0.1 ml of 10 % perchloric acid. Fructoselysine was assayed in neutralized extracts with fructoselysine 6-kinase in a pyruvate kinase and lactate dehydrogenase coupled assay [12].

### Psicoselysine assay

Psicoselysine was assayed spectrophotometrically in a mixture (1 ml) containing 25 mM Hepes, pH 7.1, 25 mM KCl, 1 mM $MgCl_2$, 0.25 mM phosphoenolpyruvate, 0.15 mM NADH, 1 mM ATP-Mg, 10 $\mu$g of rabbit muscle pyruvate kinase and 5 $\mu$g of rabbit muscle lactate dehydrogenase. After the $A_{340}$ had stabilized, 0.5 unit of fructoselysine 6-kinase was added to measure fructoselysine, and then 0.1 unit of fructoselysine 3-epimerase and 10 $\mu$M $NiCl_2$ to measure psicoselysine.

### Growth of *E. coli* on psicoselysine

*E. coli* BL21(DE3) was pre-cultured overnight in LB medium, collected by centrifugation at 700 ***g*** for 10 min at 4 °C and resuspended in the initial volume of M9 medium devoid of carbon source. Of this suspension, 50 $\mu$l was used to inoculate 2 ml of M9 medium supplemented with 20 mM sterile psicoselysine. After 72 h of growth at 37 °C under shaking, $A_{600}$ was measured and 1 ml of the culture was centrifuged at 3100 ***g*** for 10 min at 4 °C. A perchloric acid extract was prepared from the resulting supernatant to assay psicoselysine. The cell pellet was resuspended in 50 $\mu$l of buffer A containing 0.5 mM EDTA and 0.5 mM dithiothreitol, extracted by freezing and thawing as described above and centrifuged at 10 000 ***g*** for 30 min at 4 °C. The resulting supernatant was used to assay fructoselysine 3-epimerase (radiochemical assay), fructoselysine 6-kinase (pyruvate kinase/lactate dehydrogenase coupled assay), fructoselysine-6-phosphate deglycase [12] and protein [16].

The mutated strains were pre-grown similarly in LB medium before being inoculated in M9 medium containing 20 mM glucose, allose, psicoselysine or fructoselysine. Growth was followed for 96 h by measuring $A_{600}$.

## RESULTS

### Establishment of the sequence and sequence comparisons

BLAST searches indicated that ORFs yhfO and yhfP present in the *E. coli* K12 genome were homologous to one single ORF in the genome of the pathogenic strain *E. coli* 0157:H7. We therefore resequenced this region of the K12 genome to check if the existence of two separate ORFs was due to a sequencing error. Amplification of the whole yhfO–yhfP region by PCR and sequencing indeed showed that the sequence present in the database (AE000413) contained an additional cytidine at position 3377 compared with the DNA that we amplified, which therefore reads $^{3367}$CTGATTTCCGCGGCCCACGC$^{3387}$. When this correction was taken into account it appeared that this region encoded a single protein of 275 residues, predicted to be cytoplasmic by the PSORT programme [17].

BLAST searches with this protein indicated the presence of homologues sharing approx. 98, 29 and 25 % identity, in *Shigella flexneri*, *Rhodobacter spheroides* (Figure 1) and *Clostridium acetobutylicum*, respectively. In the genome of these three organisms, the ORFs homologous to yhfOP/frlC reside close to homologues of fructoselysine 6-kinase and fructoselysine-6-phosphate deglycase, indicating that they belong to putative fructosamine operons.

The alignment represented in Figure 1 also shows that YhfOP/FrlC shares ≈20 % identity with tagatose 3-epimerase, an enzyme that isomerizes D-tagatose to D-sorbose as well as D-fructose to D-psicose [18,19]. YhfOP/FrlC is more distantly related to hydroxypyruvate isomerase, which converts hydroxypyruvate into tartronate semialdehyde [20], as well as to 3-hexulose-6-phosphate isomerase, an enzyme that interconverts 3-hexulose 6-phosphate and fructose 6-phosphate [21]. Because YhfOP/FrlC is found in the fructoselysine operon, these findings suggested that it could catalyse the interconversion of fructoselysine and its C-3 epimer, psicoselysine. Such a reaction would most likely proceed through an enediol intermediate, with potential exchange of the hydrogen bound to carbon 3′ with protons from the medium. This exchange reaction forms the basis of a convenient assay used to detect the enzyme in the experiments reported below.

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FrlC-Ec   -------MKTGMFTCGHQRLPIEHAFRDASELGYDGIEIWGGRPHAFAPDL  44
Epi-Rs    MSARLSARQSIGANFAFQHFPLAEVARRMRGFGFTEIELWGVAPHLDLFHS  51
TgEpi-Pc  ---MNKVGMFYTYWSTEWMVDFPATAKRIAGLGFDLMEISLGEFHN----L  44
HpIso-Ec  -----MLRFSANLSMLFGEYDFLARFEKAAQCGFRGVEFMFPYDYD----I  42
H6Iso-Mj  -------------MSKLEELDIVSNNILILKKFYTNDEWKNKLDSL-----  33

FrlC-Ec   KAGGIKQIKALAQTYQMPIIGYTPETNGYPYNMMLGDEHMRRESLDMIKLA  95
Epi-Rs    TDRDVAEVKCVLSGEGLSVRCLTPEQVVYPINIASGDDAFRAASIDRFRRA 102
TgEpi-Pc  SDAKKRELKAVADDLGLTVMCCIGLKSEY--DFASPDKSVRDAGTEYVKRL  93
HpIso-Ec  EELKHVLASNKLEHTLHNLPAGDWAAGERGIACIPGREEEFRDGVAAAIRY  93
H6Iso-Mj  ------------------------------IDRIIKAKKIFIFGVGRSGYI  54

FrlC-Ec   MDMAKEMNAGYTLISAAHAGYLTPPNVIWG------RLAENLSELCEYAEN 140
Epi-Rs    ADIAAELGARHLFLTPGRGYESEPAARAWD------RSAEALRLIAGHAAG 147
TgEpi-Pc  LDDCHLLGAPVFAGLTFCAWPQSPPLDMKDKRPYVDRAIESVRRVIKVAED 144
HpIso-Ec  ARALGNKKINCLVGKTPAGFSSEQIHATLV---------ENLRYAANMLMK 135
H6Iso-Mj  GRCFAMRLMHLGFKSYFVGETTTPSYEKDD---------------------  84

FrlC-Ec   IGMDLILEPLTPYES--NVVCNANDVLHALALVPSPRLFSMVDICAPYVQA 189
Epi-Rs    LGLRCLLEPLQRVES--NIAHSAADTARLLEMVGADTVDVVLDTVAMACAG 196
TgEpi-Pc  YGIIYALEVVNRFEQ--WLCNDAKEAIAFADAVDSPACKVQLDTFHMNIEE 193
HpIso-Ec  EDILLLIEPINHFDIPGFHLTGTRQALKLIDDVGCCNLKIQYDIYHMQRME 186
H6Iso-Mj  --LLILISGSGRTES--------------VLTVAKKAKNINNNIIAIVXEX 119

FrlC-Ec   EPVMSYFDKLGDKLRHLHIVDSDGASDTHYIPGEGKMPLRELMRDIIERGY 240
Epi-Rs    DSVADYARLFGDRLAHVHLVD--GRPAGHLVWGEGTLPLARILADLAAAGY 245
TgEpi-Pc  TSFRDAILACKGKMGHFHLGE-----ANRLPPGEGRLPWDEIFGALKEIGY 239
HpIso-Ec  GELTNTMTQWADKIGHLQIADN----PHRGEPGTGEINYDYLFKVIENSDY 233
H6Iso-Mj  ----GNVVEFADLTIPLEVKK-----SKYLPMGTTFEETALIFLDLVIAEI 161

FrlC-Ec   EGYCTVELVT--MYMNEPRLYARQALERFRALLPEDE               275
Epi-Rs    RGALSFEPFGDGSYALDPVAAWTRHLTALAPHLDPVEARP            285
TgEpi-Pc  DGTIVMEPFMRKGGSVSRAVGVWRDMSNGATDEEMDERARRSLQFVRDKLA 290
HpIso-Ec  NGWVGCEYKP---QTTTEAGLRWMDPYR                        258
H6Iso-Mj  MKRLNLDESE----------IIKRHXNLL                       180
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**Figure 1 Alignment of YhfOP/FrlC with tagatose 3-epimerase, hydroxypyruvate isomerase and 3-hexulose-6-phosphate isomerase**

The FrlC sequence of *E. coli* (FrlC-*Ec*) is aligned with a sequence from a putative fructosamine operon in *Rhodobacter spheroides* (Epi-*Rs*; ZP_00006560), with tagatose 3-epimerase from *Pseudomonas cichorii* (TgEpi-*Pc*; sp O50580), hydroxypyruvate isomerase from *E. coli* (HpIso-*Ec*; NP_415041/BAA89011) and 3-hexulose-6-phosphate isomerase from *Methanococcus jannaschii* (H6Iso-*Mj*; 1JEOA). Residues of the last four proteins that are shared with the FrlC sequence are shown in bold.

### Expression and characterization of the protein

The sequence encoding FrlC was inserted into the pET-3a expression vector, which was used to overexpress the protein in *E. coli*. Extracts of cells induced with isopropyl $\beta$-D-thiogalactoside contained an additional band of the expected size ($\approx$30 kDa) and catalysed the release of tritium from 0.1 mM [3-$^3$H]fructoselysine at a rate of 3 nmol/min per mg of protein, as compared with $<$0.02 nmol/min per mg of protein in control extracts. This rate was considerably enhanced by bivalent cations, with the following order of decreasing potency: $Ni^{2+} > Co^{2+} > Fe^{2+} \approx Mn^{2+}$ (Figure 2). $Ca^{2+}$ and $Cu^{2+}$ were devoid of effect, whereas $Zn^{2+}$ inhibited the activity completely at 10 $\mu$M (results not shown). $Ni^{2+}$ and $Co^{2+}$ were also found to stabilize the enzyme, and $NiCl_2$ was therefore included in buffers during the purification.

The overexpressed protein was purified to near-homogeneity by chromatography on DEAE-Sepharose. As shown in Figure 3, the 30 kDa polypeptide co-eluted with the enzyme catalysing the detritiation of [3-$^3$H]fructoselysine. The enzyme displayed a specific activity of 0.4 $\mu$mol/min per mg of protein in the presence of 0.1 mM fructoselysine and 0.1 mM $NiCl_2$, corresponding to a 2-fold purification over the original extract with a 70 % yield. Gel filtration on Sephacryl S-200 in the absence or in the presence of 10 $\mu$M $CoCl_2$ indicated that the native protein had a molecular mass of $\approx$260 kDa, suggesting an octameric structure (results not shown).

### Kinetic properties and catalysed reaction

Using the radiochemical assay, the enzyme was found to display a $K_m$ of $\approx$0.5 mM for fructoselysine and a $V_{max}$ of 2.5 $\mu$mol/min per mg of protein. Using a spectrophotometric fructoselysine assay, we found that the addition of the purified enzyme to a solution containing 1 mM fructoselysine led to a decrease in the fructoselysine concentration at an initial rate of at least 6.5 $\mu$mol/min

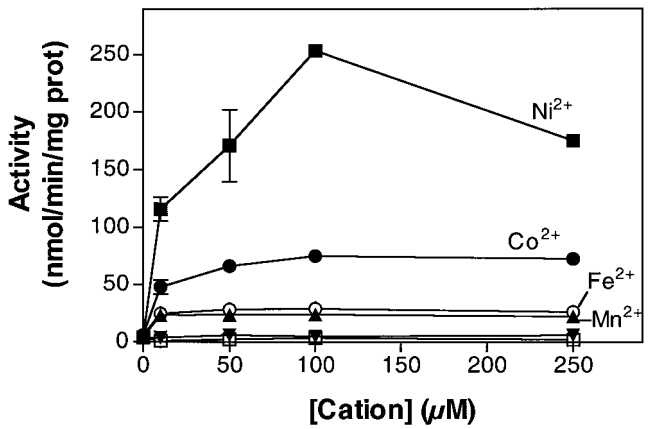


**Figure 2 Effect of bivalent cations on the release of tritium from [3-$^3$H]fructoselysine by an extract of *E. coli* overexpressing YhfOP/FrlC**

Appropriate dilutions of the bacterial extract were incubated for 10 min at 30 °C with 0.1 mM fructoselysine, 20 000 c.p.m. of [3-$^3$H]fructoselysine, 25 mM Hepes, pH 7.1, 1 mM $MgCl_2$ and the indicated concentrations of $NiCl_2$ (■), $CoCl_2$ (●), $FeSO_4$ (○), $MnCl_2$ (▲), $CaCl_2$ (▼) or $CuSO_4$ (□). The $FeSO_4$ solution was prepared in water flushed with $N_2$ to minimize oxidation of $Fe^{2+}$. Means ± S.E.M. from three experiments are shown.

per mg of protein. The fructoselysine concentration stabilized to about 75% of the initial concentration (Figure 4). No decrease was observed in the absence of enzyme.

Figure 4 also shows that the enzyme catalysed the formation of fructoselysine from 1 mM psicoselysine at a rate of 6.5 $\mu$mol/min per mg of protein. Again, the reaction reached a plateau when the [fructoselysine]/[psicoselysine] ratio was $\approx$3, indicating that this value corresponds to the equilibrium constant of the reaction. Under the same conditions, the enzyme did not catalyse any detectable (i.e. $<0.01$ $\mu$mol/min per mg of protein) conversion of psicose (tested at 1, 5 and 10 mM) into fructose (assayed with hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase). Fructoselysine 3-epimerase also did not catalyse any detectable detritiation of [3-$^3$H]fructoselysine 6-phosphate.

Using an ADP-coupled assay, we found that purified fructoselysine 6-kinase phosphorylated psicoselysine with a $K_m$ of 10 mM and a $V_{max}$ of 9 $\mu$mol/min per mg of protein. The corresponding values for fructoselysine are 18 $\mu$M and 30 $\mu$mol/min per mg of protein [12].

### Growth of *E. coli* on psicoselysine

To check the physiological role of this enzyme, we tested the ability of *E. coli* to grow on psicoselysine. BL21 cells (without

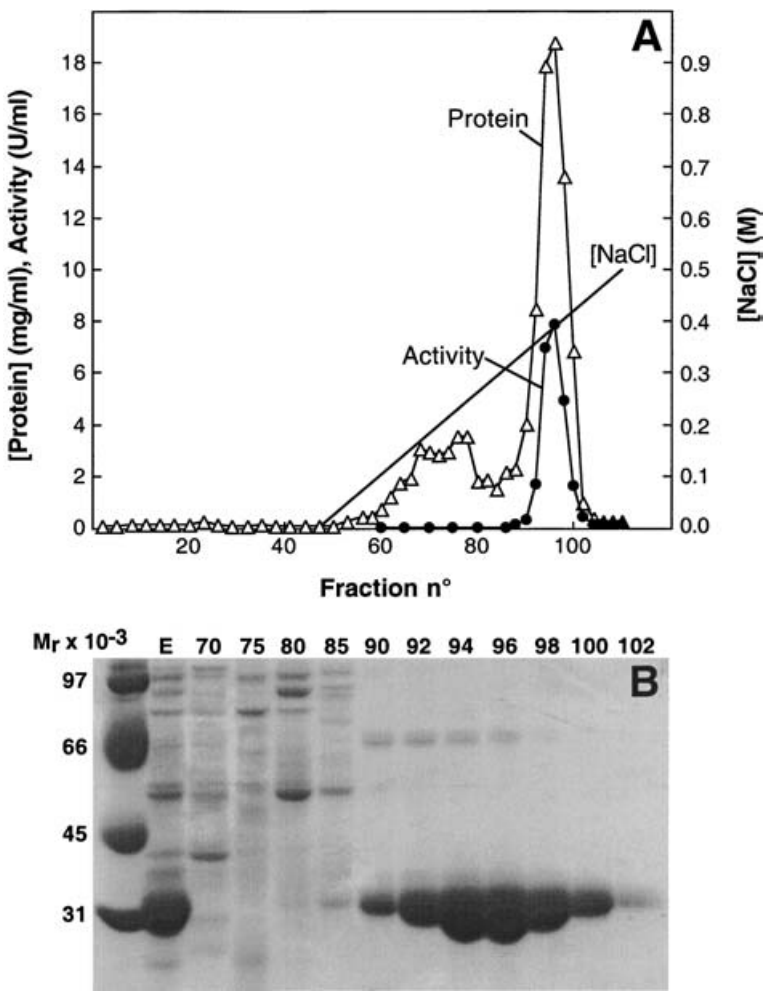


**Figure 3 Purification of the putative epimerase by chromatography on DEAE-Sepharose**

(**A**) A bacterial extract (50 ml) containing 16.5 mg of protein/ml was applied on a DEAE-Sepharose column, which was washed with buffer B. Protein was eluted with a linear NaCl gradient. The enzymic activity was determined with 0.1 mM substrate and 0.1 mM $NiCl_2$. (**B**) The original extract (10 $\mu$l; E), 20 $\mu$l of fractions 70, 75, 80 and 85, and 10 $\mu$l of fractions 90–102 were analysed by SDS/PAGE and Coomassie Blue staining. The $M_r$ is indicated to the left of the gel.

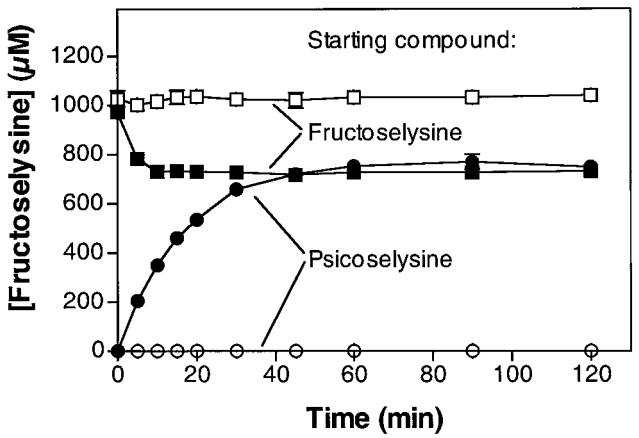


**Figure 4 Interconversion of psicoselysine and fructoselysine by the epimerase**

The purified enzyme (6 $\mu$g/ml) was incubated with 1 mM fructoselysine (■) or psicoselysine (●) for the indicated times. Fructoselysine was then assayed using a fructoselysine 6-kinase assay. Controls without enzyme (□, ○) are also shown.

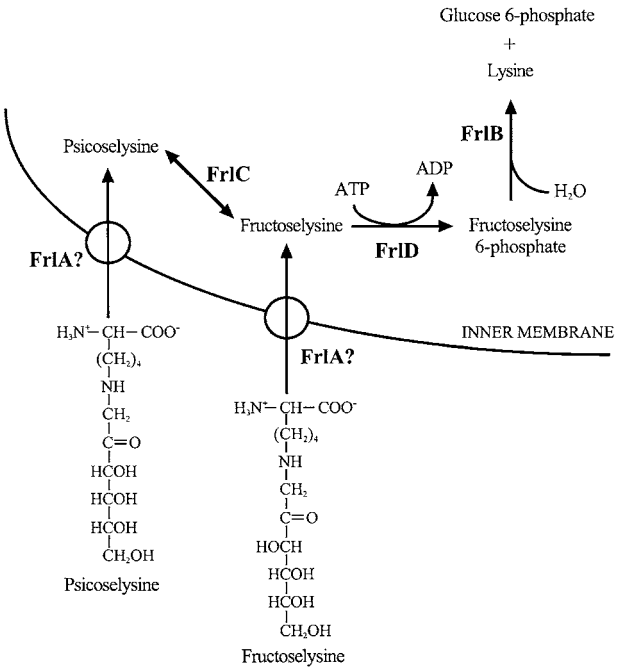


**Figure 5 Metabolism of psicoselysine in *E. coli***

Psicoselysine metabolism involves its transport to the cytoplasm, presumably via the same transporter as used by fructoselysine. Psicoselysine is then epimerized to fructoselysine, which can then be phosphorylated and converted into lysine and glucose 6-phosphate.

expression plasmid) grew on 20 mM psicoselysine at a slightly lower rate than on 20 mM fructoselysine, but eventually reached the same cell density in the stationary phase. Extracts of cells that had reached the stationary phase had an epimerase activity of $\approx$2 nmol/min per mg of protein (measured through the release of tritiated water from [3-$^3$H]fructoselysine, in the presence of 100 $\mu$M fructoselysine), a fructoselysine 6-kinase activity of $\approx$100 nmol/min per mg of protein and a deglycase activity of 20 nmol/min per mg of protein. The activities of these enzymes were undetectable if the cells had been grown on glucose.

We also checked that Tn5 mutants in which yhfM/frlA (encoding the putative transporter) or yhfN/frlB (encoding fructoselysine-6-phosphate deglycase) had been disrupted did not grow on 20 mM fructoselysine or psicoselysine, whereas they did grow on 20 mM glucose. This indicated that the frl operon is required for the metabolism of both ketosamines. By contrast, a mutant in which AlsE (presumably allulose-6-phosphate 3-epimerase), an enzyme essential for D-allose metabolism [22], had been inactivated, did grow both on fructoselysine and psicoselysine, though not on allose. This indicated that the growth of bacteria on psicoselysine does not involve the conversion of psicoselysine into free D-allose.

## DISCUSSION

The enzyme that we have characterized in the present study catalyses the reversible conversion of fructoselysine and psicoselysine. It does not act on fructose or fructoselysine 6-phosphate, and therefore appears to be a specific fructoselysine 3-epimerase. Its physiological function is to allow the metabolism of psicoselysine, which was indeed found to support the growth of *E. coli*. The specificity of the epimerase for the non-phosphorylated derivative and the poor action of fructoselysine 6-kinase on psicoselysine indicate that the epimerization of psicoselysine to fructoselysine precedes the phosphorylation of the latter to fructoselysine 6-phosphate (Figure 5). The predicted localization of the epimerase indicates that it acts in the bacterial cytoplasm. Psicoselysine has therefore to be transported across the inner membrane, and this presumably occurs via the same transporter as used by fructoselysine, most likely FrlA. The knock-out of fructoselysine-6-phosphate deglycase prevents growth on both fructoselysine and psicoselysine, indicating that the latter has to be converted into fructoselysine to be further metabolized.

The finding that fructoselysine 3-epimerase catalyses the exchange of the hydrogen bound to C-3 with protons from the medium indicates that the reaction proceeds through an enediol intermediate. This is similar to the reaction catalysed by ribulose-5-phosphate 3-epimerase [23,24], but unlike the one catalysed by UDP-glucose 4-epimerase, which involves a hydride transfer to NAD$^+$ [25]. The formation of an enediol intermediate may also account for the low similarity of fructoselysine 3-epimerase to hydroxypyruvate isomerase and 3-hexulose-6-phosphate isomerase, two enzymes that may also proceed through an enediol intermediate.

The metal-ion dependency of fructoselysine 3-epimerase is reminiscent of that observed with many isomerases acting on non-phosphorylated sugars, such as xylose isomerase [26] and L-arabinose isomerase [27]. This is probably related to the ability of transition metals to bind *cis*-diols [28]. Intriguingly, no bivalent-cation requirement has been described for tagatose epimerase [18,19] and hydroxypyruvate isomerase [20], whereas hexulose-6-phosphate isomerase has been shown to be inhibited by $Mg^{2+}$ and activated by EDTA [29]. This suggests that the bivalent cation does not participate directly in catalysis.

The existence of a fructoselysine 3-epimerase in *E. coli* and presumably also in a number of other bacteria indicates that psicoselysine and maybe other psicosamines exist in the natural environment of these organisms. Psicosamines can potentially form through the condensation of amines with D-allose, but the scarcity of this sugar, which is found essentially in some plant glycosides and glycerolglycolipids [30–33], suggests that this may not be the principal source. Incubation of proteins with glucose in the presence of inorganic phosphate leads to the formation of a substrate for fructosamine 3-kinase-related protein,

an enzyme that phosphorylates psicosamines and ribulosamines, but not fructosamines, suggesting that psicosamines are formed under these conditions [6]. Phosphate-catalysed epimerization of fructosamines may therefore be the main source of psicosamines.

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