The Mechanism of Sugar C-H Bond Oxidation by a Flavoprotein Oxidase
Occurs by a Hydride Transfer Before Proton Abstraction

Thanaporn Wongnate,*[a] Panida Surawatanawong,[b] Litavadee Chuaboon,[c] Narin Lawan,[d] and Pimchai Chaiyen[a]

[a] T. Wongnate, P. Chaiyen School of Biomolecular Science & Engineering Vidyasirimedhi Institute of Science and Technology (VISTEC) Wangchan Valley, Rayong 21210 (Thailand) E-mail: thanyaporn.w@vistec.ac.th
[b] P. Surawatanawong Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University Bangkok 10400 (Thailand)
[c] L. Chuaboon Department of Biochemistry and Center for Excellence in Protein and Enzyme Technology, Faculty of Science Mahidol University, Bangkok, 10400 (Thailand)
[d] N. Lawan Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 (Thailand)

Understanding the reaction mechanism underlying the functionalization of C–H bonds by an enzymatic process is one of the most challenging issues in catalysis. Here, combined approaches using density functional theory (DFT) analysis and transient kinetics were employed to investigate the reaction mechanism of C–H bond oxidation in D-glucose, catalyzed by the enzyme pyranose 2-oxidase (P2O). Unlike the mechanisms that have been conventionally proposed, our findings show that the first step of the C–H bond oxidation reaction is a hydride transfer from the C2 position of D-glucose to N5 of the flavin to generate a protonated ketone sugar intermediate. The proton is then transferred from the protonated ketone intermediate to a conserved residue, His548. The results show for the first time how specific interactions around the sugar binding site promote the hydride transfer and formation of the protonated ketone intermediate. The DFT results are also consistent with experimental results including the enthalpy of activation obtained from Eyring plots, as well as the results of kinetic isotope effect and site-directed mutagenesis studies. The mechanistic model obtained from this work may also be relevant to other reactions of various flavoenzyme oxidases that are generally used as biocatalysts in biotechnology applications.