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Capturing the catalytic proton of dihydrofolate reductase: implications for general acid-base catalysis

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Acid-base catalysis, which involves one or more proton transfer reactions, is a chemical mechanism commonly employed by many enzymes. The molecular basis for catalysis is often derived from structures determined at the optimal pH for enzyme activity. However, direct observation of protons from experimental structures is quite difficult; thus, a complete mechanistic description for most enzymes remains lacking. Dihydrofolate Reducatse (DHFR) exemplifies general acid-base catalysis, requiring hydride transfer and protonation of its substrate, DHF, to form the product, tetrahydrofolate (THF). Previous X-ray and neutron crystal structures coupled with theoretical calculations have proposed that solvent mediates the protonation step. However, visualization of a proton transfer has been elusive. Based on a 2.1 Å resolution neutron structure of a pseudo-Michaelis complex of E. coli DHFR determined at acidic pH, we report the direct observation of the catalytic proton and its parent solvent molecule. Comparison of X-ray and neutron structures elucidated at acidic and neutral pH reveals dampened dynamics at acidic pH, even for the regulatory Met20 loop. Guided by the structures and calculations, we propose a mechanism where dynamics are crucial for solvent entry and protonation of substrate. This mechanism invokes the release of a sole proton from a hydronium (H3O+) ion, its pathway through a narrow channel that sterically hinders the passage of water, and ultimate protonation of DHF at the N5 atom.

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