Molecular Structure of Human Galactose Mutarotase

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X-ray coordinates have been deposited in the Protein Data Bank (1SNZ) and (1SO0) and will be released upon publication.

Key Words: x-ray crystallography, protein structure, galactose mutarotase, Leloir pathway, galactosemia, enzyme mechanism
SUMMARY

Galactose mutarotase catalyzes the conversion of β-D-galactose to α-D-galactose during normal galactose metabolism. The enzyme has been isolated from bacteria, plants, and animals and is present in the cytoplasm of most cells. Here we report the x-ray crystallographic analysis of human galactose mutarotase, both in the apo-form and complexed with its substrate, β-D-galactose. The polypeptide chain folds into an intricate array of 29 β-strands, 25 classical reverse turns, and two small α-helices. There are two cis-peptide bonds at Arg 78 and Pro 103. The sugar ligand sits in a shallow cleft and is surrounded by Asn 81, Arg 82, His 107, His 176, Asp 243, Gln 279, and Glu 307. Both the side chains of Glu 307 and His 176 are in the proper location to act as a catalytic base and a catalytic acid, respectively. These residues are absolutely conserved among galactose mutarotases. To date, x-ray models for three mutarotases have now been reported, namely that described here and those from Lactococcus lactis and Caenorhabditis elegans. The molecular architectures of these enzymes differ primarily in the loop regions connecting the first two β-strands. In the human protein there are six extra residues in the loop compared to the bacterial protein for an approximate longer length of 9 Å. In the C. elegans protein, the first seventeen residues are missing thereby reducing the total number of β-strands by one.
During normal galactose metabolism, β-D-galactose is converted to glucose 1-phosphate via the action of four enzymes that constitute the Leloir pathway as shown in Scheme 1 (1). In the first step of this pathway, β-D-galactose is epimerized to α-D-galactose through the action of galactose mutarotase. The second step involves the phosphorylation of α-D-galactose to galactose-1-phosphate by galactokinase. As indicated in Scheme 1, galactose-1-phosphate uridylyltransferase catalyzes the third step by transferring a UMP group from UDP-glucose to galactose 1-phosphate, thereby generating glucose 1-phosphate and UDP-galactose. To complete the pathway, UDP-galactose is converted to UDP-glucose by UDP-galactose 4-epimerase.

Mutations in three of the enzymes of the Leloir pathway, namely galactokinase, galactose-1-phosphate uridylyltransferase, or UDP-galactose 4-epimerase, have been demonstrated to result in the diseased state known as galactosemia (2, 3). The symptoms of this genetic disease include early onset cataracts (typically within the first two years of life) and, in more severe cases, liver, kidney, and brain damage. Cataract formation is believed to be caused by the build up of unmetabolized galactose in the lens of the eye. Aldose reductase catalyzes the reduction of the sugar to galactitol (dulcitol) which is not, in contrast to galactose, readily transported across the plasma membrane (4-6). As such, the accumulation of this highly osmotically active compound draws excess water into the lens resulting in cataracts by a mechanism which is not fully understood. The more severe symptoms of galactosemia are most likely caused by a combination of these osmotic effects and the accumulation of the
intermediate α-D-galactose-1-phosphate (7) although the precise mechanism of toxicity is not understood. No known disease-causing mutations in human galactose mutarotase have been identified thus far. However, galactitol is reported to be an inhibitor of mammalian mutarotase (8) raising the possibility that its accumulation would partly block the action of mutarotase resulting in a further build up of unmetabolized galactose.

Galactose mutarotase activity was first reported in *Escherichia coli* in 1965 (9) and has since been observed in a wide range of organisms including bacteria (9-16), plants (17, 18), fungi (19), and mammals (20-23). It is present in the cytoplasm of most cells thus suggesting that the *in vivo* rate of uncatalyzed mutarotation is insufficient for the metabolic needs of the organism. Indeed, deletion of the mutarotase gene from *E. coli* results in slow growth on minimal media containing phenyl-β-D-galactopyranose as the sole carbon source (11).

The first three-dimensional structure of a dimeric mutarotase, namely that from *Lactococcus lactis*, was reported in 2002 (24). The enzyme was shown to adopt a β-sandwich motif, similar to that seen in domain 5 of β-galactosidase (25) with the sugar binding site located in a wide, shallow cleft. Five highly conserved residues (Arg 71, His 96, His 170, Asp 243 and Glu 304) were within hydrogen bonding distance to the hydroxyl groups of the bound galactose ligand. Previous kinetic studies suggested that the reaction mechanism for the enzyme proceeds *via* an acid-base mechanism with transient ring opening (26, 27) and the model of the mutarotase from *L. lactis* implicated Glu 304 as the likely catalytic base and either His 96 or His 170 as the catalytic acid (24). Accordingly, the reaction mechanism of galactose mutarotase is thought to occur via
abstraction of the proton from the C-1 hydroxyl group by Glu 304 in the *L. lactis* enzyme and protonation of the ring oxygen of the sugar by His 170, which results in ring opening. Subsequent rotation about the C-1/C-2 bond followed by a reversal of the ring opening events ultimately yields a product with an altered configuration at C-1 (28). Site-directed mutagenesis of these residues (or their equivalents in the *E. coli* enzyme) have confirmed the role of Glu 304 as the catalytic base and established His 170 as the catalytic acid (28, 29).

The gene encoding the human enzyme was identified in 2003 and the protein product characterized (30). From these investigations it was suggested that Glu 307 and His 176 fulfill the roles in acid/base catalysis in the human mutarotase (30). Interestingly, regardless of the source, mutarotases tend to show a preference for galactose over glucose and have high $k_{\text{cat}}$ and $K_m$ values with this sugar (typically $10^4$ - $10^5$ s$^{-1}$ and 10 - 100 mM respectively) (23, 30-33). The preference of these enzymes for galactose over glucose was addressed structurally with the *L. lactis* mutarotase and, at least in the case of this bacterial protein, is explained by the existence of non-productive binding modes in the active site for glucose and its derivatives (33). With respect to quaternary structure, human mutarotase appears to be monomeric (10, 30) whereas the *L. lactis* enzyme was shown to be a dimer on the basis of ultracentrifugation experiments (24). The biochemical significance, if any, of these differences in quaternary structure is not understood at the present time.

Here we describe the three-dimensional structure of human galactose mutarotase, both in the unbound state and complexed with its substrate, β-D-galactose. This study represents the first report of a eukaryotic mutarotase with
bound sugar and allows for a detailed comparison between the human and the bacterial forms of the enzyme.
EXPERIMENTAL PROCEDURES

Cloning of the Galactose Mutarotase Gene. The human galactose mutarotase gene was amplified from the originally described pET21-GALM plasmid (30) using a forward primer designed with an NdeI restriction site at the start codon and a reverse primer with a XhoI restriction site after the stop codon. This construct allowed for insertion of the PCR product into the pTYB12 expression vector which provides an N-terminal chitin binding domain followed by a DTT inducible intein (New England Biolabs). The re-cloning was done because diffraction quality crystals could not be obtained with protein expressed from the pET21-GALM plasmid construct, which contained a non-cleavable N-terminal polyhistidine tag.

Protein Expression and Purification. For protein expression, the pTYB12-GALM plasmid was used to transform E. coli HMS174(DE3) cells (Novagen). A starter culture from a single colony was grown overnight at 37 °C in LB media supplemented with ampicillin. Subsequently, 10 mL were transferred to 1000 mL of the supplemented LB media in a 2 L shaker flask and grown at 37 °C until an optical density of 0.8 was achieved at 600 nm. The cultures were cooled to 16 °C in an ice-water bath and IPTG was added to a final concentration of 1 mM. Cell growth was allowed to continue at 16 °C for an additional 15 hrs.

The cells were harvested by centrifugation at 4000 x g for 15 min and frozen in liquid nitrogen. Frozen cells (50 g) were thawed in 150 mL lysis buffer consisting of 20 mM NaH₂PO₄ and 500 mM NaCl (pH 8.0). The thawed cells were placed in an ice bath and disrupted by four rounds of sonication (1 min duration each) separated by 5 min of
cooling. Cellular debris was removed by centrifugation at 20000 x g for 25 min. The clarified supernatant was loaded onto a 100 mL column of chitin beads (New England Biolabs) that had been previously equilibrated with lysis buffer. The column was then washed with lysis buffer until the absorbance reading at 280 nm reached background level. The column was charged by washing with 200 ml lysis buffer augmented with 100 mM DTT. The charged column was subsequently moved to room temperature and allowed to stand for 18 hrs and then returned to 4 °C. The cleaved protein eluted with lysis buffer. Protein containing fractions were pooled based on purity as judged by SDS-PAGE and dialyzed against 10 mM HEPES and 200 mM NaCl (pH 7.5). The dialyzed protein was concentrated to 23.0 mg/ml based on the extinction coefficient of 0.85 cm/(mg mL) as calculated with the program Protean (DNASTAR, Inc., Madison, Wisconsin). A typical yield was between 80 to 100 mg of protein per 50 grams of cells.

**Crystallization of Apo-Galactose Mutarotase.** A search for crystallization conditions was conducted at both room temperature and at 4 °C via the hanging drop method of vapor diffusion utilizing an “in-house” designed sparse matrix screen composed of 144 conditions. The best crystals were observed growing at 4 °C from poly(ethylene glycol) 8000 (PEG-8000) at pH 5.0. Large single crystals were subsequently obtained by seeding into batch experiments with precipitant solutions of 5 - 8 % PEG-8000 buffered with 100 mM Homopipes (pH 5.0) and enzyme concentrations of 12.0 mg/ml. Diamond-shaped crystals grew to maximum dimensions of ~0.5 mm x 0.3 mm x 0.1 mm in 3 - 5 weeks. They belonged to the space group P2₁ with unit cell dimensions of \(a = 60.7 \, \text{Å}, \, b = 90.7 \, \text{Å}, \, c = 70.0 \, \text{Å}, \, \text{and } \beta = 102.5^\circ\). The asymmetric unit contained two monomers.

**Structural Analysis of Apo-Galactose Mutarotase.** An x-ray data set was collected to 2.2 Å resolution at 4 °C with a Bruker HISTAR area detector system equipped with Supper “long” mirrors. The x-ray source was CuKα radiation from a Rigaku RU200 x-
ray generator operated at 50 kV and 90 mA. The x-ray data were processed with XDS (34, 35) and internally scaled with XSCALIBRE (Rayment and Wesenberg, unpublished). X-ray data collection statistics are presented in Table I. The structure was solved by molecular replacement with the program AMORE (36) and using as a search model one subunit of the *L. lactis* enzyme (PDB 1L7J). Two peaks were readily identified in the rotation search. A subsequent translation search and rigid body refinement to 3.0 Å yielded an R-factor of 46%. To reduce model bias, the electron densities corresponding to the two monomers in the asymmetric unit were averaged with software package AVE (37, 38). From this “averaged” electron density map, a model for the complete monomer was constructed and then placed back into the unit cell for subsequent least-squares refinement with TNT (39). Alternate cycles of manual rebuilding and least-squares refinement reduced the R-factor to 17.2% for all measured x-ray data to 2.2 Å resolution. Refinement statistics are presented in Table II.

*Structural Analysis of Galactose Mutarotase Complexed with β-D-Galactose.* Apo-galactose mutarotase crystals were harvested from batch experiments and equilibrated in a synthetic mother liquor composed of 10% PEG-8000, 200 mM NaCl, 100 mM Homopipes (pH 5.0), and 100 mM D-galactose in an attempt to soak the sugar into the crystalline lattice. This, however, led to cracking of the crystals and a marked change in lattice parameters and x-ray diffraction quality. Following these initial soaking experiments, crystals were grown by seeding into batch experiments with precipitant solutions of 5 - 8% PEG-8000 buffered with 100 mM MES (pH 6.0), 200 mM LiCl, and 100 mM D-galactose, with enzyme concentrations of 12.0 mg/ml. Crystals of the apo-enzyme were used for initial nucleation. Rod-like crystals appeared after 3 - 4 weeks and achieved maximum dimensions of ~0.5 x 0.2 x 0.1 mm. They belonged to the space group P1 with unit cell dimensions of $a = 61.0 \text{ Å}$, $b = 68.7 \text{ Å}$, $c = 98.9 \text{ Å}$, $\alpha = 107.7$, $\beta =$
98.4, and \( \gamma = 102.7 \) and contained four monomers in the asymmetric unit. Most likely the initial attempts at soaking crystals of space group P2_1 in solutions containing galactose resulted in a change of space group thereby leading to the observed deterioration in the quality of the x-ray diffraction properties.

An x-ray data set from a crystal of the enzyme/sugar complex was collected to 2.3 Å resolution at 4 °C, processed with SAINT (Bruker AXS, Inc), and scaled as previously described. This protein/substrate complex structure was solved via molecular replacement with the software package AMORE (36) and employing as the search model the x-ray coordinates for the apo-enzyme. Iterative cycles of least-squares refinement with TNT (39) and manual manipulation with TURBO (40) reduced the R-factor to 16.7 % for all observed data from 30.0 to 2.30 Å resolution. Refinement statistics are presented in Table II.

Quality of the X-ray Models. The models for both the apo- and sugar-bound forms of human galactose mutarotase refined to low R-factors with excellent overall stereochemistry as indicated in Table II. Electron density was visible for all of the polypeptide chains (Met 1 to Ala 342) in the asymmetric unit, regardless of the space group. In several of the monomers, the Gly-His motifs left behind from the intein construct were visible. Only the polypeptide chain backbone from Lys 249 to Lys 252 was somewhat weaker in each monomer. A Ramachandran plot reveals only one significant outlier in either the apo-enzyme or the protein/galactose monomers, namely Asn 81 with average \( \phi, \psi \) angles of 70° and –159°, respectively. The electron density corresponding to Asn 81 is unequivocal in each monomer and, in fact, its side chain carboxamide group forms a hydrogen bond with the 2-hydroxyl group of bound \( \beta\)-D-galactose. This
strained conformation is not a function of sugar binding since it is also observed in the apo-enzyme model.

Shown in Figure 1 is electron density corresponding to one of the β-D-galactose moieties in the P1 asymmetric unit. Each of the four sugars in the asymmetric unit was modeled into the electron density map in the β-anomeric configuration. Excluding the C-6 hydroxyl oxygens, the average B-values for the sugars are between 31.0 Å² and 39.4 Å². The C-6 hydroxyl oxygens refined to high B-values of 77.1 Å², 89.3 Å², 100 Å², and 100 Å². These higher temperature factors most likely reflect the fact that there are no direct hydrogen bonding interactions between the protein and this portion of the substrate.
RESULTS AND DISCUSSION

Crystals of the apo-form of galactose mutarotase belong to the space group P2₁ with two polypeptide chains per asymmetric unit. The α-carbons for these two monomers superimpose with a root-mean-square deviation of 0.17 Å and, as such, are essentially identical within experimental error. Hence, for the sake of simplicity, the following discussion will only refer to Monomer I in the x-ray coordinate file. The chitin binding domain/intein construct employed for protein expression and purification left a residual glycine and a histidine residue at the N-terminus. These two residues are well-ordered in Monomer I but are not visible in the electron density for Monomer II.

Shown in Figure 2a is a ribbon representation of the monomeric human enzyme which has overall dimensions of ~ 60 Å x 40 Å x 55 Å. The polypeptide chain folds into a complicated series of 29 β-strands ranging in length from two to eleven amino acid residues. These strands are connected by a number of classical reverse turns including twelve Type I, four Type I’, six Type II, one Type II’, one Type III, and one Type III’. There are two α-helical regions defined by Leu 62 to Leu 66 and Leu 230 to Leu 234. Cis-peptide bonds are observed at Arg 78 and Pro 103. Arg 78 is located in a coil region connecting β-strands six and seven while Pro 103 is positioned in the loop between β-strands nine and ten. The side chain of Arg 78 points away from the active site cleft.
When crystals of human galactose mutarotase are grown in the presence of D-galactose, the space group changes from P2₁ to P1 with four molecules in the asymmetric unit. These four monomers are, however, virtually identical such that their \( \alpha \)-carbons superimpose with root-mean-square deviations of between 0.17 Å and 0.18 Å. The Gly-His motif at the N-terminus is visible in the electron density for two of the four monomers in the asymmetric unit. Again, for the sake of simplicity, the following discussion will refer only to Monomer I in the P1 asymmetric unit. Within experimental error there are no significant structural changes in the enzyme that occur upon galactose binding. All main chain plus C-\( \beta \) atoms for the apo- and substrate-bound forms of galactose mutarotase correspond with a root-mean-square deviation of 0.19 Å. The sugar moiety is situated in a shallow active site cleft with its 3-, 4-, and 6-hydroxyl groups somewhat solvent exposed as shown in Figure 2b.

A close-up view of the region surrounding the sugar ligand is depicted in Figure 3a. Those amino acid side chains responsible for anchoring the sugar to the protein include Asn 81, Arg 82, His 107, Asp 243, His 176, Gln 279, and Glu 307. A schematic diagram showing possible hydrogen bonding interactions between the protein and the ligand is presented in Figure 3b. As predicted by Timson and Reece, (30), Glu 307 is located within \(~2.8 \) Å of the 1-hydroxyl group of galactose and thus is in the proper position to serve as the catalytic base for proton abstraction. Likewise, His 176 lies at \(~2.8 \) Å from the ring oxygen and could possibly serve as a catalytic acid. The hydrogen bonding pattern displayed around the galactose moiety in the human enzyme is similar to that observed for the \( L. \ lactis \) mutarotase/sugar complex (24). The notable exceptions, however,
are those interactions observed between the sugar and Asn 81 and Gln 279 in the human protein. The structurally equivalent residues in the *L. lactis* mutarotase are Gly 70 and Val 277, respectively. Recently, the structure of the apo-form of galactose mutarotase from *Caenorhabditis elegans* was solved to 1.85 Å resolution and the x-ray coordinates deposited in the protein data bank (1LUR; Northeast Structural Genomics Target Wr66). Examination of the model for the *C. elegans* mutarotase demonstrates that the amino acid residues involved in sugar binding in the human protein, as highlighted in Figure 3b, are conserved except for the replacement of Gln 279 with a histidine residue. It can thus be postulated that galactose will bind to the *C. elegans* protein in a similar fashion to that observed for the human and *L. lactis* enzymes.

The amino acid sequence similarity and identity between the human and *L. lactis* mutarotases is 49 % and 32 %, respectively. As expected from such high primary structural homology, these two enzymes have similar overall molecular architectures as shown in Figure 4a. Indeed, the two mutarotases correspond with a root-mean-square deviation of 1.2 Å for 307 structurally equivalent α-carbons. There are five regions where the two enzymes do differ significantly, however. As indicated by the letter “A” in Figure 4a, there is six-residue insertion beginning at Glu 11 in the human protein. Another five-residue insertion, starting at Val 45, occurs in the human enzyme and is labeled “B” in Figure 4a. Likewise there are two insertions in the *L. lactis* versus the human enzyme: a four residue insertion beginning at Asp 113 (not visible in the view given in Figure 4a) and a five residue insertion starting at Met 233 of the bacterial enzyme and labeled “C.” Strikingly, the region defined by Met 233 to Val 239 in
the bacterial protein adopts an $\alpha$-helical motif, which is missing in the human protein. Apart from these insertions/deletions, the polypeptide chains for the two enzymes differ elsewhere in the area delineated by Gln 67 to Phe 72 in the human mutarotase and Glu 56 to Pro 61 in the \textit{L. lactis} enzyme as indicated in Figure 4a. The differences observed in this region arise from substitution of Pro 70 and Phe 72 with Ala 59 and Pro 61 in the human and bacterial proteins, respectively. Note that this region of polypeptide chain defines part of the active site cleft.

The mutarotase from \textit{C. elegans} demonstrates an amino acid sequence identity of 33\% with the human protein and the two eukaryotic proteins superimpose with a root-mean-square deviation of 1.0 Å for 301 equivalent $\alpha$-carbons. Perhaps the most striking difference between the human and \textit{C. elegans} enzymes occurs at the N-terminus as can be seen in Figure 4b. In the \textit{C. elegans} protein, the first seventeen residues are missing thereby reducing the number of $\beta$-strands by one. Interestingly, the cis-peptide geometry observed at position 78 of the human enzyme is conserved in the \textit{L. lactis} enzyme as cis-Pro 67 and as cis-Arg 63 in the \textit{C. elegans} protein. Furthermore, while Pro 103 in the human enzyme is in the cis-conformation, the peptide bonds for the prolines in the equivalent positions in the \textit{C. elegans} and \textit{L. lactis} mutarotases are trans.

While the maintenance of the anomeric equilibrium of galactose is likely to be a major biochemical role for galactose mutarotase, it may also function in the metabolism of glucose and other sugars. Indeed, mutarotases have been shown to turn over various sugars including D-glucose, D-fucose, D-quinovose, L-arabinose, and D-xylose (8, 23, 33). With the gene sequence for the human
enzyme now known, it will be possible to explore both its pattern of tissue expression and sub-cellular location. Undoubtedly further biochemical investigations will shed additional light on the role of this fascinating enzyme in sugar metabolism.
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FIGURE LEGENDS

Fig. 1. **Representative electron density.** Electron density corresponding to the β-D-galactose moiety and surrounding amino acid residues is shown for Monomer I in the x-ray coordinate file. The map was calculated with coefficients of the form $(2F_o - F_c)$ where $F_o$ and $F_c$ were the native and calculated structure factor amplitudes, respectively. The map was contoured at $1\sigma$.

Fig. 2. **Structure of human galactose mutarotase.** A ribbon representation of the three-dimensional architecture exhibited by the apo-enzyme is shown in (a). For clarity, only the major strands of β-sheet are displayed. Asterisks indicate the positions of cis-Arg 78 and cis-Pro 103. The location of the active site is depicted in (b) as a space-filling representation. Note that the 3-, 4-, and 6-hydroxyl groups of galactose, from left to right and indicated by the red spheres, are solvent exposed. The view is approximately the same as displayed in (a).

Fig. 3. **The active site for human galactose mutarotase.** A close-up stereo view of those amino acid residues involved in sugar binding is displayed in (a). The color-coding for the ribbon representation is the same as in Figure 2. The sugar is highlighted in grayish bonds. Possible hydrogen bonding interactions between the protein and the ligand are shown schematically in (b). The dashed lines indicate distances equal to or below 3.2 Å.
Fig. 4. **Comparison of the human, *L. lactis*, and *C. elegans* galactose mutarotases.** (a) A superposition of the α-carbons for the human and *L. lactis* enzymes is shown in black and red, respectively. The letters indicate the positions of significant differences between the two proteins. The region defined by Gln 67 to Phe 72 in the human enzyme corresponds to Glu 56 to Pro 61 in the *L. lactis* mutarotase. X-ray coordinates for the *L. lactis* enzyme were from this laboratory. Shown in (b) is a superposition of the α-carbons for the human and *C. elegans* mutarotases, displayed in black and red, respectively. X-ray coordinates for the *C. elegans* protein were obtained from the Protein Data Bank (1LUR).
Table I: X-ray Data Collection Statistics.

| Data Set                  | Resolution (Å) | Independent Reflections | Completeness (%) | Redundancy | Avg I / Avg o(I) | R\textsubscript{sym}\textsuperscript{a} |
|---------------------------|----------------|------------------------|------------------|------------|-----------------|-----------------|
| Apo Protein               | 30.0 – 2.20    | 36670                  | 94.8             | 3.4        | 8.3             | 7.1             |
|                           | 2.30 – 2.20\textsuperscript{b} | 4192                  | 90.1             | 1.6        | 1.6             | 26.9            |
| Protein/Sugar Complex     | 30.0 – 2.30    | 61156                  | 94.7             | 2.1        | 10.8            | 7.0             |
|                           | 2.40 – 2.30    | 5979                   | 77.5             | 1.4        | 2.8             | 26.4            |

\textsuperscript{a}\(R\text{sym} = \left( \frac{\sum |I - \bar{I}|}{\sum I} \right) \times 100.\)

\textsuperscript{b}Statistics for the highest resolution bin.
Table II: Relevant Refinement Statistics.

|                                 | Apo Protein | Protein/Sugar Complex |
|---------------------------------|-------------|-----------------------|
| Resolution Limits (Å)           | 30.0 – 2.20 | 30.0 – 2.30           |
| aR-factor (overall) %/ no. reflections | 17.4 / 36670 | 16.9 / 61156          |
| R-factor (working) %/ no. reflections | 17.2 / 33003 | 16.7 / 55040          |
| R-factor (free) %/ no. reflections | 20.1 / 3667 | 19.8 / 6116           |
| No. Protein Atoms               | 5111b       | 10746c                |
| No. Hetero-Atoms                | 258 (waters) | 454d                  |
| Bond Lengths (Å)                | 0.011       | 0.013                 |
| Bond Angles (deg)               | 2.34        | 2.40                  |
| Trigonal Planes (Å)             | 0.007       | 0.007                 |
| General Planes (Å)              | 0.011       | 0.012                 |
| Torsional Angles (deg)          | 19.9        | 19.5                  |

*R-factor = (\sum |F_o - F_c| / \sum |F_o|) x 100 where F_o is the observed structure-factor amplitude and F_c is the calculated structure-factor amplitude.

bThese include multiple conformations for R30, R121, and S186 in Monomer I and V75 in Monomer II.

cThese include multiple conformations for E102 and E229 in Monomer I, E229 in Monomer II, and R48 in Monomer IV.

dThese include four \(\beta\)-D-galactose moieties and 406 waters.

eThe torsional angles were not restrained during the refinement.
(1) mutarotase  
(2) galactokinase  
(3) uridylyltransferase  
(4) epimerase  

Scheme 1

Thoden et al., 2004
Thoden et al., 2004  Figure 1
Thoden et al., 2004

Figure 2
Thoden et al., 2004

Figure 3
