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Location of Active-site Hydrogen Atoms in D-Xylose Isomerase. Gerard J. Bunick[§], Amy Katz*[§], Xinmin Li[§], Jenny P. Glusker*, H.L. Carrell*, B.L. Hanson[^], Paul Langan[#], Leighton Coates[#], Benno Schoenborn[#], [§]Univ. of Tennessee, Knoxville, TN, *Fox Chase Cancer Center, Phila., PA, [^]Univ. of Toledo, Toledo, OH, [#]Biosciences Div., LANL, Los Alamos, NM.

Time-of-flight neutron diffraction has been used to locate hydrogen atoms that define the ionization states of amino acids in D-xylose isomerase (XI) from *Streptomyces rubiginosus*. XI is one of the largest enzymes studied to date at high resolution (1.8 Å) by this method. We have determined the position and orientation of a metal ion-bound water molecule located in the active site. This water is thought to be involved in the isomerization step in which D-xylose is converted to D-xylulose or D-glucose to D-fructose. Under the conditions of measurement (pH 8.0) it is found to be a water molecule rather than a hydroxyl group. One lysine appears to have an $-NH_2$ terminal group (rather than NH_3^+). The ionization state of each histidine residue has also been determined. High-resolution X-ray studies (0.94 Å) indicate disorder in some side chains when a truncated substrate is bound. This suggests how they might move during catalysis. This combination of techniques can contribute greatly to the elucidation of enzyme mechanisms.

Research supported by NIH GM-29818, CA-10925, CA-06927, NASA NAG8-1826, and the USDOE Office of Science, OBER.