The role of active-site aromatic and polar residues in catalysis and substrate discrimination by xylose isomerase

(glucose isomerase/site-directed mutagenesis/enzyme-active site/protein engineering/catalytic efficiency)

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ABSTRACT The functions of individual amino acid residues in the active site of Thermoanaerobacterium thermosulfurigenes D-xylose ketol-isomerase (EC 5.3.1.5) were studied by site-directed mutagenesis. The role of aromatic residues in the active-site pocket was not limited to the creation of a hydrophobic environment. For example, Trp-188 provided for substrate binding and Trp-139 allowed for the discrimination between D-xylose and D-glucose. The substrate discrimination was accomplished by steric hindrance caused by the side chain of Trp-139 toward the larger glucose molecule. Preference of the enzyme for the α-anomer of glucose depended on the His-101/Asp-104 pair. Wide differences observed in the catalytic constant (kcat) for α- versus β-glucose in the wild-type enzyme and the fact that only the kcat for α-glucose was changed in the His-101→Asn mutants strongly suggest that the substrate molecule entering the hydride-shift step is still in the cyclic form. On the basis of these results a revised hypothesis for the catalytic mechanism of D-xylose isomerase has been proposed that involves His-101, Asp-104, and Asp-339 functioning as a catalytic triad.

D-Xylose ketol-isomerase (EC 5.3.1.5) catalyzes the reversible isomerization of D-xylose to D-xylulose as part of the xylose metabolic pathway in microorganisms (1). Due to its ability to use D-glucose as a substrate and convert it to D-fructose this enzyme is widely used in industry for production of sweeteners. Comparison of the primary structures of xylose isomerases, deduced from the nucleotide sequences of cloned genes reveal that amino acid residues, considered to play important roles in the active site of the enzyme are conserved among all species studied to date (2–5). It is believed, therefore, that all known xylose isomerases use essentially the same mechanism of catalysis. Two classes of xylose isomerases, however, may be distinguished. Class I, represented by the enzymes from Arthrobacter, Streptomyces rubiginosus, Streptomyces olivochromogenes, and Actinoplanes missouriensis, has the N-terminal portion shorter in comparison with the class II enzymes, represented by the isomerases from Escherichia coli, Bacillus, and Thermoanaerobacterium [formerly classified as Clostridium (3)]. Crystal structures of the class I enzymes have been determined (4–9), but no three-dimensional structures of the class II isomerases have been determined to date.

On the basis of the crystal structure of the Arthrobacter isomerase and its complexes with different substrates and inhibitors a reaction mechanism for aldose isomerization has been proposed (6, 10). This mechanism included the following steps: (i) binding of α-D-pyranose substrate to the enzyme, (ii) ring-opening, presumed to be catalyzed by the His-53 residue, (iii) conformational rearrangement of substrate from pseudocyclic to an extended open-chain form, (iv) hydride shift between C1 and C2, assisted by a divalent metal at position [III], (v) conformational rearrangement, ring-closure, and release of product. Our previous work on the isotope effect of D-[2-2H]glucose on the reaction velocity (3), as well as the work of other groups (10), indicated that the transfer of hydrogen between C1 and C2 is the rate-limiting step of the isomerization pathway. Indications were also found that the substrate specificity for D-glucose is limited to the Met-87 in Arthrobacter may be a steric hindrance for accommodation of glucose in the active-site pocket of the enzyme (11).

In the present work we provide further support for the hydride-shift hypothesis formulated by Collyer et al. (6) and extend it by proposing that the hydride shift occurs in the cyclic form of the substrate rather than in the extended form. We also examine the roles of aromatic amino acid residues in the active site of Thermoanaerobacterium thermosulfurigenes, including the residue that contributes to the discrimination between D-xylose and D-glucose.

MATERIALS AND METHODS

Strains, Plasmids, and Chemicals. E. coli strain HB101 [F− hsdS20 ara-l recA13 proA12 lacY1 galK2 rpsL20 met-l y-l 5-xyl-5] (12) was used for expression of the T. thermosulfurigenes xylose isomerase gene as described (3). E. coli strain TG1 [hi supE hisD Δ(lac-proAB)/F' traD36 proA+ B' lacI9 lacZAM15] and bacteriophage M13mp19 (13) were used for site-directed mutagenesis and nucleotide-sequence determination. α-Glucose and β-glucose were from Sigma.

DNA Manipulation. Site-directed mutagenesis was performed by the method of Sayers et al. (14) using the kit from Amersham. Nucleotide sequences of the mutant genes were confirmed by the dideoxynucleotide chain-termination method (15). His-101→Asn (3), Trp-139→Tyr, and Trp-139→Phe (11) mutant enzymes were created previously.

Protein Purification and Steady-State Kinetics. The previous protocol (3) was modified to purify xylose isomerase to homogeneity (on SDS/PAGE). Briefly, crude cell extract was incubated at 75°C for 15 min, precipitate was removed by centrifugation, and supernatant was fractionated by DEAE-Sepharose followed by Sephacryl-300 chromatography (3, 11). Phe-145→Lys and Trp-188→His mutant enzymes were heated at 60°C for 20 min, and Trp-139→Lys mutant enzyme was heated at 65°C for 30 min instead of 75°C. The enzyme reactions in 1 ml contained 20 mM Mops (pH 7.0), 1.0 mM CoCl2, substrate at concentrations of 0.3–2.5 times the Km, and enzyme at 10–1500 μg. Temperature and reaction conditions are listed in the footnote to each table. Reaction products were determined by the cysteine/carbonate/

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sulfuric acid method (16). Kinetic constants were determined from both Lineweaver-Burk and Eadie-Hofstee plots (17). We defined the catalytic constant \( k_{cat} \) as the turnover number per active site of enzyme at saturating substrate concentration and determined it from the equation

\[
k_{cat}[E]_0 = V_{max},
\]

where \([E]_0\) = total active-site concentration.

### Correction for Spontaneous Mutarotation

In the determination of \( V_{max} \) and \( K_M \) for \( \beta\)-glucose, the interference from \( \alpha\)-glucose, present as impurity and formed by spontaneous mutarotation, was considered because \( K_{M(\alpha\text{-glucose})}\text{App} < K_{M(\beta\text{-glucose})}\text{App} \), where \( K_M \) values represent apparent values for \( \alpha\)-glucose and \( \beta\)-glucose, respectively. If both anomers are present, fructose may arise from two different reactions:

\[
\begin{align*}
\beta\text{-glucose} & \xrightarrow{\text{mutarotation}} \alpha\text{-glucose} \\
\alpha\text{-glucose} & \xrightarrow{\text{mutarotation}} \beta\text{-glucose}
\end{align*}
\]

The initial velocity of fructose formation from \( \beta\)-glucose \((V_\beta)\) may be calculated by subtracting the initial velocity of fructose formation from \( \alpha\)-glucose (that exists as impurity or is formed from \( \beta\)-glucose by mutarotation) \((V_\alpha)\), from the apparent velocity of fructose formation \((V_p + V_\alpha)\) determined experimentally. To account for the spontaneous mutarotation we have followed the change in optical rotation of \( \beta\)-glucose under the same conditions as were used to determine enzymatic glucose isomerization (20 mM Mops, pH 7.0/1.0 mM CoCl\(_2\); 35\(^\circ\)C; 1.5 min) with the Autopol II automatic polarimeter (Rudolph Research, Flanders, NJ). Because mutarotation is reversible and its rate constant is independent of the concentration of sugar over a wide range of concentrations, at initial reaction conditions we have

\[
\frac{d[\alpha\text{-glucose}]}{dt} = \frac{d[\beta\text{-glucose}]}{dt} = k[\beta\text{-glucose}].
\]

The reaction constant \( k \), calculated from the measurement of mutarotation and expressed in decimal logarithms and min\(^{-1}\), was 0.0073 ± 0.0009. Because the spontaneous mutarotation rate is faster than the enzyme-catalyzed rate of glucose isomerization, we assumed

\[
\frac{d[\alpha\text{-glucose}]}{dt}_{(\text{Enzyme})} = \frac{d[\alpha\text{-glucose}]}{dt}_{(\text{buffer})} = k[\beta\text{-glucose}].
\]

The content of \( \beta\)-glucose after 1.5 min of incubation under conditions used for enzymatic reaction was 96.6% of total. The content at 0 time, obtained by extrapolation of the mutarotation curve, was 99.1%. As an approximation, therefore, we can consider that during the first 1.5 min \([\beta\text{-glucose}] = \text{constant} \)

\[
\frac{d[\alpha\text{-glucose}]}{dt} = \text{constant}.
\]

It is reasonable, therefore, to use the average content of \( \alpha\)-glucose = 2.2%, present in the solution of \( \beta\)-glucose during the initial 1.5 min of incubation with the enzyme, to calculate the apparent initial velocity of fructose formation from \( \alpha\)-glucose, \( V_\alpha \), from the equation:

\[
V_\alpha = \frac{V_{max(\alpha\text{-glucose})}}{[\alpha\text{-glucose}] + K_{M(\alpha\text{-glucose})}},
\]

where we assumed \( V_{max(\alpha\text{-glucose})} \approx V_{max(\alpha\text{-glucose})}\text{App} \) and \( K_{M(\alpha\text{-glucose})} \approx K_{M(\alpha\text{-glucose})}\text{App} \). The velocity of fructose formation from \( \beta\)-glucose at a given concentration of \( \beta\)-glucose, \( V_\beta \), could thus be calculated and used to calculate \( V_{max(\beta\text{-glucose})} \) and \( K_{M(\beta\text{-glucose})} \). To show the corrections obtained by this method one set of data for the wild-type enzyme is presented in Table 1.

The same method was used to correct for mutarotation in the determination of the \( V_{max(\alpha\text{-glucose})} \) and \( K_{M(\alpha\text{-glucose})} \). It was found that these corrections were very small.

### RESULTS AND DISCUSSION

#### The Role of Aromatic Residues in the Active Site of Xylose Isomerase

In the active site of xylose isomerase from *Arthrobacter* (Fig. 1), the substrate analogue (\( \alpha\)-5-thio-D-glucose) is sandwiched between Trp-15 and Trp-136. In the *T. thermosulfurigenes* enzyme these residues correspond to Trp-49 and Trp-188. In addition, the residue Met-87 in the *Arthrobacter* enzyme is conserved as tryptophan in all class II enzymes [Trp-139 in the enzyme from *T. thermosulfurigenes* (3)]. This residue is very close to the C6-OH group of glucose and may constitute a steric hindrance in the discrimination between D-xylose and D-glucose as substrate.

![Fig. 1. Stereostructure of the active site of D-xylose isomerase from *Arthrobacter* containing the substrate analog \( \alpha\)-5-thio-D-glucose [from the coordinates of Collyer et al. (6)]. The metal ion sites are represented by crosses. Amino acids are indicated in one-letter code.](image-url)
Table 2. Kinetic constants for wild-type and mutant d-xylose isomerases substituted in the active-site aromatic amino acids

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Glucose</th>
<th>Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{cat}$, s$^{-1}$</td>
<td>$K_M$, mM</td>
</tr>
<tr>
<td>Wild type</td>
<td>11 ± 2.0</td>
<td>110 ± 8</td>
</tr>
<tr>
<td>Trp-139 → Tyr</td>
<td>9 ± 0.4</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>Trp-139 → Phe</td>
<td>16 ± 1.0</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Trp-139 → Met</td>
<td>11 ± 1.0</td>
<td>55 ± 2</td>
</tr>
<tr>
<td>Trp-139 → Leu</td>
<td>8 ± 0.4</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>Trp-139 → Val</td>
<td>5 ± 0.2</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Trp-139 → Ala</td>
<td>8 ± 0.2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Trp-139 → Lys</td>
<td>3 ± 0.2</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Trp-49 → Phe</td>
<td>10 ± 0.3</td>
<td>330 ± 14</td>
</tr>
<tr>
<td>Trp-49 → Ala</td>
<td>7 ± 0.3</td>
<td>710 ± 48</td>
</tr>
<tr>
<td>Trp-49 → Arg</td>
<td>10 ± 1.0</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>Wild type (37°C)*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Trp-188 → His (37°C)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Phe-145 → Lys (37°C)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.

*aReactions were performed at 37°C, as indicated in parentheses; all other reactions were run at 65°C. ND, not determined.

As shown in Table 2, substitution of Trp-139 by residues with smaller side chains resulted in lower $K_M$ (glucose). Most mutant enzymes thus obtained exhibited higher catalytic efficiency ($k_{cat}/K_M$) for glucose and lower catalytic efficiency for xylose than the wild-type enzyme. The correlation between the water-accessible surface of the hydrophobic residue in position 139 and the $K_M$ (glucose) (Fig. 2) suggests that, at least, part of the side chain of Trp-139 protrudes into the cavity of the active-site pocket and constitutes a steric hindrance against the binding of glucose. Reduction of this steric hindrance creates mutant enzymes that accommodate glucose better than the wild-type enzyme. The Trp-139 → Lys mutant fails out of the proportionality rule. The polar and positively charged side chain may cause a local structure change and thus affect the affinity of substrate indirectly or increase the affinity directly by hydrogen-bonding to the substrate. A methionine residue is present in the corresponding position of the enzymes from *Arthrobacter* (5, 6), *Streptomyces* (4, 8), and *Actinoplanes* (9). It is possible that the $K_M$ (glucose) may be reduced for these enzymes if this methionine is replaced with a smaller hydrophobic residue.

Substitution of Trp-49 in the *Thermosulfurigenes* enzyme (Trp-15 in the *Arthrobacter* enzyme) with phenylalanine or alanine residues resulted in a 3- and 6.5-fold increase in $K_M$ (glucose), respectively, with no appreciable change in $k_{cat}$. Surprisingly, the substitution Trp-49 → Arg did not change appreciably either $K_M$ or $k_{cat}$ (Table 2). According to the structure of the *Streptomyces* enzyme the N$^\bullet$ of this indole residue contributes to the construction of the active-site hydrogen-bonding network (8). The loss of one hydrogen bond in the network is expected to affect indirectly the binding affinity for the substrate. It is possible that the N$^\bullet$ of arginine may take a position in which it can perform the same function as the N$^\bullet$ of Trp-49.

Mutant enzymes obtained by substitution of Trp-188 (Arthrobacter equivalent, Trp-136) with Lys, Asp, or Glu exhibited no detectable activity with either D-glucose or D-xylose, although the proteins remained soluble under the conditions of assay (65°C). The mutant Trp-188 → His showed a low activity only with D-xylose. The $K_M$ (xylose) of this mutant enzyme at 65°C was >2 M, and thus the kinetic constants could not be measured precisely. They could, however, be determined at 37°C. Under these conditions the $K_M$ (xylose) was 800-fold higher than that of the wild-type enzyme, whereas the $k_{cat}$ was only lower by a factor of 2 (Table 2). These results are consistent with the prediction, deduced from the crystal structure of xylose isomerase, that the hydrophobic interactions between the indole of Trp-188 and the carbon backbone of pyranose contribute strongly to substrate binding in the active site. The role of tryptophan residues in binding substrates has been demonstrated in maltose-binding protein (19), arabinose-binding protein, and galactose-binding protein (20). Substitution of Phe-145 (Arthrobacter equivalent, Phe-93) with lysine resulted in a 50-fold increase of $K_M$ (xylose) and an insignificant change in $k_{cat}$ (Table 2). This result suggests that Phe-145 also plays an important role in substrate binding. Phe-145 may orient the indole group of Trp-188 in an optimal position for substrate interaction.

**Anomeric Specificity of Xylose Isomerase.** The models of catalytic isomerization suggested by the crystallographic data include a ring-opening step, presumed to be catalyzed by His-53 residue acting as a base (6, 8). We have, therefore, tested, by mutagenesis, the role of the corresponding residue, His-101, and Asp-104, a residue that may assist the function of His-101, in *Thermosulfurigenes* xylose isomerase. As was shown in the previous work (3) and here in Table 3, substitution of the His-101 by residues incapable of acting as a base, but capable of accepting hydrogen bonds, resulted in mutant enzymes exhibiting considerable residual activity with insignificant change of $K_M$. Similar results have been reported for the *A. missouriensis* enzyme (21).
The activity of the wild-type and mutant enzymes was also
determined for the α- and β-anomers of D-glucose. In the
wild-type enzyme $k_{\text{cat}}(\alpha\text{-glucose})$ was 5-fold lower than the
$k_{\text{cat}}(\beta\text{-glucose})$. The substitution His-101 → Asn reduced the
$k_{\text{cat}}(\alpha\text{-glucose})$ to 12% of the wild-type value, whereas $k_{\text{cat}}(\beta\text{-}
\text{glucose})$ did not change. The $K_M$ for either anomer remained
essentially unchanged (Table 3). If the hydrogen transfer,
shown previously to be the rate-limiting step (3), occurred
after the opening of the pyranose ring, it would be expected
that $k_{\text{cat}}(\beta\text{-glucose})$ and $k_{\text{cat}}(\alpha\text{-glucose})$ would be of the same
order of magnitude because anomers do not exist in the open-chain
form. Moreover, it would be expected that both constants
would be affected to the same extent by the His-101 → Asn
mutation. The results presented in Table 3 suggest that the
hydrogen transfer occurs when the structure of the substrate
molecule is cyclic rather than linear. This conclusion is
consistent with the results of crystallographic studies done
under steady-state conditions in a flow-cell by Farber et al.
(4). The electron densities observed by these authors indicated
that the rate-limiting step was preceded by a cyclic form
of the substrate. We hypothesize that hydrogen transfer and
ring opening occur as a concerted single-step reaction. Two
possible mechanisms for this step may be considered. In one
of them, a base attracts the proton from the C2 carbon of the
pyranose; this results in the formation of a cis-enediol inter-
mediate and ring opening during the transfer of proton (22).

In the second mechanism, a base attracts the proton from the
C2-OH, and this is followed by a hydride shift and ring
opening.

Two arguments can be raised against the cis-enediol inter-
mediate mechanism: (i) no residue capable of acting as a
general base has been seen near the C2 hydrogen in the
available crystal structures; (ii) no exchange of proton with
the medium occurs during the isomerization reaction (23).
Therefore, we propose the second mechanism, involving the
hydride shift particularly because the crystal structure of the
enzyme from Streptomyces (8) indicated that Asp-287 residue
(corresponding to Asp-339 of T. thermosulfurigenes enzyme)
is close enough to C2-OH of the substrate to attract its
proton.

If the position of His-101 in the T. thermosulfurigenes
isomerase is indeed equivalent to the position of His-53 of the
Arthrobacter enzyme, its major role would be as hydrogen-
bond acceptor to stabilize the transition state of the rate-
limiting step (3). The Asp-104 residue could assist this
function by stabilizing the His-101 residue. As shown in
Table 3, substitution Asp-104 → Asn resulted in a drop of $k_{\text{cat}}$
by ≈50%. The mutant obtained by the Asp-104 → Ala
substitution, exhibited a $k_{\text{cat}}(\beta\text{-glucose})$ of only 6% of the
wild type, whereas the $k_{\text{cat}}(\beta\text{-glucose})$ remained unchanged.
This result suggested that anomic specificity of xylose
isomerase depends not only on the presence of His-101 but
also depends on the position of this residue. With Asp-104 in
place, either oxygen of its carboxyl group can function as
hydrogen-bond acceptor. By providing a hydrogen bond to
His-101, Asp-104 locks His-101 at one of the two possible
tautomeric forms, thus ensuring that N4 of His-101 is always
a hydrogen-bond acceptor. In the Asp-104 → Asn mutant
only the oxygen of the amide group can provide this function
(Fig. 3). Without the hydrogen bond provided by Asp-104
(e.g., in Asp-104 → Ala mutant) the imidazole of His-101
could rotate or take up the tautomeric form that is unfavor-
able for the formation of the hydrogen bond to the transition
state.

In the previously proposed models, the extended-chain
molecules of the substrate, identified in the crystal structures
of the enzyme, were interpreted as being intermediates that
precede the hydride shift (6, 8). It is possible, however, that
these extended-chain sugar molecules were not intermediates
that preceded the rate-limiting step. They could be, for
example, the free ketose of xylose because it is present as
a 20.2% fraction in the aqueous solutions of xylose at

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{\text{cat}}$, s⁻¹</th>
<th>$K_M$, mM</th>
<th>$k_{\text{cat}}/K_M(\alpha)$</th>
<th>$k_{\text{cat}}/K_M(\beta)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>23 ± 1.0</td>
<td>9.3 ± 2.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Asp-309 → Asn</td>
<td>2.7 ± 0.1</td>
<td>5.8 ± 1.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Asp-296 → Asn</td>
<td>1.0 ± 0.1</td>
<td>140 ± 20</td>
<td>28 × 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>Asp-339 → Asn</td>
<td>0.08 ± 0.01</td>
<td>70 ± 10</td>
<td>4 × 10⁻⁴</td>
<td></td>
</tr>
</tbody>
</table>

Reactions were performed at 65°C for 30 min.
equilibrium (24). Whitlow et al. (8) actually suggested that the extended species in the enzyme–xylose–MnCl₂ structures observed in their diffraction pictures may be better described as xyulose.

The Role of Metal-Coordinating Residues. To test the function of each of the two metal ions in the active-site pocket, we have substituted Asp-309, -296, and -339 residues (Arthrobacter equivalents -256, -244, and 292, respectively; Fig. 1). Asp-309 corresponds to the residue binding the metal at position [II], whereas Asp-296 and Asp-339 correspond to residues that bind the metal in position [I]. In addition to its metal-coordinating function, Asp-257 in the Streptomyces and Actinoplanes isomerases (corresponding to Asp-256 in the Arthrobacter and Asp-309 in Thermoanaerobacterium) was proposed to act as a general base that initiates the hydride shift on the linear form of the substrate (8, 25).

Substitution of each of these aspartate residues with asparagine resulted in enzymes that still required Co²⁺ for maximal activity and thermostability suggesting that the metal-binding site still exists in these mutants. The Asp-309 → Asn mutant enzyme exhibited a 20% loss of the wild-type catalytic efficiency (kcat/KM; Table 4). This result argues against the hypothesis that Asp-309 might be the essential catalytic base that initiates the hydride shift.

Substitutions Asp-296 → Asn or Asp-339 → Asn caused drastic decreases in catalytic efficiency resulting from both an increase in KM and decrease in kcat (Table 4). Thus, these two residues, or the metal [I], seem to play an important role in the stabilization of the substrate and the transition state. The four order-of-magnitude decrease in kcat/KM due to the Asp-339 → Asn substitution supports the hypothesis that Asp-339 is the essential base in the catalytic mechanisms of xylose isomerase.

The Proposed Catalytic Mechanism of Xylose Isomerase. In the previously proposed models it was suggested that the hydride shift is catalyzed by the metal in site [II] (6). Results of this work indicate that substitution of amino acid residues coordinating to metal site [I] has a much more drastic effect on the activity than the substitution of the residues coordinating to metal site [II]. It is possible, therefore, that metal [I] stabilizes the substrate and the transition state by coordination as well as by electrostatic interaction with the developing negative charge of the transition state. Studies of the coordination sphere of the two metal-binding sites by spectroscopic methods have also suggested that metal site A, corresponding to the metal site [I] identified in the x-ray studies, is the site responsible for catalysis (26, 27).

We would like, therefore, to propose, in Fig. 4, another model for the reaction catalyzed by xylose isomerase. In this model, His-101 is locked in one tautomeric form by interaction with Asp-104, and it acts as a proton donor, playing a role in stabilizing the substrate and the transition state. Asp-339, acting as a base, attracts the proton from C2-OH of the substrate. This attraction facilitates the subsequent hydride shift from C1 to C2 and simultaneously induces the ring opening. Metal [I] stabilizes the substrate and the transition state by coordination and, perhaps, provides the electrostatic force to stabilize the developing negative charge at the C5-O. The involvement of three different amino acid residues in a catalytic triad is another hypothesis for the xylose isomerase mechanism. Nonetheless, the involvement of a specific catalytic triad has been reported for different hydrolytic saccharidases, such as α-glucanases (28).

We thank Paul Johnson for advice on mathematical correction for mutarotation of anomic substrate and David Blow for the discussions on the structure of xylose isomerase. This work was supported by a grant from the U.S. Department of Agriculture (90-34189-5014 to Michigan Biotechnology Institute) and Research Excellence Fund from the State of Michigan.

Fig. 4. Proposed catalytic mechanism for D-xylose isomerase involving the cyclic substrate, amino acid catalytic triad, and divalent metal in position [I].