Crystal structure of bovine milk xanthine dehydrogenase and its conversion to the oxidase form by proteolytic cleavage

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Xanthine oxidoreductase is an archetype enzyme, which was originally described as aldehyde oxidase. The enzyme has been isolated from a wide range of organisms, from bacteria to man, and it undertakes the hydroxylation of a wide variety of purine, pyrimidine, pterin, and aldehyde substrates. All of these proteins have similar molecular weights and composition of redox centers. The mammalian enzymes (which catalyze the hydroxylation of hypoxanthine and xanthine, the two last steps in the formation of urate) exist originally as the dehydrogenase forms (XDH), but can be converted to the oxidase forms (XO) by modification of the protein molecule. This conversion occurs either reversibly by oxidation of sulfhydryl residues or irreversibly by proteolysis. XDH shows a preference for NAD^+ reduction at the FAD reaction site, while XO exclusively uses dioxygen as its substrate, leading to the formation of superoxide anion and hydrogen peroxide. The enzyme is a target of drugs against gout and hyperuricemia and the conversion of XDH to XO has been implicated in diseases characterized by oxygen-radical-induced tissue damage (e.g., postischemic reperfusion injury and Lou Gehrig's disease). Recent work suggests that XO might also be associated with blood pressure regulation.

Using data collected at beamline X8C of the National Synchrotron Light Source (NSLS) and at beamline 14-BM-D of BioCARS at the Advanced Photon Source (APS), we determined the crystal structure of the dimeric (MWr 290,000) bovine milk XDH at 2.1 Å resolution. Each subunit of XDH is composed of an N-terminal 20 kDa domain containing two iron sulfur centers, a central 40 kDa FAD domain, and a C-terminal 85 kDa molybdopterin-binding domain with the four redox centers aligned in an almost linear fashion. Cleavage of a surface-exposed loop of XDH causes major structural rearrangements of a stretch of protein chain close to the flavin ring. This leads to a change in its accessibility for oxidant substrate, as well as in its electrostatic environment reflecting the switch of substrate specificity observed for the XDH and XO forms of this enzyme.

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