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Review

CO, NO and O_2 as vibrational probes of heme protein interactions

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ABSTRACT

The gaseous XO molecules (X = C, N or O) bind to the heme prosthetic group of heme proteins, and thereby activate or inhibit key biological processes. These events depend on interactions of the surrounding protein with the FeXO adduct, interactions that can be monitored via the frequencies of the Fe—X and X—O bond stretching modes, νFeX and νXO. The frequencies can be determined by vibrational spectroscopy, especially resonance Raman spectroscopy. Backbonding, the donation of Fe d_π electrons to the XO π^* orbitals, is a major bonding feature in all the FeXO adducts. Variations in backbonding produce negative νFeX/νXO correlations, which can be used to gauge electrostatic and H-bonding effects in the protein binding pocket. Backbonding correlations have been established for all the FeXO adducts, using porphyrins with electron donating and withdrawing substituents. However the adducts differ in their response to variations in the nature of the axial ligand, and to specific distal interactions. These variations provide differing vantages for evaluating the nature of protein–heme interactions. We review experimental studies that explore these variations, and DFT computational studies that illuminate the underlying physical mechanisms.

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1. Introduction

Heme proteins are nature's receptors for the gaseous XO molecules (X = C, N, O), which play vital roles in many biological processes. By binding to the heme, the XO molecules can serve as signals, triggering a protein conformation change that initiates DNA binding or an enzymatic reaction. Also the bound XO can be activated for redox reactions through electron transfer to or from the heme. Interactions with the surrounding protein are critical for binding discrimination among the XO molecules, and for guiding the subsequent protein conformation change or the XO reactivity. These interactions involve the protein sidechains that line the heme pocket. Steric contacts can directly impede ligand binding, and can also distort the porphyrin ring, influencing the Fe—XO electronic properties. In addition, electric fields and H-bonds associated with sidechains distal to the bound XO can polarize the Fe—XO bond. Also critical is the nature of the proximal sidechain, generally histidine or cysteine, that serves as a *trans* axial ligand to the heme. The donor strength of the axial ligand also influences the Fe—XO bond.

Characterizing these interactions is fundamental to understanding how heme proteins operate, and vibrational spectroscopy offers a powerful tool for characterization.

The Fe—X and X—O stretching force constants are proportional to the bond strengths, and are connected (albeit somewhat indirectly, as discussed in Section 2.1) to the frequencies of the associated vibrational modes. These modes can be detected by IR or Raman spectroscopy. The latter is particularly useful because tuning the Raman laser into resonance with the heme electronic transitions enhances heme vibrational modes [1], providing sensitivity and selectivity with respect to the background spectra of the protein and of the water solvent. Most FeXO vibrational data have been obtained from resonance Raman spectra [2–6]. However, IR spectroscopy can also be useful, particularly for CO adducts, since the CO stretching vibration occurs in an uncrowded spectral region, and binding to the heme induces a large transition dipole for the CO stretch [7–13].

The structures of the heme—XO adducts, as well as the vibrational frequencies can be modeled via DFT computations [14–29]. The structures agree well with available structural data, while the frequencies are somewhat overestimated, as is usual with ab initio methods. However, computed trends in the frequencies agree with observations, giving confidence in the analysis of protein interactions with heme-XO adducts via DFT modeling. In the ensuing sections we describe the patterns that have been revealed in the correlations of Fe—X and X—O stretching frequencies, νFeX and νXO, from experiment and computer modeling.

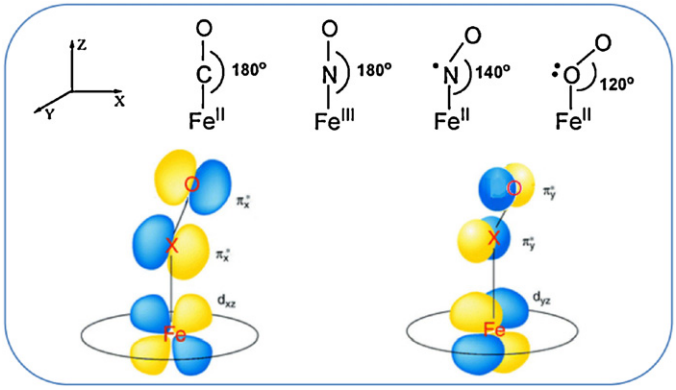

Fig. 1. Backbonding schematic for FeXO adducts (X = C, N or O), showing the orbitals available for π backdonation from Fe to XO.

2. The backbonding pattern

The heme group is electronically tuned to bind CO, NO and O_2, because the XO molecules have vacant π^* orbitals that are well matched to the filled d_π orbitals of the Fe(II) ion in the middle of the porphyrin ring (Fig. 1). This arrangement is optimal for Fe—XO π backbonding. Electrons shift from Fe to XO in the d_π–π^* system, and are donated back to Fe in the σ system (synergic bonding). Backbonding strengthens the Fe—X bond while weakening the X—O bond. Consequently, the dominant motif in FeXO is a negative correlation of νFeX and νXO. As backbonding increases, νFeX increases while νXO decreases [16,18].

In CO, both π^* orbitals are empty, and FeCO is a linear adduct, with d_π–π^* overlaps in both perpendicular directions (*x* and *y*, with *z* as the FeCO axis, Fig. 1). However, NO and O_2 have one and two π^* electrons, and their adducts are forced to bend ($\sim$140° for FeNO, $\sim$120° for FeO_2) in order to accommodate a π antibonding interaction. The bonding can be thought of in terms of sp^2 hybridization at the X atom, with one or two electrons in the lobe pointing away from the direction of bending (Fig. 1). Meanwhile, the π_y^* orbital remains available for backbonding with the Fe d_{yz} orbital.

For bent FeXO adducts there is an issue in determining the Fe—X stretching frequency, because the Fe—X stretching and Fe—X—O bending motions have comparable frequencies and the coordinates are allowed to mix. (This mixing is symmetry-forbidden in the linear FeCO.) The mixing produces vibrational modes with variable contributions from stretching and bending. However, only one of the modes is significantly enhanced in RR spectra, and it has the larger frequency shift upon isotopic substitution at X [30]. For convenience, this mode has been labeled 'νFeX', and, as we shall see, it correlates with νXO in the same way that νFeC correlates with νCO for FeCO adducts.

2.1. Five-coordinate MXO adducts

The backbonding correlation is clearly seen in νFeX/νXO plots for 5-coordinate M(II)XO porphyrin adducts (Fig. 2); Co(II)NO adducts, isoelectronic with Fe(II)O_2 adducts, are included for comparison. Backbonding in these adducts was modulated by electron-donating or withdrawing substituents on a

Abbreviations: IR, infrared; RR, resonance Raman; DFT, density functional theory; QM/MM, quantum mechanical/molecular mechanical; H-bond, hydrogen bond; HOMO, highest occupied molecular orbital; cyt P450cam, cytochrome P450 camphor hydroxylase; P450nor, nitric oxide reductase cytochrome P450; swMb, sperm whale myoglobin; H-NOX, Heme-Nitric oxide/OXygen binding domain; sGC, soluble guanylate cyclase; Hb, hemoglobin; Hb_{ASC}, *Ascaris* hemoglobin; HbN, HbO, hemoglobin I and II from *Mycobacterium tuberculosis*; *Chl*Hb, *Chlamydomonas* hemoglobin; *Sc*Hb, *Synechocystis* hemoglobin; HemDGC, heme-containing diguanylate cyclase; Ctb, hemoglobin III from *Campylobacter jenjuni*; hHO-1, human Heme oxygenase isoform 1; NOS, nitric oxide synthase; iNOS, nNOS, inducible and neuronal nitric oxide synthases; saNOS, *Staphylococcus aureus* NOS; NIR, NOR, nitrite and nitric oxide reductase; CPO, chloroperoxidase; HRP, horseradish peroxidase; CCP, cytochrome *c* peroxidase; CCO, cytochrome *c* oxidase; AXCP, cytochrome *c*′ from *Alcaligenes xylosoxidans*; BjFixLH, *Bradyrhozobium japonicum* FixL heme-PAS; MtDosH, *Methanobacterium thermoautotrophicum* Dos heme-PAS; CLOCK PAS-A, mammalian circadian protein CLOCK; NP, nitrophorin; CooA, carbon monoxide oxidation activator protein; Arg, arginine; Trp, tryptophan; TMCH, 3,3,5,5-tetramethylcyclohexanone; CPRQ, camphoroquinone; VCA0720, an H-NOX protein from *Vibrio cholerae*; *Tt*Tar4H, *Thermoanaerobacter tengcongensis* Tar4 protein heme domain; TPP, TMP, OEP, tetraphenyl-, tetramethyl- and octaethyl- porphyrins; Pc, phthalocyanine; PPDME or PPIXDME, protoporphyrin IX dimethylester; $PP(ProP^-)_2$, $PP(Prop^-)(PropH)$, $PP(PropH)_2$, adducts of protoporphyrin IX with 0, 1 or 2 protons on the propionate substituents; TpivP, picket fence porphyrin; N-MeIm, N-methylimidazole; ImH, MeImH, imidazole, 4-methylimidazole; SR, model Fe(III)-NO porphyrin-alkanethiolate complex; SR-HB, model Fe(III)-NO porphyrin-alkanethiolate complex with intramolecular NH-S hydrogen bond; YC-1, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole; C_2Cap, 5,10,15,20-[pyromellitoyl(tetrakis-*o*-oxyethoxyphenyl)]porphyrin; CH_2Cl_2, dichloromethane; DMF, dimethylformamide; Bz, benzene.