Final report

NKFIH (OTKA) project **PD 108955**

Activation of Strong Covalent Bonds in Metalloenzymes: Theoretical Studies

1. Introduction

Metalloenzymes, exploiting their highly fine-tuned metal centers in conjunction with their multifunctional protein scaffold, catalyze a broad diversity of reactions, including the surprisingly facile activation of chemically inert species and bonds. The work carried out within the frames of the present project was aimed at elucidating and understanding the mechanisms of such reactions via the application of quantum mechanical (QM) or combined quantum mechanical and molecular mechanical (QM/MM) methods. The focal point of the work was the activation of dioxygen and the oxidative transformation of substrates within iron-containing enzymes.

2. Results and Publications

The key achievements of the project can be summarized in the following points.

a. A program called `gaumber' was developed that allows data interchange among the Amber, Gaussian, and Turbomole software suites including the handling of QM/MM partitioning. One of its functions was to link the structure preparation (in Amber) with the QM/MM geometry optimizations and potential energy surface scans (in Gaussian), as well as to process the output of the latter. It allows to combine several strengths of the packages: MM topology generation and dynamics within Amber, efficient QM/MM geometry optimizations with Gaussian 09 using its quadratic macroiteration algorithm, and fast DFT calculations with Turbomole using the multipole accelerated resolution of identity approximation. It furthermore provided a platform for the implementation of several enhancements of the `textbook' QM/MM protocol proposed in the literature, including the iterative updating of QM charges within a mechanical embedding calculation and the data processing for a large middle layer calculation within a QM/QM/MM scheme. The capabilities of `gaumber' were described as part of our paper on benzoyl coenzyme A epoxygenase.[1]

b. Oxygenation of aromatic rings using O_2 in non-heme diiron enzymes was studied on the representative example of benzoyl coenzyme A epoxidase, BoxB, which converts the benzoyl ring of its substrate to the corresponding 2,3-epoxide. We identified a large number of possible reaction pathways for the attack on the aromatic ring, which could be grouped into four mechanistically

distinct families: (a) electrophilic ($2e^-$) attack by a bis(μ-oxo)-diiron(IV) species (**Q** pathway); (b) electrophilic ($2e^-$) attack via the σ* orbital of a μ-η^2:η^2-peroxo-diiron(III) intermediate (**P**σ* pathway); (c) radical ($1e^-$) attack via the π*-orbital of a superoxo-diiron(II,III) species (**P**π* pathway); (d) radical ($1e^-$) attack of a partially quenched bis(μ-oxo)-diiron(IV) intermediate (**Q**' pathway). This grouping conveys information about general reactivity features of the diiron-dioxygen core, and as a conceptual framework, it is expected to be valid for aromatic oxygenation reactions of other members of the non-heme diiron enzyme family. It furthermore allowed literature works (de Visser, Liao–Siegbahn) to be fit into a unified framework. Specifically for the BoxB enzyme, the **Q** pathway was found to be the most preferred, but the corresponding bis(μ-oxo)-diiron(IV) species is significantly destabilized and not expected to be directly observable. Epoxidation via the **P**σ* pathway represents an energetically somewhat higher lying alternative; we estimated the isotope effects on the two routes, which we consider as possible measures for experimental discrimination. Using isolated models of the active site, various substrate analogues, and an analysis of the electron donation processes, we showed that the selectivity toward epoxidation is inherent in the electronic properties of the thioacyl substituent, although it is further supported by enzymatic constraints. These results were published in a paper[1] and presented as poster in two conferences (10th congress on Electronic Structure: Principles and Applications (ESPA2016); 13th European Biological Inorganic Chemistry Conference).

c. Together with collaboration partners in the Institute of Organic Chemistry and Biochemistry (IOCB), Prague, and in the Institute for Molecular Science, Okazaki, Japan, I participated in studies on the reaction mechanism of the stearoyl acyl carrier protein Δ^9 desaturase enzyme also having a carboxylate-bridged diiron core. This enzyme is capable of inserting a double bond into an alkyl chain by double hydrogen atom abstraction using molecular O_2. On top of QM(DFT)/MM geometries, large-scale multireference calculations were done using the density matrix renormalization group (DMRG) approach. DMRG allows unprecedentedly large active spaces for the explicit correlation of electrons in the large part of the chemically important valence space, and to our knowledge, this was the first time it was employed in a bioinorganic computational mechanistic study. The derived reaction mechanism involves protonation of the previously characterized 1,2-μ-peroxo-diiron(III) (P) intermediate to a 1,1-μ-hydroperoxy species, which abstracts an H atom from the C^{10} site of the substrate. An Fe(IV)-oxo unit is generated concomitantly, supposedly capable of the second H atom abstraction from C^9. We furthermore investigated the performance of several popular DFT functionals against the DMRG-CASPT2 data. While the applied DMRG-CASPT2 level may still be too low to serve as a gold standard in multireference calculations, this comparison

highlighted its capability to provide a qualitatively correct description of the variations in the electronic structure of these highly open shell species. Notably, many of the compared exchange–correlation functionals predicted completely wrong mechanism due to an erroneous preference for heterolytic C–H cleavage instead of radical H atom abstraction. These results were published as an original article.[2]

d. Together with collaboration partners in IOCB and in the Jaroslav Heyrovský Institute of Physical Chemistry, Prague, we prepared a review article on the chemistry of mononuclear and binuclear non-heme iron systems from a computational perspective. The review,[3] extending into 26 pages and containing 242 references, provides an account of the current state-of-the-art of the theoretical treatment of these highly challenging systems. We put the emphasis on the concerted progress on both theoretical and experimental sides, and we focused on the correlations between various experimental data (spectroscopic, kinetic, thermodynamic, electrochemical) and computations.

3. Unpublished results, abandoned studies, and differences with respect to the original aims

a. Development of the `gaumber' software (discussed above) was decided after a detailed survey of the literature. While these efforts significantly increased the originally planned time of the preparatory phase of the project, the tool allowed efficient, state-of-the-art computations to be carried out, which paid off where mapping of large number of reaction pathways was necessary.

b. The original aims included two enzymes capable of oxygenating aromatic rings, BoxB (discussed above) and phenylacetyl coenzyme A epoxidase (Paa). Understanding of a particularly interesting feature of Paa, deoxygenation of its own product, was considered a key point. However, as the available crystal structures of Paa did not contain the iron ions, its preparation for computation was more demanding, and emphasis was put more on BoxB. In the meantime, a publication appeared in the literature discussing both BoxB and the deoxygenation pathway in Paa;[4] hence, studies on Paa were abandoned after protein preparation and optimization of a handful of structures as they were not considered to yield significant additional results on top of BoxB.

c. For the [Fe]-hydrogenase, a detailed computational study appeared in 2014,[5] providing exhaustive answer on my questions about the roles of the iron atom and the cofactor, before I would have started any research activity. Hence, no work on [Fe]-hydrogenase was done.

d. As my studies on Paa and [Fe]-hydrogenase were cancelled, I accepted the invitation to participate in preparing a review article (discussed above) to appear in the special issue of the Journal of Biological Inorganic Chemistry dedicated to Prof. Edward I. Solomon on the occasion of his ACS Alfred Bader Award in Bioinorganic or Bioorganic Chemistry.

e. For the same reason, I decided to include a new enzyme in my studies, lactate racemase. This enzyme was found recently to contain an unprecedented sulfur-carbon-sulfur nickel pincer complex as cofactor, derived from nicotinamide mononucleotide.[6] We targeted the role of the nickel center in the activation of the C–H bond of lactate. According to the preliminary results, this probably lies in the immobilization of the cofactor and in tuning its hydride affinity. Unfortunately, this project could not be brought to conclusion before the termination of the OTKA project.

f. Due to lack of time, no studies were done on the originally planned N-oxygenating enzyme AurF.

4. Comments on expenses and participants

Besides myself, in agreement with my original goals, two university students participated in the research described above. They made minor contributions to the aromatic oxygenation and to the lactate racemization projects. The expenses largely followed the original plans. Due to the financial situation of the Research Centre of Natural Sciences and to personal reasons, the number of conference participations was smaller than expected; the resources were—with the approval of OTKA—reallocated to an extension of the computational resources.

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[1] Rokob, T. A. *Pathways for Arene Oxidation in Non-Heme Diiron Enzymes: Lessons from Computational Studies on Benzoyl Coenzyme A Epoxidase*, *J. Am. Chem. Soc.* **2016**, *138*, 14623.

[2] Chalupsky, J., Rokob, T. A., Kurashige, Y., Yanai, T., Solomon, E. I., Rulisek, L., Srnec, M. *Reactivity of the Binuclear Non-Heme Iron Active Site of D9 Desaturase Studied by Large-Scale Multireference* Ab Initio *Calculations, J. Am. Chem. Soc.* **2014**, *136*, 15977.

[3] Rokob, T.A., Chalupsky, J., Bim, D., Andrikopoulos, P. C., Srnec, M., Rulisek, L., *Mono- and Binuclear Non-Heme Iron Chemistry from a Computational Perspective, J. Biol. Inorg. Chem.* **2016**, *21*, 619.

[4] Liao, R.-Z., Siegbahn, P. E. M., *Mechanism and Selectivity of the Dinuclear Iron Benzoyl-Coenzyme A Epoxidase BoxB*, *Chem. Sci.* **2015**, *6*, 2754.

[5] Finkelmann, A. R., Senn, H. M., Reiher, M., *Hydrogen-Activation Mechanism of [Fe]-Hydrogenase Revealed by Multi-Scale Modeling, Chem. Sci.* **2014**, *5*, 4474.

[6] Desguin, B., Zhang, T., Soumillion, P., Hols, P., Hu, J., Hausinger, R. P., *A Tethered Niacin-Derived Pincer Complex With a Nickel-Carbon Bond in Lactate Racemase, Science* **2015**, *349*, 66.