

Ionization of the LpxC deacetylase metal cofactor influences catalytic activity and product binding

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The enzyme UDP-3-*O*-(R-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase (LpxC) catalyzes the second and committed step in the biosynthesis of lipid A. LpxC deacetylase requires a single, essential divalent metal ion cofactor for catalytic activity while a second metal ion inhibits catalytic activity. Measurement of the pH dependence of the catalytic activity with various metal ions indicates that the enzyme activity decreases as the metal-bound water ionizes to form a metal-hydroxide. Additionally, at the pH-optimum the rate of the Zn-LpxC catalyzed reaction is significantly faster than the Co-LpxC catalyzed reaction. The active site metal ion is proposed to both lower the pK_a of the zinc-water for activation by a general base catalyst and to stabilize the oxyanion intermediate and flanking transition states in the reaction. We have also examined interactions between the metal ion(s) on product (myrUDPGlcNH₂) and fatty acid binding using pH and metal substitution studies, in combination with binding experiments. While fatty acid binding affinity is dependent on the identity and number of metal ions bound to the active site, it is relatively independent of metal-water ionization. X-ray crystal structures of the LpxC-palmitate complex, indicates that there are different conformations of the bound fatty acid in the mononuclear and binuclear zinc enzymes. However, ionization of the metal-water influences myrUDPGlcNH₂ binding affinity; in contrast to the effect observed on catalytic activity, product binding is enhanced by ionization to form the bound metal-hydroxide. These findings may suggest that following the deacetylation reaction regeneration of the zinc-water is not only necessary for catalysis, but also serves to facilitate product dissociation. Together these data suggest that the metal cofactor of LpxC deacetylase may play complementary roles in catalysis, initially functioning to enhance the rate of the chemical step in the reaction and in a later step facilitating product release.