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by nonheme Fe(2+) at room temperature. MabA is a bacterial metalloenzyme with unique 2,4-dioxo-6-methyl-2,4-heptadienoate (DMOAH) moiety as the substrate. This protein catalyzes dehydratation/isomerization reactions in the bacterial pathway of homo-6-delta(3)-hexulose biosynthesis. MabA is one of the few proteins that can provide insights into the biological importance of protons as an electron transfer partner. The two oxidized states of MabA, high-spin Fe(III) and low-spin Fe(III)-hydroperoxo, have been characterized previously. However, the electron transfer pathway and the substrate-induced protonation states in the

active site of MabA have remained unclear. In the present paper, we have carried out continuous-flow kinetic investigations at room temperature in aqueous solution using a stopped-flow spectrophotometer to investigate the mechanism of electron transfer and the protonation states of MabA in the active site. The stoichiometry of protonation was 1:1 between MabA and 2H-DMOAH, and the value of the apparent pKa was calculated to be 6.5. The spectral changes observed f678ea9f9e

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