UDPgalactose 4-epimerase from *Saccharomyces cerevisiae*
A bifunctional enzyme with aldose 1-epimerase activity

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UDPgalactose 4-epimerase (epimerase) catalyzes the reversible conversion between UDPgalactose and UDPglucose and is an important enzyme of the galactose metabolic pathway. The *Saccharomyces cerevisiae* epimerase encoded by the *GAL10* gene is about twice the size of either the bacterial or human protein. Sequence analysis indicates that the yeast epimerase has an N-terminal domain (residues 1–377) that shows significant similarity with *Escherichia coli* and human UDPgalactose 4-epimerase, and a C-terminal domain (residues 378–699), which shows extensive identity to either the bacterial or human aldose 1-epimerase (mutarotase). The *S. cerevisiae* epimerase was purified to > 95% homogeneity by sequential chromatography on DEAE-Sephacel and Resource-Q columns. Purified epimerase preparations showed mutarotase activity and could convert either α-ᴅ-glucose or α-ᴅ-galactose to their β-anomers.

Induction of cells with galactose led to simultaneous enhancement of both epimerase and mutarotase activities. Size exclusion chromatography experiments confirmed that the mutarotase activity is an intrinsic property of the yeast epimerase and not due to a copurifying endogenous mutarotase. When the purified protein was treated with 5′-UMP and ʟ-arabinose, epimerase activity was completely lost but the mutarotase activity remained unaffected. These results demonstrate that the *S. cerevisiae* UDPgalactose 4-epimerase is a bifunctional enzyme with aldose 1-epimerase activity. The active sites for these two enzymatic activities are located in different regions of the epimerase holoenzyme.

Keywords: aldose 1-epimerase; bifunctional enzyme; reductive inhibition; *Saccharomyces cerevisiae*; UDPgalactose 4-epimerase.

UDPgalactose 4-epimerase (henceforth called epimerase) is an essential enzyme of the galactose metabolic pathway. This enzyme catalyses a freely reversible reaction between UDP-galactose and UDPglucose, and is responsible for both catabolism and anabolism of galactose in all cell types studied so far. The reaction mechanism involves abstraction of the 4′-hydroxyl hydrogen by an enzymatic base and hydride transfer from C4 of the sugar to the nicotinamide ring of NAD^+. Subsequent formation of UDP 4-keto sugar and NADH as transient intermediate on the enzyme surface, followed by stereospecific return of hydride from NADH to the opposite face of the keto sugar results in epimerization of the substrate and regeneration of NAD^+ [1].

Epimerase has been purified and characterized from *Escherichia coli* to humans. Both the *E. coli* [2] and human epimerase [3] are homodimeric proteins, each with a molecular mass of approximately 80-kDa, and contains one tightly bound NAD^+ as a cofactor in each subunit. Most of the mechanistic and crystallographic studies have been carried out with the *E. coli* and human protein [1]. Epimerase has also been purified and analyzed from the yeast *Kluyveromyces fragilis* [4–6] and *Saccharomyces cerevisiae* [7]. Both the yeast proteins are homodimers with an apparent molecular mass of 156-kDa and contain enzyme bound NAD^+. Why do yeast epimerases have twice the molecular mass of either the *E. coli* or human protein? A BLAST search [8] using the *S. cerevisiae* epimerase as the query sequence revealed that the 699 amino acid protein has two domains. The N-terminal domain (residues 1–377) showed a high degree of sequence identity with either the *E. coli* or human epimerase. The C-terminal domain (residues 378–699) showed significant identity to bacterial or human aldose 1-epimerase sequence.

Aldose 1-epimerase (henceforth called mutarotase) catalyzes the equilibration of α- and β-anomers of aldoses [9,10]. Mutarotase plays a key role in linking lactose and galactose metabolism. Hydrolysis of lactose by β-galactosidase generates α-ᴅ-glucose and β-ᴅ-galactose. While α-ᴅ-glucose is phosphorylated by glucokinase for glycolysis, β-ᴅ-galactose needs to be transformed to α-ᴅ-galactose before being phosphorylated by galactokinase. Mutarotase catalyzes the interconversion of β-ᴅ-galactose to α-ᴅ-galactose, which is then converted to the metabolically useful glucose 1-phosphate by the concerted action of three enzymes of

the Leloir pathway: galactokinase, galactose 1-phosphate uridylyltransferase, and UDPgalactose 4-epimerase.

A multiple-sequence alignment of the C-terminal domain of the *S. cerevisiae* epimerase (residues 378–699) with the complete sequence of *E. coli* [11], *Lactococcus lactis* [12], and human [13] mutarotase is shown in Fig. 1. These mutarotases show 24–31% identity and 45–48% similarity with the C-terminal half of the yeast protein, indicating that the yeast epimerase might have additional mutarotase activity. To resolve this question, we cloned, expressed and purified *S. cerevisiae* epimerase to homogeneity, and assayed for mutarotase activity. We report that the purified yeast protein does indeed have both epimerase and muta-

rotase activities. We also report that these two enzymatic activities are located in different regions of the protein.

Materials and methods

Materials

All biochemicals unless otherwise stated were purchased from Sigma. Restriction enzymes and *Taq* DNA polymerase were from Invitrogen. Amicon centrifugal filter units were from Millipore while DEAE-Sephacel and Sephacryl S-200 HR was purchased from Amersham Biosciences.
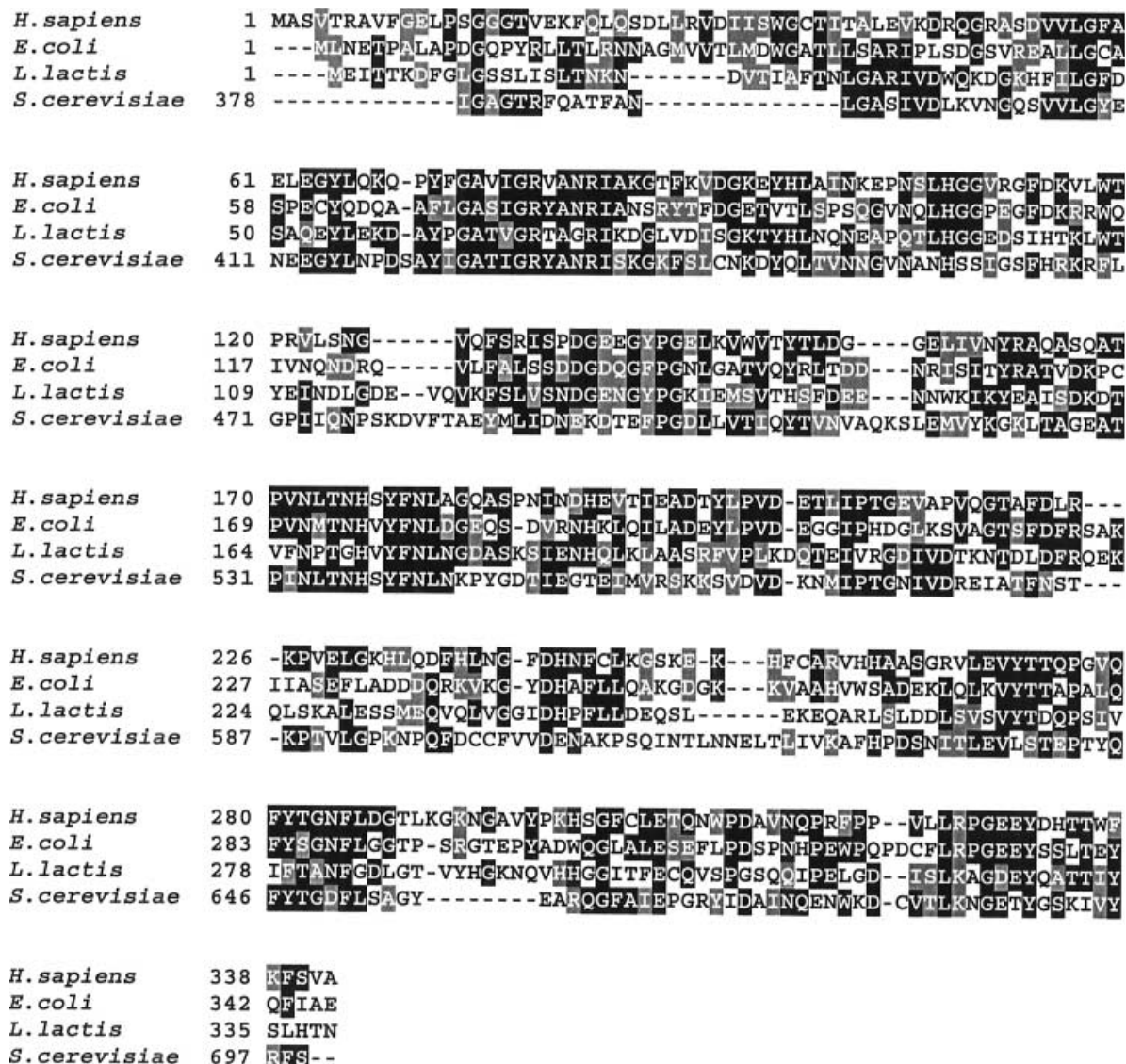

Fig. 1. Sequence alignment of the C-terminal domain (residues 378–699) of the *S. cerevisiae* epimerase with the complete amino acid sequence of *Homo sapiens*, *E. coli*, and *L. lactis* mutarotase. The GenBank Accession numbers are *S. cerevisiae* (NP_009575), *H. sapiens* (NP_620156), *E. coli* (P40681), and *L. lactis* (CAB44215). Multiple alignments were carried out using the BCM Search launcher (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). Amino acids marked with black or gray boxes indicate sequence identity or similarity, respectively. The dashes indicate the gaps introduced to maximize sequence alignment.

Strains and growth conditions

E. coli strain DH-5α (F⁻ Φ80 d*lacZ*ΔM15 Δ(*lacZYA-arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r$_k^-$, m$_k^+$) *pho*A *sup*E44 λ⁻ *thi*-1 *gyr*A96 *rel*A1) (Invitrogen) was used for cloning experiments. *E. coli* strains were grown in LB medium [14] supplemented with 100 μg·mL⁻¹ ampicillin. The diploid *S. cerevisiae* strain (wild-type) used in the purification of endogenous epimerase was derived from *S. cerevisiae* strains 8534-10A (*MATa leu2-3, 112 ura3-52 his4*Δ34) and 6460-8D (*MAT*α *met3*) [15]. Yeast strain PJB5 with a disrupted *gal10* locus (*MATa ade2-101 ile ura3-52 leu2-3112 trp1-HIII his3*Δ-1 *MEL1 gal10::LEU2*) [16] was used for expression of the recombinant protein. *S. cerevisiae* strains were grown at 30 °C in either YEP medium (1% bacto-yeast extract, 2% bacto-peptone, and either 2% glucose or galactose; w/v) or synthetic minimal medium containing either 2% glucose or galactose [17].

Cloning and expression

A 2.1-kb fragment containing the complete *GAL10* gene along with 54 bp of its upstream sequence was amplified by PCR from *S. cerevisiae* genomic DNA using a sense primer 5′-TCAGGATCCACTTCTTTGCGTCCATCC-3′ that introduced a *Bam*HI restriction site and an antisense primer 5′-CCACTGCAGTCAGGAAAATCTGTAGAC-3′ that introduced a *Pst*I restriction site. The PCR fragment was ligated with pBluescriptIIKS⁺ vector (Stratagene) creating pBluescript-GAL10. The absence of any mutation was confirmed by complete sequencing of the PCR product using the ABI Prism-377 DNA sequencer (Applied Biosystems). pBluescript-GAL10 was digested with *Bam*HI and *Pst*I and the gel-purified DNA fragment containing the coding region and termination signal of *GAL10* was ligated into *Bam*HI-*Pst*I digested *S. cerevisiae* centromeric expression vector pUS234, creating pUSGAL10. pUS234 was generated (by Uttam Surana, Institute of Molecular and Cell Biology, Singapore) after cloning an *Eco*R1/*Bam*H1 fragment containing *GAL1-10* promoter into *S. cerevisiae* shuttle vector Ycplac33 [18]. The plasmid pUSGAL10 was introduced into *gal10*-deficient strain PJB5 (henceforth called transformed PJB5) by following the method of Gietz *et al.* [19]. To test for complementation of the *gal10* mutant, transformed PJB5 cells were grown on synthetic minimal medium containing 2% galactose.

Purification of epimerase

Both wild-type and transformed *S. cerevisiae* cells were grown and harvested as described by Fukasawa *et al.* [7]. A modification of the previously reported purification procedure [7] was used. Unless otherwise mentioned, all steps in the protein purification protocol were performed at 4 °C. Frozen cells (15–20 g) were thawed quickly and suspended in 3 mL per gram of wet cells of buffer A (20 mM Tris/HCl, pH 7.4 containing 1 mM EDTA, 1 mM phenylmethane-sulfonyl fluoride, and 5 mM DL-dithiothreitol). The cells were lysed by two passages through a French pressure cell at 20 000 p.s.i. Unbroken cells and cell debris were removed after centrifugation at 12 000 *g* for 30 min and the supernatant was retained (crude extract). The crude extract was treated with 35–55% ammonium sulfate and the precipitated protein was dissolved in buffer B (20 mM Tris/HCl, pH 7.4 containing 1 mM EDTA and 5 mM dithiothreitol). The protein was desalted and concentrated using Amicon Ultra-15 (50-kDa cut-off) centrifugal filter. The concentrated protein was applied at a flow rate of 0.3 mL·min⁻¹ to a DEAE-Sephacel column (20 × 2 cm) equilibrated with buffer B. The column was washed with 150 mL of buffer B and the protein eluted from the column with a 400 mL linear gradient of 20 mM to 500 mM Tris/HCl, pH 7.4 at a flow rate of 0.2 mL·min⁻¹. Fractions of 3 mL were collected and analyzed by SDS/PAGE [20] as well as assayed for epimerase activity. The most active fractions were pooled, desalted and concentrated using Amicon Ultra-15 (50-kDa cutoff) centrifugal filter. The concentrated protein was applied at a flow rate of 1 mL·min⁻¹ on a 1 mL Resource-Q column (Amersham Biosciences) equilibrated with buffer B. The column was washed with 10 mL of buffer B and the protein eluted from the column by a step gradient of 0–1 M NaCl in buffer B. Fractions containing the epimerase protein were quickly frozen in a dry ice/ethanol bath and stored at −70 °C in aliquots. Epimerase purified from wild-type cells will be referred to as wild-type protein while that purified from transformed PJB5 cells will be described as recombinant protein. Protein concentration in crude preparations were measured by the method of Lowry *et al.* [21], while the concentration of epimerase in purified preparations was determined by the absorption at 280 nm using a molar extinction coefficient of 85 260 [22].

Epimerase assay

Epimerase activity was assayed using an NADH-coupled assay developed by Wilson and Hogness [23]. In this case, UDP-glucose, the product of epimerization, is immediately converted to UDP-glucuronic acid by coupling the reaction with UDP-glucose dehydrogenase and NAD⁺. The assay mixture consisted of 0.1 M glycylglycine buffer, pH 8.8, 0.25 mM NAD⁺, 0.16 units of UDP-glucose dehydrogenase, and 0.5 μg of epimerase. The reaction was started by the addition of 0.35 mM UDP-galactose, and the increase in absorbance due to formation of NADH was measured at 340 nm over a linear range of 2–5 min.

Mutarotase assay

Mutarotase activity was measured with a DIP-360 polarimeter (Jasco). This assay is based upon the change in optical rotation of the substrate (α-D-glucose or α-D-galactose) during an enzyme catalyzed mutarotation reaction [10]. α-D-Glucose (65 mM) was dissolved in 5 mM Tris HCl, pH 7.4 buffer containing 1 mM EDTA, immediately before addition of the enzyme. The solution was rapidly introduced into the polarimeter tube, and readings for optical rotation were taken at 1-min intervals for 6 min. The rate of the nonenzymatic turnover was subtracted from the initial rate of the enzymatic reaction.

Mutarotase activity was also assayed using the NAD⁺ and β-D-glucose dehydrogenase coupled assay [11,24]. In this method, the conversion of α-D-glucose to β-D-glucose is coupled to oxidation of β-D-glucose by β-D-glucose

dehydrogenase and reduction of NAD^+. The assay mixture consisted of 0.1 M Tris/HCl buffer, pH 7.2, 3 mM NAD^+, 10 units of β-D-glucose dehydrogenase, and 5 μg of epimerase. The reaction was initiated by the addition of 5 mM freshly dissolved α-D-glucose, and the increase in absorbance was measured at 340 nm over a linear range.

Sephacryl S-200 chromatography

The wild-type strain was grown in YEP medium containing either 2% (w/v) glucose or galactose. The *gal10* strain was grown in 3% (v/v) glycerol containing 10 mM α-D-fucose. Cells (1 g) were suspended and lysed as described above. The crude extract was treated with 0–70% ammonium sulfate and the precipitated protein was dissolved in 2 mL of buffer B. Approximately 20–30 mg of the protein was loaded on a Sephacryl S-200 column (20 × 2 cm) pre-equilibrated with buffer B. Elution was carried out with buffer B at a flow rate of 0.15 mL·min^{-1}. Fractions of 2 mL were collected and analyzed for both epimerase and mutarotase activities as well as for their protein content.

Reductive inhibition

Epimerase (10 μg) was incubated at room temperature with 2 mM uridine 5′-monophosphate (5′-UMP) and 10 mM L-arabinose in 10 mM potassium phosphate buffer, pH 8.0. Aliquots of the reaction mixture were removed at intervals and passed through a spin column [25] to remove the nucleotide and free sugar. The eluate was then assayed for both epimerase and mutarotase activity.

Results

Purification of UDPgalactose 4-epimerase

The purification procedure for UDPgalactose 4-epimerase from *S. cerevisiae* is described in the Materials and methods section and also summarized in Table 1. Either the wild-type or the recombinant epimerase was purified from *S. cerevisiae* cytosol using sequential chromatography on DEAE Sephacel and Resource-Q columns. Figure 2 shows a sharp, single protein peak following elution from the Resource-Q column. The purified epimerase preparation was judged to be > 95% homogeneous by Coomassie blue staining of samples separated by SDS/PAGE (Fig. 2, inset).
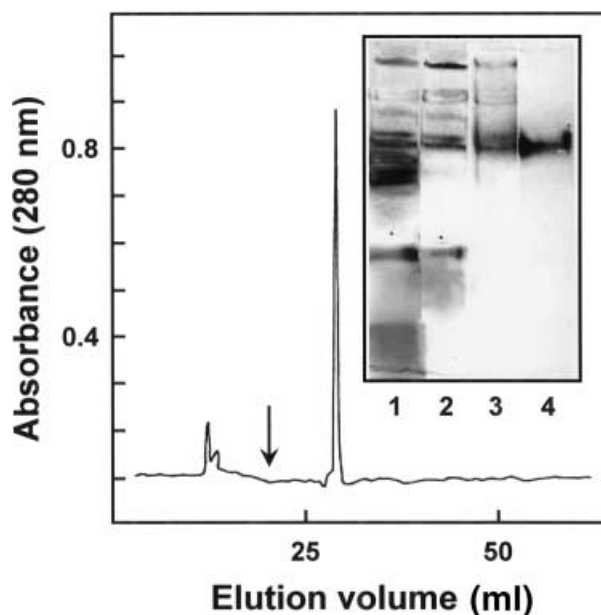

Fig. 2. Purification of *S. cerevisiae* epimerase. Elution profile of the protein from a Resource Q column. Arrow indicates initiation of ionic gradient. Inset: SDS/PAGE analysis at each step of purification. Lane 1, crude extract (cytosol); lane 2, ammonium sulfate precipitated and Amicon ultramembrane filtered fraction; lane 3, pooled fraction from DEAE-Sephacel column; lane 4, pooled fraction from Resource-Q column.

The specific activity of the purified epimerase preparation was in excess of 25 units·mg^{-1} of protein. This method is faster and more convenient than the purification procedure described by Fukasawa *et al.* [7].

Aldose-1-epimerase activity of UDPgalactose 4-epimerase

Purified epimerase preparations were analyzed for mutarotase activity by polarimetric method. The change in optical rotation of the substrate (glucose or galactose) was measured as the α-anomer was converted to the equilibrium mixture of isomers. Although glucose undergoes spontaneous mutarotation with a first-order rate constant of 0.032 min^{-1} at 25 °C [10], the addition of the

Table 1. Purification of UDPgalactose 4-epimerase from *S. cerevisiae*.

| Steps	Total protein (mg)	Total activity (units)		Specific activity (units·mg^{-1})		Ratio of Epimerase : Mutarotase Activity	Fold purification	
		Epimerase	Mutarotase	Epimerase	Mutarotase		Epimerase	Mutarotase
Crude extract	2460	718	8610	0.3	3.5	1 : 12	1	1
Ammonium sulfate fractionation	760	671	7100	0.9	9	1 : 10	3	3
DEAE-Sephacel chromatography	44	557	5200	13	118	1 : 9	43	34
Resource-Q chromatography	9	225	2026	25	225	1 : 9	83	64
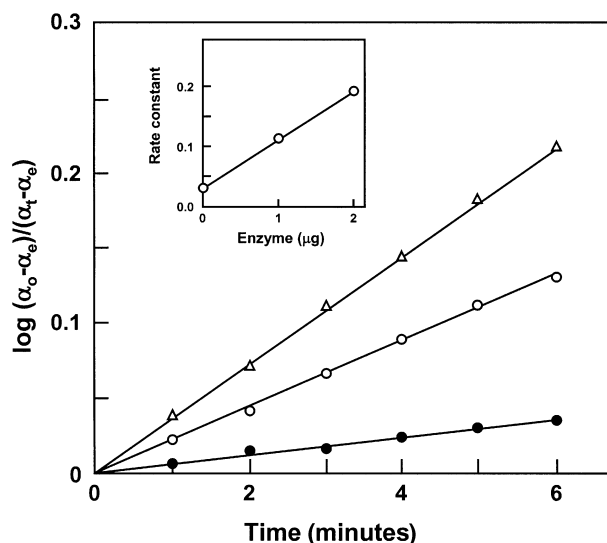
Fig. 3. Polarimetric assay of mutarotase activity of *S. cerevisiae* epimerase. First-order mutarotation reactions for α-D-glucose alone and in the presence of increasing amount of epimerase. ●, spontaneous mutarotation (only α-D-glucose); ○, 1 μg epimerase; △, 2 μg epimerase. Inset: Plot of rate constant vs. micrograms of epimerase.

protein resulted in an even greater increase in optical rotation. The first-order rate constant for the catalyzed mutarotation reaction was acquired from the slope of the straight line plot obtained by plotting $\ln(\alpha_o - \alpha_e)/(\alpha_t - \alpha_e) = k_t$, where α_o, α_t, and α_e are the observed angular rotations at time zero, t and equilibrium, respectively, and k is the calculated rate constant [10]. A linear increase in the first order rate constant was obtained with increasing quantities of the purified enzyme (Fig. 3). A similar kinetics was observed when α-D-galactose was used as the substrate. Additionally, a coupled assay method using β-D-glucose dehydrogenase as the coupling enzyme was also employed to confirm the presence of mutarotase activity in epimerase. Using α-D-glucose as a substrate, the enzymatic assay was linear over the first five minutes, and an increase in enzyme concentration proportionately increased the rate of reaction (data not shown). Mutarotase activity was also assayed for glucose or galactose induced cell lysate. The rate of conversion of α-D-glucose to β-D-glucose in induced and uninduced cell lysates are 3.5 μmol·min^{-1} and 2.2 μmol·min^{-1}·mg^{-1} of protein, respectively. These rates are much higher than the observed spontaneous rotation rate of 0.1 μmol·min^{-1}.

To determine that the mutarotase activity is an intrinsic property of *S. cerevisiae* epimerase, the ratio of epimerase to mutarotase activity was monitored during each stage of purification (Table 1). Crude cytosolic extract showed an epimerase: mutarotase activity of 1 : 12. After an ammonium sulfate precipitation and Amicon centrifugal filtration step the ratio changed to 1 : 10. The ratio of epimerase to mutarotase activity attained a constant value of 1 : 9 following DEAE–Sephacel chromatography. This suggested that the ion-exchange column might be sep-

arating a copurifying constitutive mutarotase from epimerase.

To examine the possibility that the mutarotase activity of epimerase was not due to a copurifying constitutive mutarotase, a gel filtration chromatography experiment was performed. The presence of a constitutive mutarotase in *S. cerevisiae* has been reported earlier by Sammler *et al.* [26]. Moreover, a BLAST search showed two *S. cerevisiae* open reading frames (ORFs) YHR210c and YNR071c, with putative aldose 1-epimerase activity. These ORFs encode for proteins, each with a predicted molecular mass of 38-kDa, and exhibit 99% identity with the human aldose 1-epimerase [13]. Both the *E. coli* and human aldose 1-epimerase have been shown to exist as a monomer in solution [13,27]. However, the crystal structure of *L. lactis* enzyme indicates the protein to be a dimer [28]. On the other hand, the yeast epimerase is present as a 156-kDa dimeric species [7]. Therefore, size exclusion chromatography experiments were performed to separate the yeast epimerase from any copurifying constitutive mutarotase species.

Cells were grown in YEP medium in presence of 2% glucose, harvested and lysed as described in Materials and methods. The cytosolic proteins were collected by saturated ammonium sulfate (0–70%) precipitation, dissolved in minimum volume of buffer and loaded on a Sephacryl S-200 column. The fractions were monitored for epimerase and mutarotase activities as well as for protein content. Figure 4A shows the elution profile of epimerase that is distinctly separated from a constitutively expressed mutarotase. The fractions containing epimerase activity also showed mutarotase activity. If *S. cerevisiae* epimerase were a truly bifunctional enzyme, then induction with galactose should induce both epimerase and mutarotase activity. When a similar experiment was performed after inducing the cells with galactose, there was 3.5-fold increase in epimerase activity (Fig. 4B) while the constitutive mutarotase activity remained the same. More importantly, with the increase in epimerase activity, the coeluting mutarotase activity was also induced nearly 3.5-fold. This indicated that yeast epimerase also has additional mutarotase activity. This conclusion was further confirmed when *gal10* strain (PJB5), totally lacking epimerase activity was used as a control. PJB5 cells were grown in 3% glycerol and 10 mM α-D-fucose. α-D-Fucose is an inducer for *gal* operon that acts by inactivating the *gal* repressor [11]. In this case, apart from the constitutive mutarotase, no other mutarotase activity was detected (Fig. 4C). Absence of epimerase activity in the *gal10* strain also led to simultaneous lack of epimerase associated mutarotase activity. The *gal10* strain (PJB5) cannot grow on media containing galactose as the sole carbon source due to lack of epimerase activity. This observed sensitivity could be complemented by expression of *GAL10* from a plasmid. When transformed cells were grown in presence of galactose, lysed, and similarly fractionated as the wild-type cells, both epimerase and mutarotase activities coeluted in the same fractions (data not shown). These set of experiments clearly indicate the presence of both epimerase and mutarotase activities in the same protein.
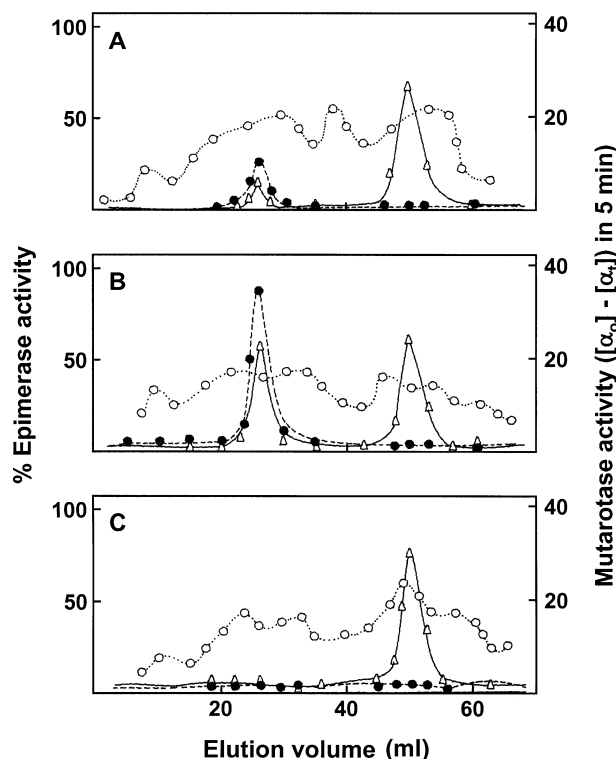
Fig. 4. Separation of epimerase from constitutive mutarotase in a Sephacryl S-200 column. Cells were grown, harvested and lysed as described in Materials and methods. Fractions were monitored for both epimerase (●) and mutarotase (△) activity as well as for their protein content (○). Wild-type strain grown in 2% glucose (A) or 2% galactose (B) and *gal10* strain grown in 3% glycerol (C).

Fig. 5. Effect of reductive inhibition on epimerase and mutarotase activity. Epimerase (10 μg) was incubated with 2 mM 5′-UMP and 10 mM L-arabinose at room temperature. Aliquots were taken at intervals and assayed for both epimerase (●) and mutarotase (○) activity.

Distinct active site for aldose-1-epimerase in UDPgalactose 4-epimerase

The participation of NAD^+ as an initial reductant is essential for the epimerization process [1]. The question arises whether NAD^+ is also critical for mutarotase activity. Bhaduri *et al.* [29] had earlier shown that upon incubation of yeast epimerase with 5′-UMP and a free sugar such as D-glucose or L-arabinose, the NAD^+ bound form of the enzyme is slowly but irreversibly reduced to NADH. The formation of NADH on the enzyme surface is accompanied by progressive enhancement of fluorescence along with a corresponding decrease in enzymatic activity and the process was termed as reductive inhibition. Upon incubation of purified epimerase with 5′-UMP and L-arabinose, the epimerase activity was progressively lost, while the mutarotase activity of the enzyme remained completely unaffected (Fig. 5). This clearly indicated that NAD^+ is not essential for mutarotase activity and mutarotation did not proceed through an oxidation–reduction mechanism. Therefore, epimerase and mutarotase activities are located in different regions of the epimerase holoenzyme.

Discussion

The identification of mutarotase activity in *S. cerevisiae* epimerase is supported by several lines of evidence. First, the protein was purified to > 95% homogeneity and shown to
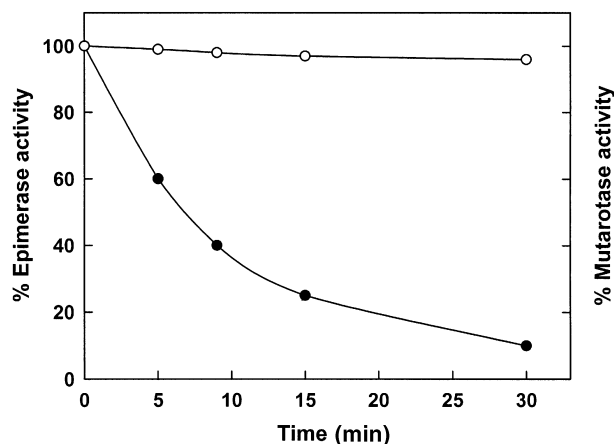
possess mutarotase activity by two independent assay methods. Either α-D-glucose or α-D-galactose could serve as the substrate. Second, size exclusion chromatography experiments showed that epimerase and mutarotase activities coeluted in the same fractions, and were conveniently separated from constitutive mutarotases. Finally, induction of cells with galactose led to a simultaneous enhancement of epimerase and mutarotase activity, whereas both activities were absent in the *gal10* strain. Reductive inhibition experiments clearly showed that the catalytic centers of epimerase and mutarotase activity are independent of each other.

It has been shown that *E. coli* mutarotase do not require either metal ions or cofactors for activity [27]. A possible catalytic mechanism was first suggested by Hucho and Wallenfels [9], which involved the abstraction of a proton from the C1 hydroxyl group of the sugar by an active base and donation of a proton to the C5 ring oxygen by an active site acid, thereby leading to ring opening. Subsequent rotation of 180° about the C1–C2 bond followed by abstraction of the proton on the C5 oxygen and donation of a proton back to the C1 oxygen generated the product. The crystal structure of *L. lactis* mutarotase indicates that Glu304 serves as the active site base to abstract the C1 hydroxyl hydrogen and His170 functions as the active site acid to protonate the C5 ring oxygen [1,30]. A similar mechanism has been proposed for the *E. coli* mutarotase where His175 has been suggested to be involved in catalysis [27]. Also, site-directed mutagenesis and kinetic experiments implicates His176 and Glu307 as active site acid and base, respectively, for the human mutarotase [13]. A multiple sequence alignment of yeast epimerase with *E. coli*, *L. lactis*, and human mutarotase (Fig. 1) indicates that His537 and Glu665 of the *S. cerevisiae* epimerase are most likely to play a role in acid-base catalysis. Site-directed mutagenesis experiments are currently in progress to investigate the role of these residues in epimerase-associated mutarotase activity.

A BLAST analysis shows that UDPgalactose 4-epimerase from *Kluyveromyces lactis* (687 amino acids), *Pachysolen tannophilus* (689 amino acids), and *Schizosaccharomyces pombe* (713 amino acids) exhibit 52–56%

identity and 68–72% similarity with *S. cerevisiae* epimerase. We would therefore hypothesize that *K. lactis*, *P. tannophilus*, and *S. pombe* epimerase to have an intrinsic mutarotase activity. However, not all fungal epimerases are likely to have mutarotase activity. Seiboth *et al.* [31] have shown that the *GAL10* gene of the filamentous fungi *Hypocrea jecorina*, codes for a 370-amino acid protein that does not contain the C-terminal mutarotase domain. Similarly, *Neurospora crassa* Gal10p has 375-amino acids in its primary structure and lacks the C-terminal extension of the *S. cerevisiae* epimerase. The evolutionary history of the fusion of epimerase and mutarotase activity in the yeast enzyme remains entirely speculative at this moment and further work is needed before we begin to appreciate its biological significance.

Acknowledgements

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