RESEARCH PAPERS

Acta Cryst. (1994). D**50**, 113–123

Modes of Binding Substrates and their Analogues to the Enzyme D-Xylose Isomerase

By H. L. Carrell, Helga Hoier† and Jenny P. Glusker*

The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, PA 19111, *USA*

(*Received* 1 *March* 1993; *accepted* 3 *September* 1993)

Abstract

Studies of binding of substrates and inhibitors of the enzyme D-xylose isomerase show, from X-ray diffraction data at 1.6–1.9 Å resolution, that there are a variety of binding modes. These vary in the manner in which the substrate or its analogue extend, on binding, across the carboxy end of the $(\beta\alpha)_8$-barrel structure. These binding sites are His54 and the metal ion (magnesium or manganese) that is held in place by Glu181, Asp245, Glu217 and Asp287. Possible catalytic groups have been identified in proposed mechanisms and their role in the binding of ligands is illustrated.

Introduction

The enzyme D-xylose isomerase (E.C. 5.3.1.5) catalyzes the interconversion of five- and six-carbon aldoses and ketoses. For example, D-xylose is converted to D-xylulose and D-glucose to D-fructose. The reaction involves the movement of an H atom between C(1) and C(2) of the substrate. A divalent metal is required by the enzyme and the metal cations Mg^{2+}, Mn^{2+} and Co^{2+} are found effective. The molecular weight of the enzyme is 172 000 and it contains four equivalent subunits.

The three-dimensional structure of the enzyme from *Streptomyces rubiginosus* was shown, by an X-ray diffraction analysis at 4 Å resolution (Carrell, Rubin, Hurley & Glusker, 1984), to be a $(\beta\alpha)_8$-barrel with the active-site carboxy terminus at the base of the barrel. Since that time several other xylose isomerases from the same and different bacteria (Henrick, Blow, Carrell & Glusker, 1987; Farber, Petsko & Ringe, 1987; Rey, Jenkins, Janin, Lasters, Alard, Claessens, Matthyssens & Wodak, 1988; Carrell, Glusker, Burger, Manfre, Tritsch & Biellmann, 1989; Henrick, Collyer & Blow, 1989; Farber, Glasfeld, Tiraby, Ringe & Petsko, 1989; Dauter, Dauter, Hemker, Witzel & Wilson, 1989; Whitlow, Howard, Finzel, Poulos, Winborne & Gilliland, 1991; Jenkins, Janin, Rey, Chiadmi, van Tilbeurgh, Lasters, De Maeyer, Van Belle, Wodak, Lauwereys, Stanssens, Mrabet, Snauwaert, Matthyssens & Lambeir, 1992; Lambeir, Lauwereys, Stanssens, Mrabet, Snauwaert, van Tilbeurgh, Matthyssens, Lasters, De Maeyer, Wodak, Jenkins, Chiadmi & Janin, 1992; van Tilbeurgh, Jenkins, Chiadmi, Janin, Wodak, Mrabet & Lambeir, 1992; Collyer, Goldberg, Viehmann, Blow, Ramsden, Fleet, Montgomery & Grice, 1992; Blow, Collyer, Goldberg & Smart, 1992) have been studied in the native state, with bound substrates or inhibitors, in the presence of different metals and after specific mutations of selected amino-acid residues.

The enzyme binds two metal ions, one, which we call *M*1, *via* Glu181, Glu217, Asp245 and Asp287 and the other, which we call *M*2, *via* Glu217 (which it shares with *M*1), His220, Asp255 and Asp257. The metal ion *M*2 is bound in a bidentate manner by the carboxyl group of Asp255. At the other end of the active site lies His54, held in a rigid orientation by three hydrogen bonds to Asp57.

The catalytic mechanism is still under investigation. The hydrogen transfer is stereospecific and seems to involve a planar intermediate with both oxygen atoms in the *cis* position. An H atom is transferred from one side of this plane only and it does not exchange with solvent protons (Feather, Deshpande & Lybyer, 1970; Schray & Rose, 1971). The mechanism was originally thought to involve base-catalyzed proton transfer with a *cis* ene diol intermediate (Rose, O'Connell & Mortlock, 1969), but the stereochemistry of the active site has suggested that a hydride shift mechanism is more likely (Farber *et al.*, 1989; Collyer, Henrick & Blow, 1990), possibly mediated by the metal ions (Whitlow *et al.*, 1991). Fig. 12 illustrates the various proposed mechanisms for isomerization.

We present here some new data on the binding of some substrate analogs and show that there are a

* Author to whom correspondence should be addressed.

† Permanent address: Institut für Organische Chemie und Isotopenforschung der Universität Stuttgart, Pfaffenwaldring 55, D-7000 Stuttgart 80, Germany.

© 1994 International Union of Crystallography
Printed in Great Britain – all rights reserved

ISSN 0907-4449 © 1994

Table 1. *X-ray diffraction data for* D-*xylose isomerase crystals*

DAHS = 1,5-dianhydrosorbitol. 3DFMG = 3-deoxy-3-fluoromethyleneglucose.

Data set	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Metal added*	—	Mg^{2+}	—	—	Mn^{2+}/Mg^{2+}	—	Mg^{2+}	—	Mg^{2+}
Concentration (m*M*)	—	—	—	—	1/2		1		1
Ligand	—	—	—	—	D-Xylose	D-Xylose	D-Xylulose	D-Sorbitol	Xylitol
Concentration (m*M*)					~1000	~1500	300	100	100
pH	7.4	7.4	8.0	9.0	7.4	9.0	8.0	9.0	7.4
Resolution (Å)	1.65	1.6	1.6	1.6	1.7	1.6	1.7	1.7	1.7
No. unique measured data	54234	60132	60630	61209	49384	62185	51327	52652	47002
% of available data	93	94	95	96	93	97	96	99	88
B_{av} of ligand ($Å^2$)	—	—	—	—	32	28	15	11	10

Data set	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
Metal added	Mn^{2+}/Mg^{2+}	—	—	Mg^{2+}	—	—	Mg^{2+}/Mn^{2+}	Mg^{2+}/Mn^{2+}
Concentration (m*M*)	1/2	—	—	1.0	—	—	1/1	1/1
Ligand	D-Glucose	D-Fructose	DAHS	L-Ascorbic acid	Threonate	2DFMG	Ribose	L-Sorbose
Concentration (m*M*)	500	500	200	25	20	~2	500	500
pH	8.0	8.0	8.0	7.4	9.0	7.4	8.0	8.0
Resolution (Å)	1.62	1.6	1.7	1.7	1.67	1.7	1.7	1.6
No. unique measured data	58695	63064	50406	51915	54827	45771	52675	62502
% of available data	95	99	95	97	98	86	99	98
B_{av} of ligand ($Å^2$)	14	14	23	27	16	13	—	—

* This refers to metal ion added at the time of the experiment. The crystals of native enzyme were grown from a solution containing 2 Mn^{2+} per subunit of enzyme and no unbound cation.

variety of ways by which this enzyme can bind a sugar of the appropriate size.

Materials

The threonate used in this study was prepared and kindly provided by Dr Steven Seeholzer. The 3-deoxy-3-fluoromethylene glucose was provided by Dr Jean-Francois Biellmann of the University of Strasbourg, France. Other materials used were reagent-grade chemicals from Sigma Company.

Experimental

Preparation of crystals and data collection

The crystals used in these studies were prepared from 0.2 ml solutions containing purified D-xylose isomerase at ~25 mg ml^{-1}, 0.76 *M* $(NH_4)_2SO_4$. Crystals were grown at three different pH conditions, pH 7.4, 8.0 and 9.0 using 0.01 *M* PIPES buffer at pH 7.4, 0.01 *M* HEPES at pH 8.0 and 0.01 *M* CHES at pH 9.0. All stock solutions were prepared using the appropriate buffer for the particular pH condition. The solutions were refrigerated overnight at 277 K and crystals ranging up to ~2 mm in the largest dimension were grown. The crystals grown in this manner were used for all of the studies involving substrate, substrate analogues and inhibitors and other sugar analogs. In all cases, crystals for the diffraction experiments were prepared by placing crystals from the appropriate stock batch in a droplet containing the compound of interest. Where appropriate, the crystals were allowed to equilibrate for at least 2 d at 277 K before the data collection was performed. The crystals of the enzyme containing the active-site-directed inhibitor, 3-deoxy-3-fluoromethylene-D-glucose (DFMG), were prepared by placing crystals from the stock batch in a droplet of crystallization mother liquor containing the inhibitor at a concentration of ~2 m*M* along with both Mn^{2+} and Mg^{2+} at 2 m*M*. The crystals were allowed to soak in this manner at 277 K for 4 d before the diffraction data were collected.

The X-ray diffraction data were measured at 285–287 or 277–279 K using the Nicolet/Xentronix (now Siemens) area detector mounted on a Rigaku rotating-anode generator using Ingersoll focusing nickel mirrors with Cu $K\alpha$ radiation. The data were processed using the *XENGEN* software package (Howard, Gilliland, Finzel, Poulos, Ohlendorf & Salemme, 1987) or the *XDS* package (Kabsch, 1988*a*,*b*) to obtain structure amplitudes. Table 1 contains a summary of the results of the data collection and conditions for the crystals used in this study. In all cases, data were measured to a maximum resolution of 1.55 Å with the goal of obtaining at least 1.7 Å resolution data that are nearly complete. In all cases, the data were truncated at the stated resolution (Table 1) since for that particular data set, the average intensity fell below $2\sigma(I)$.

Refinement of structures

The native enzyme structure has been reported previously by this laboratory (Carrell *et al.*, 1984, 1989) as well as by other investigators. The high-resolution structure obtained in this laboratory is

virtually identical to the structure as published by Dauter and by Whitlow and their co-workers (Dauter, Terry, Witzel & Wilson, 1990; Whitlow *et al.*, 1991). In our studies we have used the coordinates from our own studies including the 381 solvent molecules placed by us. In this case, the water molecules near the binding/substrate-binding sites are well characterized. The refinements were carried out using restrained least-squares refinement as described by Knossow, Lewis, Rees, Wilson, Skehel & Wiley (1986) utilizing the fast Fourier transform (FFT) acceleration of the process (Ten Eyck, 1977) using *PROLSQ* (Hendrickson & Konnert, 1980; Hendrickson, 1985). The refinements led to R factors ranging between 0.131 and 0.15 for the structures. The general strategy for the crystals soaked in substrate or analog was to compute difference maps with all active-site solvent molecules and metal ions removed. The appropriate metal ions were then placed in the resulting electron-density map, as well as any clearly indicated solvent atoms and any indicated adjustments to positions of the side chains of the protein. This rebuilding of the model was carried out by use of the computer program *FRODO* (Jones, 1985) with both $F_o - F_c$ and $2F_o - F_c$ density maps. A refinement of the appropriate structure was then carried out, omitting the coordinates of ligand molecules. The bound ligand molecule was then

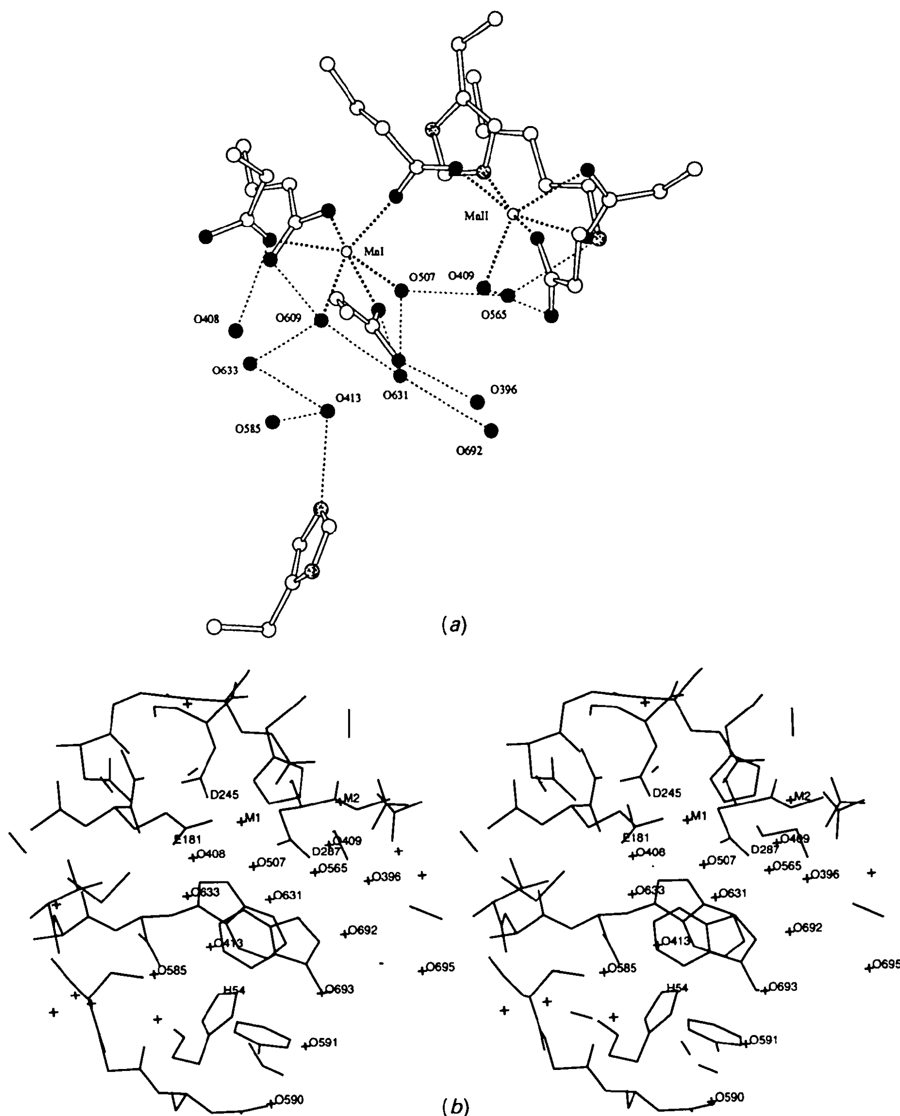


Fig. 1. The native enzyme. (*a*) In this and subsequent diagrams O atoms are black and N atoms are stippled. This and similar diagrams were drawn by the UNIX program *ICRVIEW* (Erlebacher & Carrell, 1992). In Figs. 2–10 the ligand bonds are black. (*b*) Stereoview of the binding region in D-xylose isomerase.

fitted to the difference electron density as well as to the density in the $2F_o - F_c$ map. The structure was then refined with the bound molecule included in the refinement. In all cases we checked the overall difference electron-density map for any other changes, including solvent molecules. The only changes observed were those occurring in or near the active site. The largest changes in atomic positions in the 'active' site occurred with the covalently bound inhibitor, 3-deoxyfluoromethyleneglucose. In all of the refinements, the results were virtually identical in terms of r.m.s. error. The r.m.s. errors for bond distances ranged from 0.019 to 0.025 Å: 0.30–0.35 Å for 1–3 (angle) distances and 0.45–0.60 Å for 1–4 distance restraints. The number of restrained distances which deviated by more than 3σ ranged between 21 and 35 for all the refinements. The Luzzati plots (Luzzati, 1952) indicated that the coordinate errors in all of the structures lie between 0.1 and 0.15 Å.

Results

The compounds for which we have obtained binding data are D-xylose, D-xylulose, D-glucose, D-fructose, D-sorbose, D-ribose, D-xylitol, D-sorbitol, 1,5-dianhydro-D-sorbitol, 3-deoxy-3-fluoro-D-glucose,

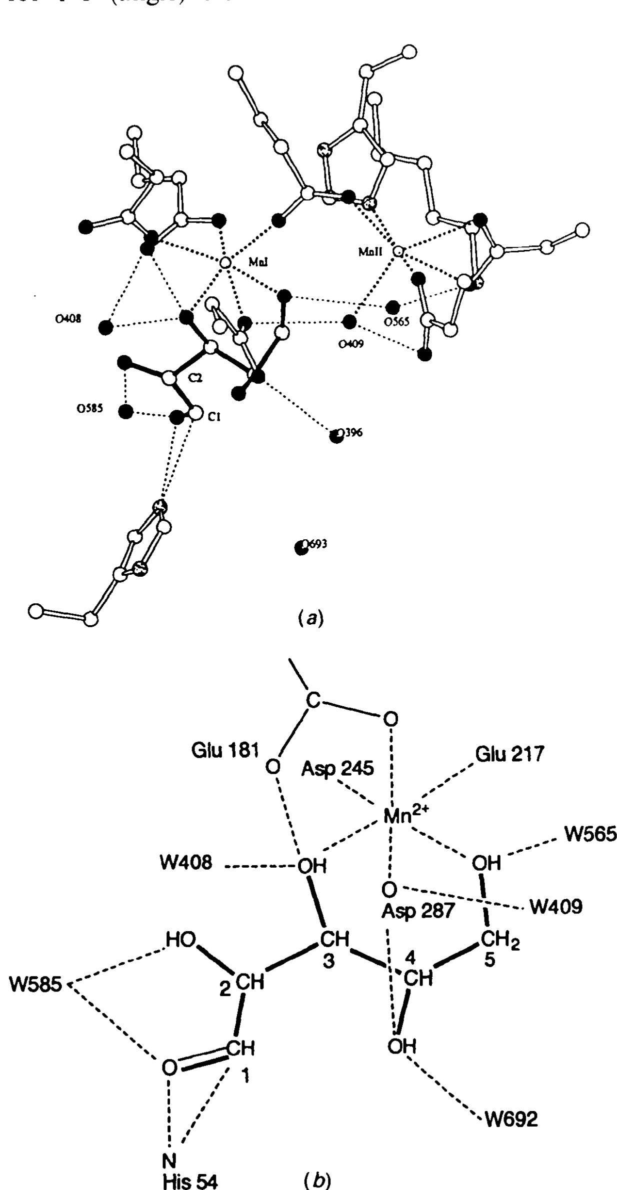


Fig. 2. Binding of D-xylose. (a) Ball-and-stick view and (b) identification of some binding groups.

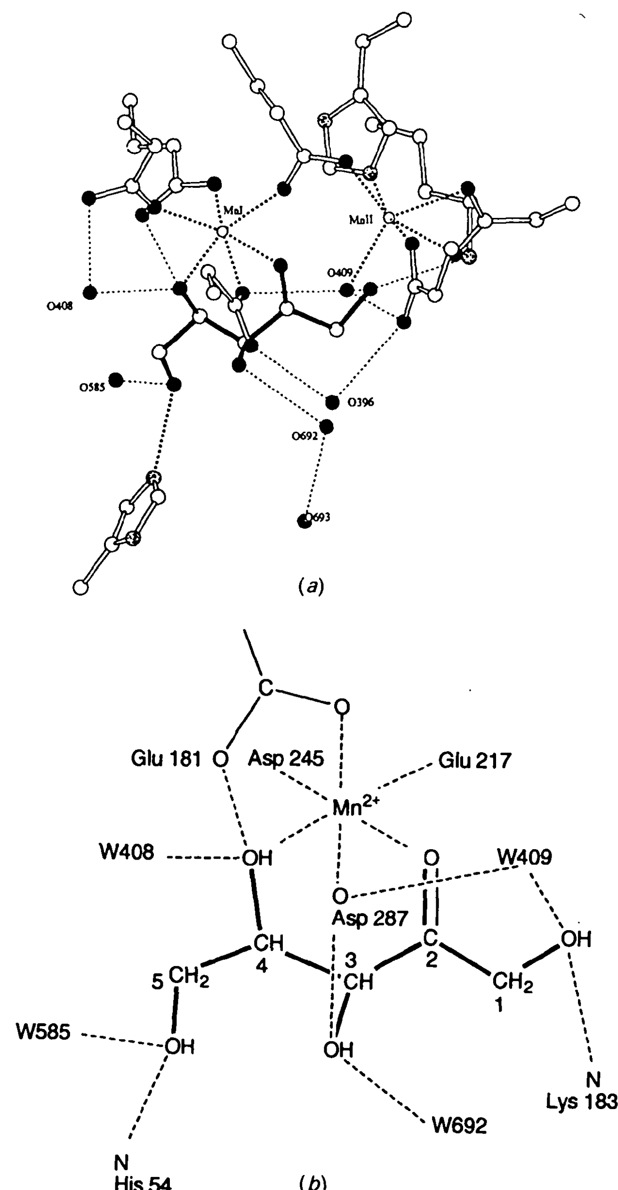


Fig. 3. Binding of D-xylulose. (a) Ball-and-stick view and (b) identification of some binding groups.

L-ascorbic acid, threonate and the active-site-directed irreversible inhibitor DFMG that alkylates His54 (Carrell *et al.*, 1989). Five water molecules are displaced by these sugars, two of which (water molecules 609 and 507) are bound to $M1$ and three of which are bound to other water molecules and/or His54 (water molecules 633, 413 and 631). The crystal structures of the complexes with xylose and the alkylating agent have already been published (Carrell *et al.*, 1989).

When a crystal of D-xylose isomerase was soaked in D-fructose solution, the resulting electron-density map showed an α-D-glucopyranose molecule, indicating that catalysis had occurred. When 2-deoxy-3-fluoroglucose was soaked into the crystals, α-D-glucose was also found in the electron-density map to be bound to the enzyme. This is, apparently, a non-enzymatic conversion; it was found to occur also in buffer in the absence of enzyme. Two electron-density maps were uninterpretable – those with bound sorbose and ribose.

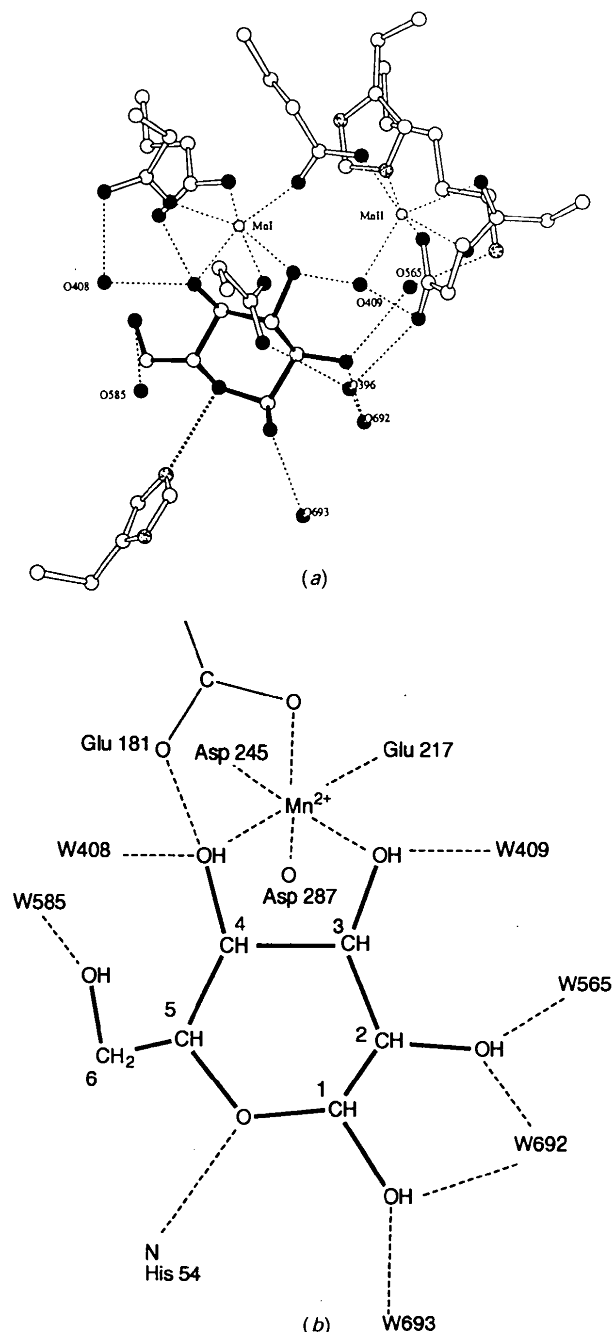


Fig. 4. Binding of D-glucose. (*a*) Ball-and-stick view and (*b*) identification of some binding groups.

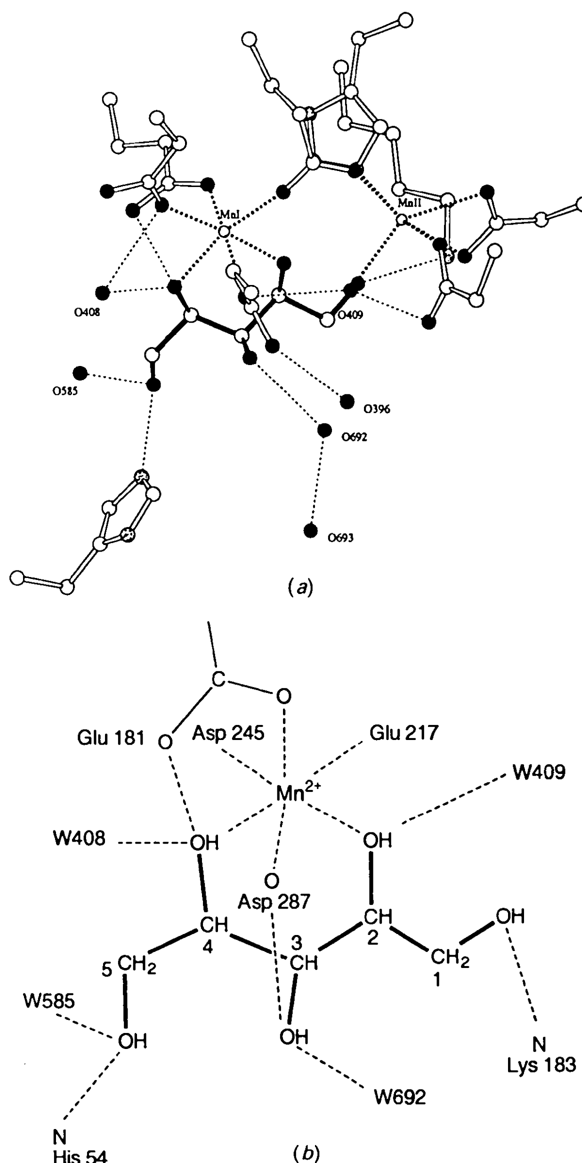


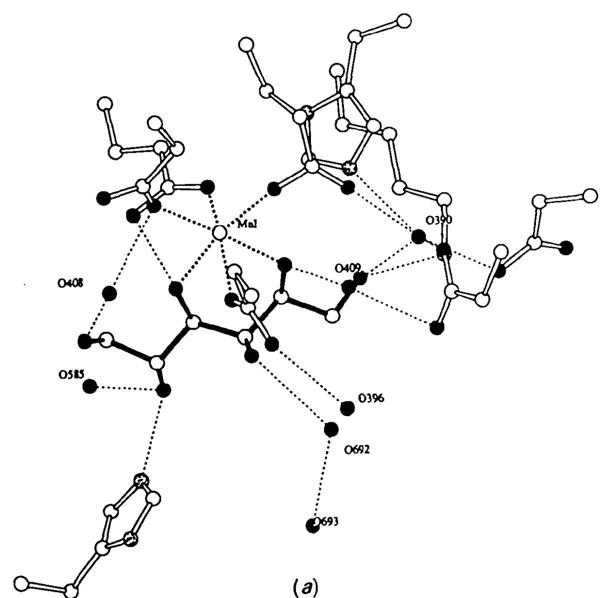
Fig. 5. Binding of D-xylitol. (*a*) Ball-and-stick view and (*b*) identification of some binding groups.

The binding is shown as a ball-and-stick view, drawn with the UNIX program *ICRVIEW* (Erlebacher & Carrell, 1992), and by line diagrams for clarity. Shown are: native enzyme (Fig. 1), D-xylose (Fig. 2), D-xylulose (Fig. 3), D-glucose (Fig. 4), D-xylitol (Fig. 5), D-sorbitol (Fig. 6), threonate (Fig. 7), L-ascorbic acid (Fig. 8), 1,5-dianhydrosorbitol (Fig. 9), and the alkylated enzyme (Fig. 10). Fig. 11(*a*) shows the 'omit' electron density for native + 1.5 *M* D-xylose (crystal 6). Fig. 11(*b*) shows the 'omit' density for native + D-xylulose (crystal 7, Table 1).

Discussion

The mode of catalytic action of this enzyme is still under active investigation. Various possible mechanisms have been proposed (Fig. 12) and they include a *cis* ene diol intermediate with proton transfer and at least two mechanisms involving a hydride shift. The current work described here cannot be used to distinguish among these, but it demonstrates the many binding modes that this enzyme can accommodate.

The role of His54 is still being debated. Originally it had been suggested by us (Carrell *et al.*, 1989),



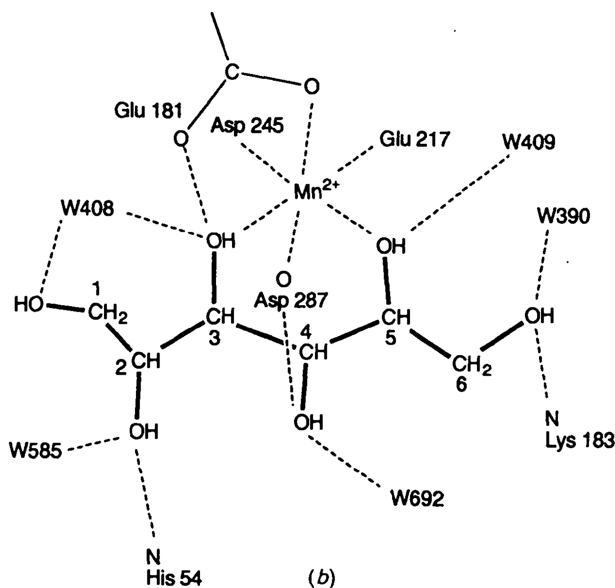
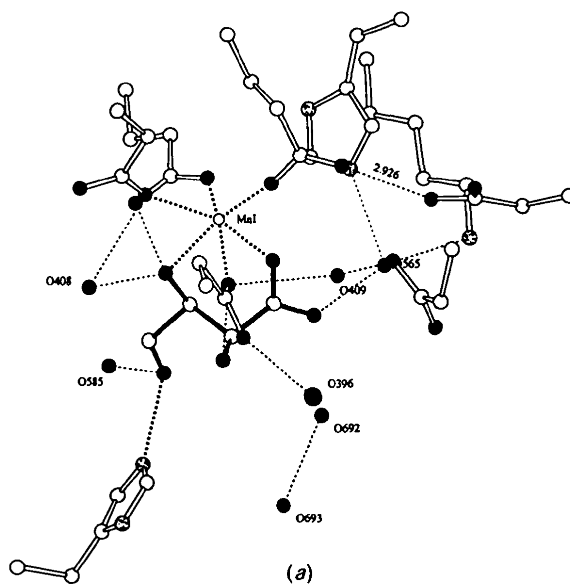


Fig. 6. Binding of D-sorbitol. (*a*) Ball-and-stick view and (*b*) identification of some binding groups.



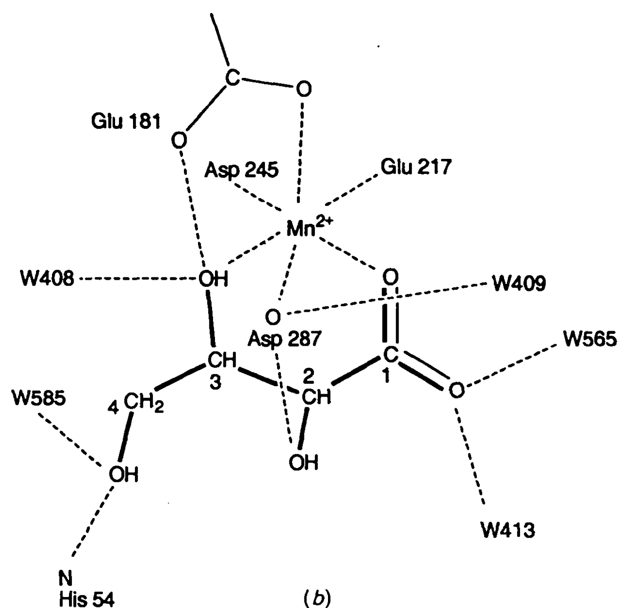
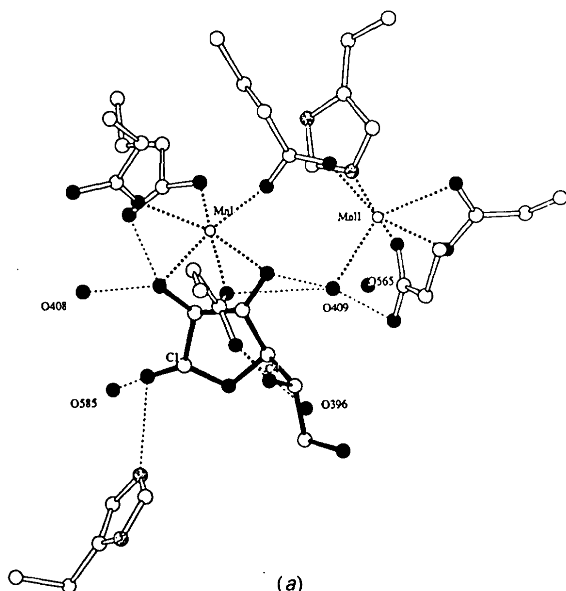


Fig. 7. Binding of D-threonate. (*a*) Ball-and-stick view and (*b*) identification of some binding groups.

based on the electron-density map of bound D-xylose (see Fig. 2*b* and Fig. 11*a* which show an interaction between C1 and His54), that His54 might be the catalytic base that abstracted a proton from the substrate. Whitlow *et al.* (1991), however, point out that His54 is not in an environment where any transferred proton could be protected from exchange with solvent. This binding mode was reproduced in this work with crystal 7 (Table 1) and in Fig. 11(*b*). There are three hydrogen bonds between His54 and Asp57.

His54	N	Asp57	OD1
His54	O	Asp57	N
His54	ND1	Asp57	OD1
His54	NE2	Water 413	

These interactions presumably serve to maintain the ring of His54 in a fixed orientation. Whitlow *et al.*



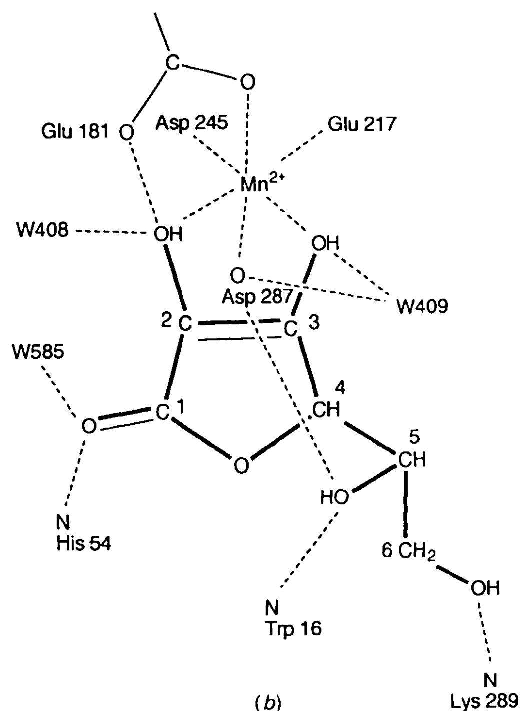
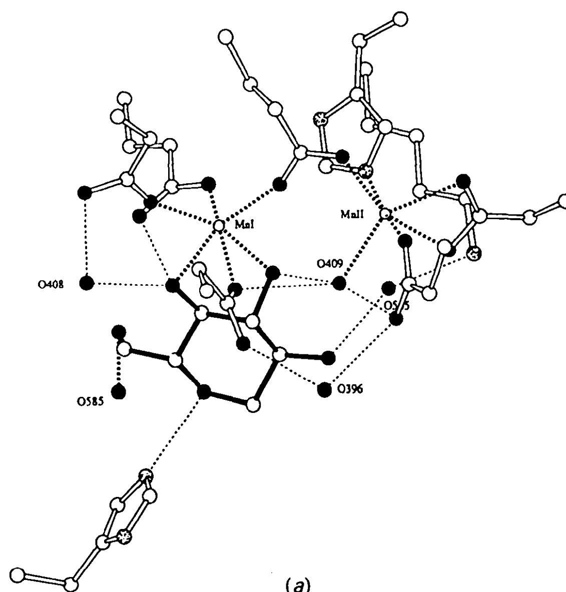


Fig. 8. Binding of L-ascorbic acid. (*a*) Ball-and-stick view and (*b*) identification of some binding groups.



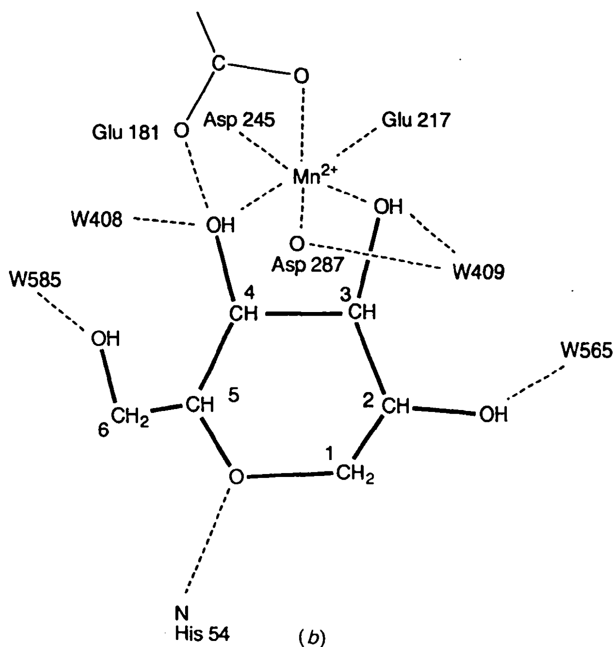


Fig. 9. Binding of 1,5-dianhydrosorbitol. (*a*) Ball-and-stick view and (*b*) identification of some binding groups.

(1991) suggest that His54 may act as an acid–base catalyst by extracting a proton from Asp57 so that ND1 becomes protonated. His54 can then shuttle a proton between the O1 hydroxyl group and the ring O atom of α-D-xylose.

Whitlow *et al.* (1991) envision a mechanism similar to that of Collyer & Blow (1990), in which *M*2 is the 'catalytic metal'. A water molecule that is hydrogen bonded to O1 and O2 of substrate, to OD1 of Asp257, and coordinated to *M*2, transfers a proton to OD1 of Asp257. This leaves a metal-bound hydroxyl group which removes the O2 hydroxyl proton from D-xylose. *M*2 then moves 1.76 Å so that it can coordinate directly to O1 and O2 of the ligand, displacing the water carrying the O2 proton. The proton of D-xylose moves to the *pro-R* position on C1 to give D-xylulose by a 1,2-hydride shift. The metal-mediated hydride-shift mechanism was proposed because there did not seem to be as suitable base in the vicinity for proton abstraction and because the proton that is transferred between C1 and C2 does not exchange with solvent. In this proposed mechanism Trp16 excludes solvent. The negative charge on O2 migrates to O1 during the hydride shift and the water donates a proton to O1. OD1 of Asp257 transfers a proton to the hydroxyl group. The product, D-xylulose, is then cyclized.

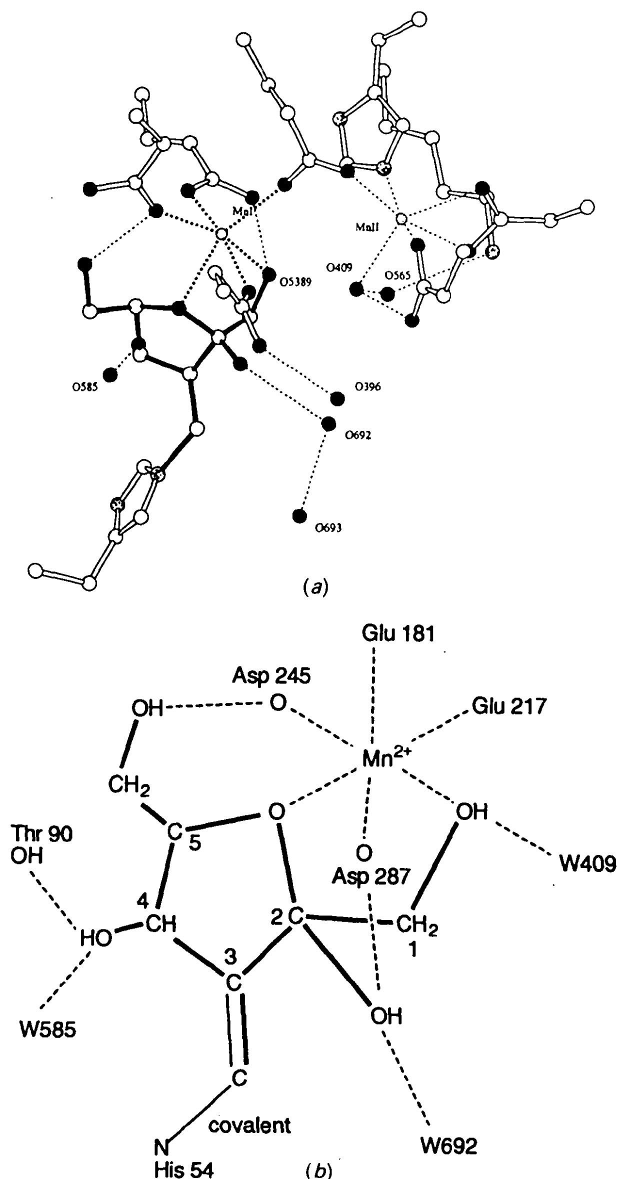


Fig. 10. Binding of D-alkylation product (Carrell *et al.*, 1989). (*a*) Ball-and-stick view and (*b*) identification of some binding groups.

The results we obtain are of interest in light of this proposed mechanism. There is no evidence that any of the compounds reported here by us are ever directly bonded to *M*2. This is different from the situation described by Lambeir *et al.* (1992) for the *A. missouriensis* enzyme in which *M*2 binds the C1 hydroxyl of D-xylitol. In the crystalline enzyme–ligand complexes we have studied there is always an intervening water molecule. Also, we always find the metal ion to be hexa-coordinated, unlike the findings of a lower coordination number by Jenkins *et al.* (1992). On the other hand, we find that the C1 hydroxyl group of D-xylitol binds to the N atom of Lys183. Lambeir *et al.* (1992) point out that this may aid in stabilizing the binding of substrate in the open-chain conformation. They also suggest that the ε-amino group of Lys183 may help polarize the C=O bond of aldose substrates and lower the pK_a of the hydroxyl group of ketoses.

The binding of substrates and their analogues has also been studied by other workers. Jenkins *et al.* (1992) studied D-xylose, D-xylitol and D-sorbitol, and found each bound in the open-chain form. They also studied the cyclic compounds 1,5-anhydroxylitol (no hydroxyl group at position 2), α-D-pseudoxylose and β-D-pseudoglucose (ring oxygen replaced by methylene). They found that the enzyme from *Actinoplanes missouriensis* binds D-xylitol, D-sorbitol and D-xylose/D-xylulose to the metal through O2 and O4. These studies were at 2.2–2.6 Å resolution. The binding of D-sorbitol is described by Henrick *et al.* (1989). The binding of 5-thioglucose is described by Collyer & Blow (1990) as involving the metal binding to O3 and O4 with the ring atom bound to His54. When a ring oxygen binds to His54 it seems that the histidine must be protonated at NE2.

The binding of substrates and their analogs (see Fig. 13 for formulae), illustrated in Figs. 2–10, show the importance of Glu181, Lys183, Asp287, waters 408 and 409, and His54 in the binding. The ligands in the octahedral coordination of *M*1 and *M*2 in each structure are listed in Table 2. Note that we find

that the metal is lost in the structures containing D-sorbitol (Fig. 6) and threonate (Fig. 7). Each metal ion is part of a motif that also involves a carboxyl group, and a water molecule, as illustrated in Table 3. The water molecule 409 (bound to $M2$), important in the metal-mediated hydride-shift mechanism, is not replaced by any of the ligands we have used. The water molecule, 609 (bound to $M1$), is always replaced as is water molecule 507 which is hydrogen bonded to water 409. Whitlow *et al.* (1991) point out that this $M2$-bound water molecule (409 in our numbering) can, together with Lys183, position the substrate so that the O1—C1—C2—O2 group is in the *cis* conformation (Schray & Rose, 1971). The enzyme moves the protons on O2 and C2 of D-xylose to O1 and C1 of D-xylulose.

Our D-xylose binding electron-density map (Fig. 11*a*) differs from that published by others. Collyer & Blow (1990) suggest that our electron density consists of a mixture of cyclic and extended forms of D-xylose. We have tried, without success, to fit such models to our electron-density maps (1.6 and 1.7 Å resolution) and conclude that we do not have such a mixture.

Concluding remarks

We have shown that the binding site of D-xylose from *Streptomyces rubiginosis* is fairly flexible and can accommodate substrate analogs in a variety of ways (summarized in Table 4). Some notable consistencies in most of the cases we present here are the role of the $M1$-bound Glu181 carboxyl group in positioning what is generally O3 or O4 of the substrate (see Table 3), the binding of the ε-amino group of Lys183 to the hydroxyl group on C1 in D-xylulose, D-xylitol and D-sorbitol, the role of water 409 which is not replaced by any of the compounds studied here, and the ever-present carboxyl group of Asp287 positioned over C3. This aspartic acid (287) side

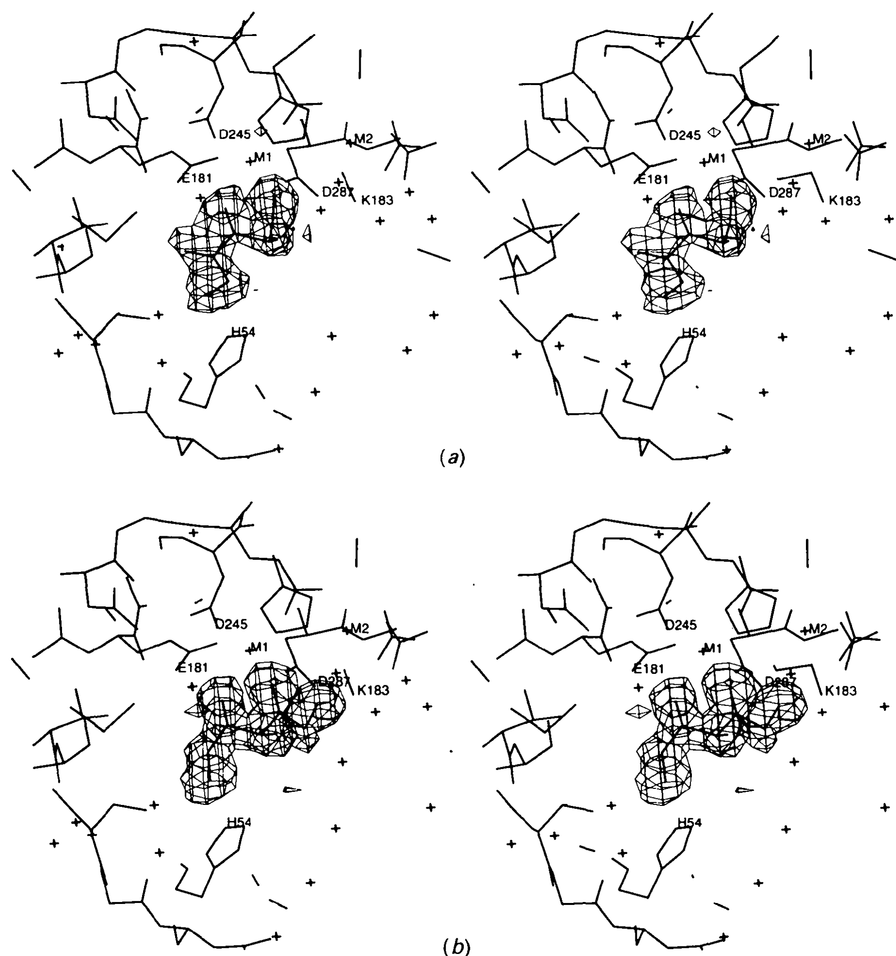


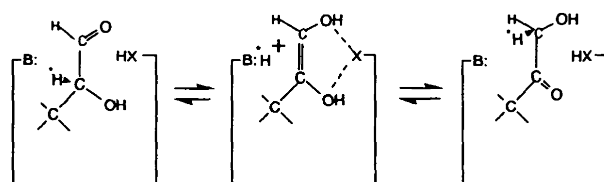
Fig. 11. (*a*) Stereoview of 'omit' difference density for enzyme + 1.5 *M* D-xylose. Contours are drawn at $3\sigma(F_o - F_c)$. (*b*) Stereoview of 'omit' difference density for enzyme + 300 m*M* D-xylulose. Contours are drawn at $3\sigma(F_o - F_c)$.

chain is often hydrogen bonded to water 409 (as is Asp257).

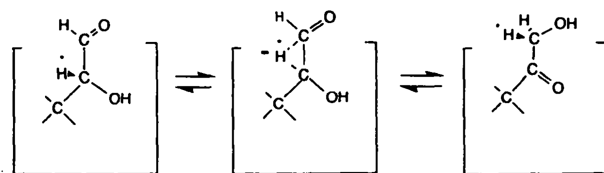
This analysis of the binding of substrates and inhibitors to D-xylose isomerase has led to the identification of three structural motifs (Fig. 14)

(1) The metal ion may take part in a six-membered chelate ring by binding hydroxyl, carbonyl, carboxyl, or ring-ether O atoms. This motif is seen on binding of xylose, xylulose, xylitol, sorbitol and threonate.

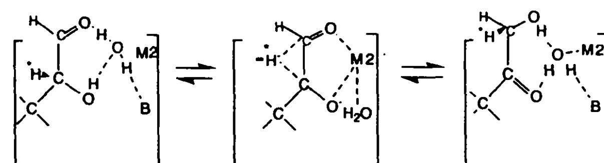
(2) The metal ion may, alternatively, take part in a five-membered chelate ring, binding hydroxyl or ring-ether O atoms. This motif is seen on binding of



Base-catalyzed proton transfer *via* ene diol



Simple hydride shift



Metal-assisted hydride shift

Fig. 12. Proposed isomerization mechanisms.

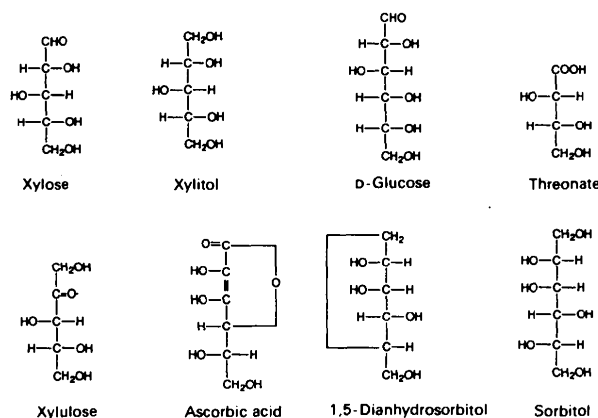


Fig. 13. Formulae of binding substrates.

Table 2. *Octahedral coordination of M1 and M2 in each enzyme–ligand structure studied*

*M*1	1	2	3	4	5	6
Native	E217(OE2)	D245	E181	D287	*W*609	*W*507
D-Xylose	E217(OE2)	D245	E181	D287	O3x	O5x
D-Xylulose	E217(OE2)	D245	E181	D287	O4x	O2x
D-Glucose	E217(OE2)	D245	E181	D287	O4x	O3x
D-Xylitol	E217(OE2)	D245	E181	D287	O4x	O2x
D-Sorbitol	E217(OE2)	D245	E181	D287	O3x	O5x
Threonate	E217(OE2)	D245	E181	D287	O3x	O1x
L-Ascorbic acid	E217(OE2)	D245	E181	D287	O2x	O3x
1,5-Dianhydrosorbitol	E217(OE2)	D245	E181	D287	O4x	O3x
Covalent adduct	E217(OE2)	D245	E181	D287	Ring Ox	O1x

*M*2	1	2	3	4	5	6
Native	E217(OE1)	H220	D255	D255	D257	*W*409
D-Xylose	E217(OE1)	H220	D255	D255	D257	*W*409
D-Xylulose	E217(OE1)	H220	D255	D255	D257	*W*409
D-Glucose	E217(OE1)	H220	D255	D255	D257	*W*409
D-Xylitol	E217(OE1)	H220	D255	D255	D257	*W*409
D-Sorbitol	No Mn	No Mn	No Mn	No Mn	No Mn	No Mn
Threonate	No Mn	No Mn	No Mn	No Mn	No Mn	No Mn
L-Ascorbic acid	E217	H220	D255	D255	D257	*W*409
1,5-Dianhydrosorbitol	E217	H220	D255	D255	D257	*W*409
Covalent adduct	E217	H220	D255	D255	D257	*W*409

Table 3. *Binding of a carboxyl–water–metal motif to D-xylose isomerase*

Added ligand	Replacement* for water 609	Replacement* for water 409	Replacement* for water 507
Native enzyme	—	—	—
D-Xylose	O3x	No	O5x
D-Xylulose	O4x	No	O2x
D-Glucose	O4x	No	O3x
Xylitol	O4x	No	O2x
Sorbitol	O3x	No	O5x
Threonate	O3x	No	O1x
Ascorbic acid	O2x	No	O3x
1,5-Dianhydrosorbitol	O4x	No	O3x
Covalent adduct	Ring Ox	No	O1x

* Refers to bound ligand.

α-D-glucopyranose, ascorbic acid, 1,5-dianhydrosorbitol and the alkylated enzyme.

(3) The metal ion and a metal-bound hydroxyl group may bind both O atoms of the side-chain carboxyl group of Glu181. This motif requires formation of two metal ion···oxygen bonds and one hydrogen bond. It is found in all structures reported here. In this way the enzyme assures the binding of a hydroxyl group at at least one of the two possible metal-binding positions and may, in this way, serve to control its p*K* value.

(4) In all crystal structures reported here Asp287 lies above the C—C portion of the metal chelate rings.

Table 4. *Directionality of binding of substrates and their analogues*

→ indicates binding of O1 end of substrate to His54 while ← indicates binding of O1 end of substrate to *M*1.

	His54	Metal	Direction of binding
D-Xylose	O_1	O_3,O_5	→
D-Xylulose	O_5	O_2,O_4	←
D-Glucose	Ring O	O_3,O_4	⇆
Xylitol	O_5	O_2,O_4	←
Sorbitol	O_2	O_3,O_5	→
Threonate	O_4	O_1,O_3	←
Ascorbic acid	O_1	O_2,O_3	→
1,5-Dianhydrosorbitol	Ring O	O_3,O_4	⇆
Covalent adduct	Covalent	Ring O	⇄

(5) In no case is there direct binding of metal *M*2 to any atom in any of the liganding compounds described in this work.

From the studies reported here it has become clear that further investigation of the role of Asp287 in the catalytic action of the enzyme is necessary.

We thank Drs Steven Seeholzer and Jean-Francois Biellmann for compounds used in this study. The work was supported mainly by a grant GM-44360 from the National Institutes of Health, also by grants CA-10925 and CA-06927 from the National Institutes of Health, by an appropriation from the Commonwealth of Pennsylvania and by grant Ste-230 from the Deutsche Forschungsgemeinschaft.

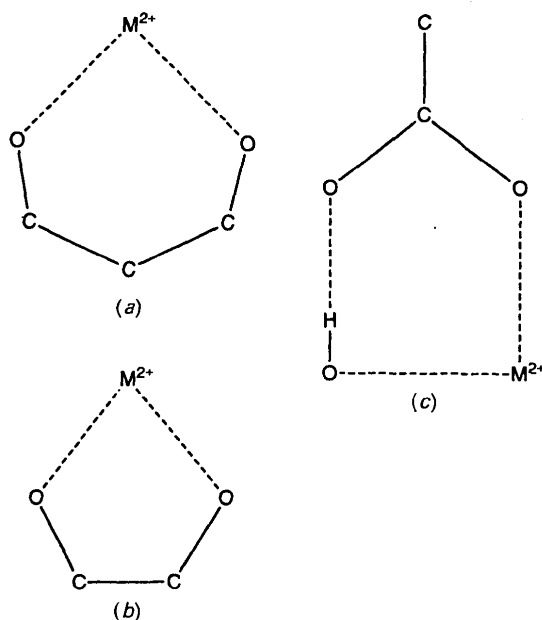


Fig. 14. Motifs found in these structure determinations.

References

Blow, D. M., Collyer, C. A., Goldberg, J. D. & Smart, O. S. (1992). *Faraday Discuss.* **93**. In the press.

Carrell, H. L., Glusker, J. P., Burger, V., Manfre, F., Tritsch, D. & Biellmann, J.-F. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 4440–4444.

Carrell, H. L., Rubin, B. H., Hurley, T. J. & Glusker, J. P. (1984). *J. Biol. Chem.* **259**, 3230–3236.

Collyer, C. A. & Blow, D. M. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 1362–1366.

Collyer, C. A., Goldberg, J. D., Viehmann, H., Blow, D. M., Ramsden, N. G., Fleet, G. W. J., Montgomery, F. J. & Grice, P. (1992). *Biochemistry*, **31**, 12211–12218.

Collyer, C. A., Henrick, K. & Blow, D. M. (1990). *J. Mol. Biol.* **212**, 211–235.

Dauter, Z., Dauter, M., Hemker, J., Witzel, H. & Wilson, K. (1989). *FEBS Lett.* **247**, 1–8.

Dauter, Z., Terry, H., Witzel, H. & Wilson, K. S. (1990). *Acta Cryst.* **B46**, 833–841.

Erlebacher, J. & Carrell, H. L. (1992). *ICRVIEW*. Institute for Cancer Research, Philadelphia, PA, USA.

Farber, G. K., Glasfeld, A., Tiraby, G., Ringe, D. & Petsko, G. A. (1989). *Biochemistry*, **28**, 7289–7297.

Farber, G. K., Petsko, G. A. & Ringe, D. (1987). *Protein Eng.* **1**, 459–466.

Feather, M. S., Deshpande, V. & Lybyer, M. J. (1970). *Biochem. Biophys. Res. Commun.* **38**, 859–863.

Hendrickson, W. A. (1985). *Methods Enzymol.* **115**, 252–270.

Hendrickson, W. A. & Konnert, J. H. (1980). In *Computing in Crystallography*, edited by R. Diamond, S. Ramaseshan & K. Venkatesan. Banglore: Indian Academy of Sciences.

Henrick, K., Blow, D. M., Carrell, H. L. & Glusker, J. P. (1987). *Protein Eng.* **1**, 467–469.

Henrick, K., Collyer, C. A. & Blow, D. M. (1989). *J. Mol. Biol.* **208**, 129–157.

Howard, A. J., Gilliland, G. L., Finzel, B. C., Poulos, T. L., Ohlendorf, D. H. & Salemme, F. R. (1987). *J. Appl. Cryst.* **20**, 383–387.

Jenkins, J., Janin, J., Rey, F., Chiadmi, M., van Tilbeurgh, H., Lasters, I., De Maeyer, M., Van Belle, D., Wodak, S. J., Lauwereys, M., Stanssens, P., Mrabet, N. T., Snauwaert, J., Matthyssens, G. & Lambeir, A.-M. (1992). *Biochemistry*, **31**, 5449–5458.

Jones, T. A. (1985). *Methods Enzymol.* **115**, 158–171.

Kabsch, W. (1988*a*). *J. Appl. Cryst.* **21**, 67–71.

Kabsch, W. (1988*b*). *J. Appl. Cryst.* **21**, 916–924.

Knossow, M., Lewis, M., Rees, D., Wilson, I. A., Skehel, J. J. & Wiley, D. C. (1986). *Acta Cryst.* **B42**, 627–632.

Lambeir, A.-M., Lauwereys, M., Stanssens, P., Mrabet, N. T., Snauwaert, J., van Tilbeurgh, H., Matthyssens, G., Lasters, I., De Maeyer, M., Wodak, S. J., Jenkins, J., Chiadmi, M. & Janin, J. (1992). *Biochemistry*, **31**, 5459–5466.

Luzzati, V. (1952). *Acta Cryst.* **5**, 802–810.

Rey, R., Jenkins, J., Janin, J., Lasters, I., Alard, P., Claessens, M., Matthyssens, G. & Wodak, S. (1988). *Proteins Struct. Funct. Genet.* **4**, 165–172.

Rose, I. A., O'Connell, E. L. & Mortlock, R. P. (1969). *Biochem. Biophys. Acta*, **178**, 376–379.

Schray, K. J. & Rose, I. A. (1971). *Biochemistry*, **10**, 1058–1062.

Ten Eyck, L. F. (1977). *Acta Cryst.* **A33**, 486–492.

Tilbeurgh, H. van, Jenkins, J., Chiadmi, M., Janin, J., Wodak, S. J., Mrabet, N. T. & Lambeir, A.-M. (1992). *Biochemistry*, **31**, 5467–5471.

Whitlow, M., Howard, A. J., Finzel, B. C., Poulos, T. L., Winborne, E. & Gilliland, G. L. (1991). *Proteins Struct. Funct. Genet.* **9**, 153–173.