D-xylose isomerase (XI; D-xylose ketol-isomerase, EC 5.3.1.5) is a metal-ion dependent bacterial enzyme that catalyses the conversion of D-glucose to D-fructose, a reaction extensively used in the industry for the production of high-fructose corn syrup from starch. In addition, XI is used for ethanol production in a process that involves conversion of xylose to xylulose and then to ethanol by fermentation.

Based on structural similarities with triose isomerase, it was first assumed that XI may have an analogous catalytic mechanism involving an ene-diol intermediate. However, xylose isomerase has an absolute requirement for divalent metal ions, preferably Mg$^{2+}$ or Mn$^{2+}$, whereas triose isomerase does not require co-factors for catalysis. Moreover, the substrate for triose isomerase, triose phosphate, is an open chain compound. In contrast, xylose isomerase binds the $\alpha$-anomers of D-xylose or D-glucose in the closed-ring conformation [1]. Isotope exchange experiments and crystallographic studies have led to the currently accepted mechanism that involves 1) preferential binding of the $\alpha$-anomer 2) ring opening, 3) extension of the substrate and, finally 4) metal-mediated hydride-shift. According to this mechanism, the hydride shift follows the ring-opening step and occurs at the extended form of the sugar. However, an alternative mechanism has been proposed [2] where the hydride-shift step precedes the ring-opening step and occurs on the cyclic form of the sugar.

Despite the extensive crystallographic studies on the catalytic mechanism of XI, only the linear forms of glucose or xylose have been observed so far in substrate-soaked crystals. Insights into the orientation of the substrate have been obtained by using thio-$\alpha$-D-glucopyranose (THG), a closed-ring sugar analogue. However, as the ring-opening step in THG is very slow compared to that of glucose, the information provided is limited [3]. No crystal structure has been reported for the closed-forms of the natural substrates (glucose and xylose) of xylose isomerase. In order to gain further insight into the catalytic mechanism of the enzyme, particularly of what is happening during the first moments of the catalytic reaction we have collected a high resolution data-set (1.4 Å) from a single crystal flash-soaked with a glucose solution and quickly cooled in a liquid nitrogen stream. Data were collected on station X11 at EMBL Hamburg using the MARCCD detector. A difference electron density map showed binding of glucose in its cyclic form. However due to some discontinuity in the electron density, the soaking experiment was repeated by increasing the soaking time. A new data set to 1.2 Å showed continuous density for the cyclic-form of glucose. The structure is currently under refinement using SHELX. A data set to 1.3 Å resolution for the unliganded structure was also collected at the crystallographic beamline X11. An MPD molecule was located at the active site. A preliminary model of the native structure at this stage is shown in Fig. 1 (Rcryst=13.2%, Rfree=15.6% after anisotropic refinement). Detailed structural comparison of the two structures is currently underway. Data from xylose-soaked crystals have been collected on stations X11 and X13 under different conditions but no binding of the cyclic form has been seen yet.
Figure 1. Ribbon diagram of the current XI model at 1.3 Å resolution.

References