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X-ray Absorption Spectroscopy of Intermediates of Oxygen Activation in Nonheme Iron Enzymes

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Methane monooxygenase intermediate T: In recent years the oxidative mechanism of the enzyme methane monooxygenase (MMO) has drawn considerable attention, both because of its intrinsic enzymological interest and because of the insight it offers in developing processes for the controlled oxidation of hydrocarbons. One of the strategies to obtain mechanistic information at atomic detail involved studying the structure of the diferric protein-product complex [1]. The basis of this approach was the belief that the atomic arrangement in the enzyme-product complex is closely related to that of the transition state during oxygen transfer, whereas differences arise mainly from the distribution of electron density between the metal cluster and the substrate/product molecule. We decided to test this postulate by comparing the structure of the enzyme-product complex with that of the transient species, termed intermediate T, that was identified by fast kinetics immediately after the decay of the high-valent intermediate Q.

Methods and Results: Intermediate T was generated from the reaction of Q with nitrobenzene, using a double rapid mixing experiment, and trapped by rapid freezing. Fe K-edge XAS data were recorded for both intermediate T and the complex of MMO with nitrophenol, corresponding to the enzyme-product complex. FT analysis of the EXAFS oscillations shows that differences exist between the two structures, suggesting that the enzyme-product complex is formed only after relaxation of intermediate T. Therefore its structure bears no direct connection to the mechanism by which the oxygen insertion process occurred. Further experiments are being carried out to repeat this comparison on a variety of substrates and to obtain additional structural details on intermediate T.

Human H ferritin peroxo intermediate: The process of Fe oxidation and biomineralization by the protein ferritin (Human H chain) was studied by following Fe K-edge XAS data over the oxidative cycle. The study was aimed at confirming structural conclusions on the putative diiron μ -1,2-peroxo intermediate of the cycle. This species had previously been characterized by another group using XAS on the ferritin from bullfrog [2]. These earlier studies had provided an unusual description of the μ -1,2-peroxo species, having a surprisingly short 2.5 Å Fe–Fe bond, with consequences on the physiological activity of the protein itself. It was of interest to determine whether the same structural, and therefore physiological, conclusions could be drawn for the corresponding human protein.

Methods and Results: We recorded the Fe K-edge XAS data for the peroxo intermediate of Human H ferritin and the first intermediate in the process of peroxo decay. In contrast to the bullfrog case, EXAFS analysis did not reveal any Fe shells at 2.5 Å for the peroxo intermediate. Instead, only weak contributions from Fe shells were obtained at > 3 Å. This is in agreement with what has been observed for related synthetic diferric μ -1,2-peroxo complexes. We conclude that differences at the atomic level exist in the processes of peroxo formation and decay between ferritin proteins from these two different sources.

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References:

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[2]. J. Hwang, C. Krebs, B. H. Huynh, D. E. Edmondson, E. C. Theil, J. E. Penner-Hahn, *Science* **2000**, *287*, 122-125.