X-ray Absorption Spectroscopy of Recombinant Alkane Hydroxylase (AlkB) and Rubredoxin-2 (AlkG) from *Pseudomonas putida* GPo1

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Abstract

AlkB from *Pseudomonas putida* GPo1, an integral membrane non-heme iron oxygenase, is a medium-chain-length (C_5 to C_{12}) alkane hydroxylase. It is of vast interest due to its high turnover frequency in the conversion of alkanes to alcohols. In this study, we overexpress recombinant AlkB and its redox component, AlkG (rubredoxin-2), in *E. coli* and purify them into homogeneity. AlkG is also a metallo-protein identified to contain a pair of tetrahedral cores of FeS_4. We subjected these two proteins for the study of X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). Our data support that electrons can be transferred from the reduced AlkG (rubredoxin-2, the redox partner of AlkB) to AlkB in a two-phase manner. Moreover, both of the active AlkB and AlkG were subjected to EXAFS study for revealing ligand types, the number of coordination and the geometry of irons. Based on these spectroscopic studies, an approach for the electrocatalytic conversion from alkanes to alcohols mediated by AlkB using an AlkG immobilized screen-printed carbon electrode (SPCE) is developed. The framework distortion of AlkB-AlkG adduct on SPCE surface might create promiscuity toward gaseous substrates. Hence, small alkanes including propane and *n*-butane can be accommodated in the hydrophobic pocket of AlkB for C–H bond activation. Taken together, our results provide insights to unravel the reaction mechanisms required for alkane hydroxylation by AlkB.

Keywords – Alkanes, Alcohols, Iron, X-ray absorption spectroscopy, iron-sulfur cofactor

References

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