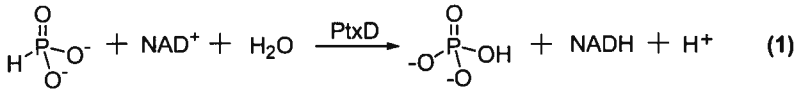
Kinetic Analysis and pH Dependence of the Phosphite Dehydrogenase Reaction

Heather A. Relyea and Wilfred A. van der Donk

The oxidation of hydrogen phosphonate (phosphite, HPt) to inorganic phosphate is catalyzed by the NAD^+-dependent enzyme phosphite dehydrogenase (PtxD) from Gram-negative soil bacteria (equation 1).



PtxD is homologous to the enzymes in the D-2-hydroxyacid dehydrogenase family both in amino acid sequence and in chemical reaction. Therefore, we can propose an analogous active site for PtxD utilizing the conserved catalytic residues (Figure 1). As drawn, histidine serves as the catalytic base, the pK_a of which is modulated by glutamate, and an arginine residue anchors the negatively charged substrate in the active site. During the course of our studies, we have found that this proposed complex is likely *not* the catalytically competent form of PtxD. This presentation will focus on the kinetic and pH-dependence data that has led to us propose an alternative substrate binding mode and potential mechanisms will be discussed.

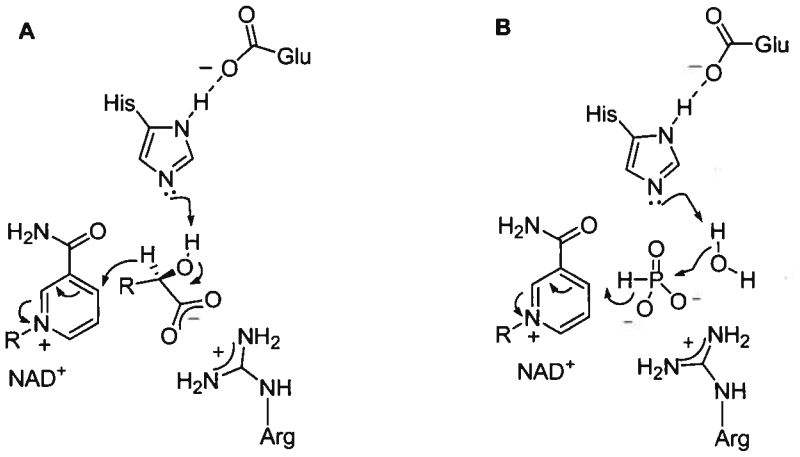


Figure 1. (A) Active site architecture of D-hydroxyacid dehydrogenases performing an oxidation reaction, and (B) initial proposed active site structure of PtxD.