Effective Intermediate-Spin Iron in O₂-Transporting Heme Proteins

Nils Schuth¹, Stefan Mebs¹, Dennis Huwald², Pierre Wrzolek³, Matthias Schwalbe³, Anja Hemschemeier³, Michael Haumann¹*

¹Freie Universität Berlin, Department of Physics, 14195 Berlin, Germany
²Ruhr-Universität Bochum, Department of Plant Biochemistry, Photobiotechnology, 44801 Bochum, Germany
³Humbold-Universität zu Berlin, Department of Chemistry, 12489 Berlin, Germany

* michael.haumann@fu-berlin.de

Proteins carrying an iron-porphyrin (heme) cofactor are essential for biological O₂ management. The nature of the Fe-O₂ bonding in hemoproteins has been debated for long. We used energy-sampling and rapid-scan X-ray Kβ emission and K-edge absorption spectroscopy at synchrotrons as well as quantum chemistry to determine molecular and electronic structures of unligated (deoxy), CO-inhibited (carboxy), and O₂-bound (oxy) hemes in myoglobin (MB) and hemoglobin (HB) solutions and in porphyrin model compounds at 20-260 K. Similar metrical and spectral features revealed analogous heme sites in MB and HB and the absence of low-spin (LS) to high-spin (HS) conversion. Amplitudes of Kβ main-line emission spectra were directly related to the formal unpaired Fe(d) spin count, indicating HS Fe(II) in deoxy and LS Fe(II) in carboxy. For oxy, two effective Fe(d) spins were revealed by our static and kinetic X-ray spectroscopy data, as supported by (TD)DFT and CASSCF calculations. The emerging Fe-O₂ bonding situation includes in essence a ferrous iron center, minor superoxide character of the non-innocent ligand, significant double-bond properties of the interaction, and three-center electron delocalization as in ozone. It resolves the classical and apparently contradictory models of Pauling, Weiss, and McClure/Goddard into a unifying view of O₂-bonding tuned towards reversible oxygen transport in the heme proteins [1].

Figure 1: Effective unpaired Fe(d) spins from Kβ emission spectroscopy (left) and molecular orbitals of the Fe-O₂ bonding (right; numbers: spin densities, Fe(d)/O(p) characters, EUEDs).

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