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Forum

Preface Forum: "Functional Insight from Physical Methods on Metalloenzymes"

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Inorganic Chemistry has traditionally been the venue for the publication of studies on inorganic complexes that model active sites of metallobiomolecules. The emphasis of model studies was initially on structure, either its elucidation or, in the context of protein crystallography, its correlation to the chemical and physical properties of active sites. Now much of the focus of model studies is on function. The molecular basis of reactivity can often best be studied on structurally defined small-molecule analogues, which also have the potential for being developed into new catalysts. *Inorganic Chemistry* has also been the primary literature repository for studies on the physical properties of inorganic systems, initially in the fields of coordination and organometallic complexes and now in such timely areas as inorganic photochemistry; photophysics and electron transfer; and the electric and magnetic properties of materials, clusters, and nanoparticles. The purpose of this Forum is to emphasize that parallel physical methods are having a huge impact in the study of metallobiomolecules.

A fairly complete set of spectroscopic and magnetic methods for the study of inorganic systems (complexes, clusters, materials, metallobiomolecules, etc.) is summarized in Table 1, which is adapted from ref 1. In the context of the general energy level diagram in the associated figure, ground-state methods study the unpaired electron(s) in the valence orbitals perturbed by a magnetic field. The energy range is $0.1-10\ cm^{-1}$ depending on the source and magnetic field. At low resolution, magnetic susceptibility and Mössbauer (for ^{57}Fe) spectroscopy provide the redox and spin states of a metal ion. At higher resolution, magnetic Mössbauer and multifrequency EPR methods provide insight into the nature of the ground state, that is, the half-occupied orbitals and their delocalization onto the ligands. At still higher resolution are double resonance (electron nuclear double resonance, ENDOR) and pulsed (electron spin−echo envelope modulation, ESEEM) EPR methods, which directly probe the delocalization of the unpaired electron spin onto the ligands through their nuclear spin superhyperfine couplings with the electron spin.

In the near-infrared−visible spectral region, are the d → d or ligand field transitions whose energy splittings and intensities probe the ligand environment around the metal center. These are parity-forbidden and thus relatively weak in absorption spectroscopy, but can be studied at high sensitivity by circular dichroism (CD, for optically active sites as in metalloproteins) and, in particular, magnetic circular dichroism (MCD) spectroscopies at low temperature (for paramagnetic complexes). The relatively high intensity of d → d transitions in CD and MCD reflects the different selection rules for different spectroscopic methods, where CD intensity requires a magnetic-dipole-allowed transition and low-temperature MCD intensity involves a C-term mechanism for paramagnetic systems, both of which can be large for metal-centered transitions.

At still higher energy (visible−ultraviolet) are the ligand-to-metal charge-transfer transitions (metalloproteins mostly have donor ligands and therefore exhibit LMCT transitions), which involve a shift of electron density from the ligand to the metal. The transitions are thus electric-dipole-allowed and can be intense in the absorption spectrum. Charge-transfer transitions are important probes of the detailed nature of the ligand−metal bond, particularly for highly covalent ligands, which produce low-energy, intense CT transitions. Their electric dipole transition moments are oriented along the specific ligand−metal bonds and so can be studied by polarized single-crystal electronic absorption spectroscopy and, for randomly oriented frozen solutions, by variable-temperature variable-field MCD (VTVH MCD). The high intensity of charge-transfer transitions allows resonance enhancement of Raman vibrations when the laser source is tuned into the absorption band. Resonance Raman spectroscopy provides vibrational information on the metal site as well as insight into the nature of the electronic transition

(1) Solomon, E. I.; Hanson, M. A. Bioinorganic Spectroscopy. In *Inorganic Electronic Structure and Spectroscopy*; Solomon, E. I., Lever, A. P. B., Eds.; Wiley: New York, 1999; Vol. II, p 1.

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Table 1. Spectroscopic Methods

method	parameter(s)	information content
	Ground State	
magnetism	molar magnetic susceptibility (χ_m)	spin of ground state
	effective magnetic moment ($\mu = 2.83\sqrt{\chi T}$)	
	axial (D) and rhombic (E) zero-field splitting (ZFS)	low-symmetry splitting of d orbitals and their relative covalency
	exchange coupling constant (J)	covalent interaction of bridging ligand with two or more metal ions
Mössbauer	isomer shift (δ)	oxidation and spin state of ^{57}Fe
	metal quadrupole splitting (E_Q)	
	metal hyperfine (A^M)	orbital angular momentum and metal covalency of ground state
	electron Zeeman effect (g_i, $i = x, y, z$)	
	ZFS	see above
electron paramagnetic resonance (EPR)	g_i, A_i^M, D, E	see above
	ligand superhyperfine coupling (A^{Li})	covalency electron spin density on Li
electron nuclear double resonance (ENDOR)	A^{Li}	see above
	nuclear Zeeman effect ($g_N\beta_N H$)	identification of ligand
	ligand quadrupole coupling (P)	electric field gradient at ligand nucleus
electron spin−echo envelope modulation (ESEEM)	same as ENDOR but for smaller A^{Li}	see above
	Ligand Field Excited States	
electronic absorption (ABS)	energies	LF splitting of d orbitals = geometry
	intensities (f = oscillator strength)	distortion of site from centrosymmetric
	band shapes	excited-state distortion—probes change in bonding on excitation
polarized single-crystal absorption	parallel (π), perpendicular (σ), and axial (α) polarized spectra	electric dipole selection rule for transition assignment
circular dichroism (CD)	energies/band shapes	same as above
	intensities (R = rotational strength)	resolution of bands, magnetic dipole selection rule
magnetic circular dichroism (MCD)	energies/band shapes	same as above
	intensities (A, B, C terms)	probes nature of excited states, spin−orbit coupling intensity mechanism
	VTVH MCD	probes ground-state parameters as described above allows polarized single-crystal-like data from frozen solution
	Charge-Transfer Excited States	
ABS/CD/MCD	as above	nature of L−M bond ABS intensity quantitates ligand donor strength
resonance Raman (rR)	depolarization ratio (ρ)	assigns vibrations
	enhancement profiles (A and B terms)	as with band shapes, gives excited-state distortion
	Core Excited States	
metal K-edge X-ray absorption spectroscopy (XAS)	energy	charge on a metal ion in a complex
	intensity	quadrupole and 4p mixing due to distortion of site from centrosymmetric
L-edge XAS	energy dependence of intensity	multiplet splitting gives oxidation and spin state
	intensity	covalency—metal d character in valence orbitals
ligand K-edge XAS	energy	effective charge on ligand
	intensity	covalency—ligand p character in valence orbitals
	Photoelectron Final States	
core levels	energy	charge on a metal ion in a complex
	intensity distribution (into shake-up satellites)	electronic relaxation (i.e., change in electronic structure upon ionizaiton)
valence band	energy	atomic orbital composition of molecular orbitals
	photoionization cross section	change in electronic structure upon valence ionization

associated with resonance (its excited-state distortion due to the change in bonding upon excitation).

In the higher-energy vacuum UV and soft X-ray regions, many transitions overlap, and these energy regions are best studied through photoelectron methods (vide infra). However, in the X-ray region, the absorption spectrum simplifies as core levels are excited that are well separated in energy. In the 500−1000 eV region, one excites metal 2p (for 3d metal complexes) core electrons. p → d transitions are electric-dipole-allowed and, because the 2p orbital is localized on the metal center, the intensity of these transitions provides a direct probe of the metal character in the half-occupied and unoccupied valence d molecular orbitals and, hence, the covalency of the complex. This is strongly complimented by ligand K-edge X-ray absorption spectroscopy (XAS). This involves the ligand 1s → metal 3d transition, and because the 1s orbital is localized on the ligand and s → p is electric-dipole-allowed, the intensity of this transition reflects the ligand p character covalently mixed into the metal d orbitals. For sulfur, an important biological ligand donor that is highly covalent, the sulfur K-edge is at 2470 eV. At still higher X-ray energies, one studies metal K-edge XAS. Most chemists are familiar with EXAFS (extended X-ray absorption fine structure). This involves excitation of the metal 1s electron into the continuum and the constructive and destructive interferences of the de Broglie waves of the electron (associated with different kinetic energies) due to scattering by adjacent ligand atoms that gives structural information.

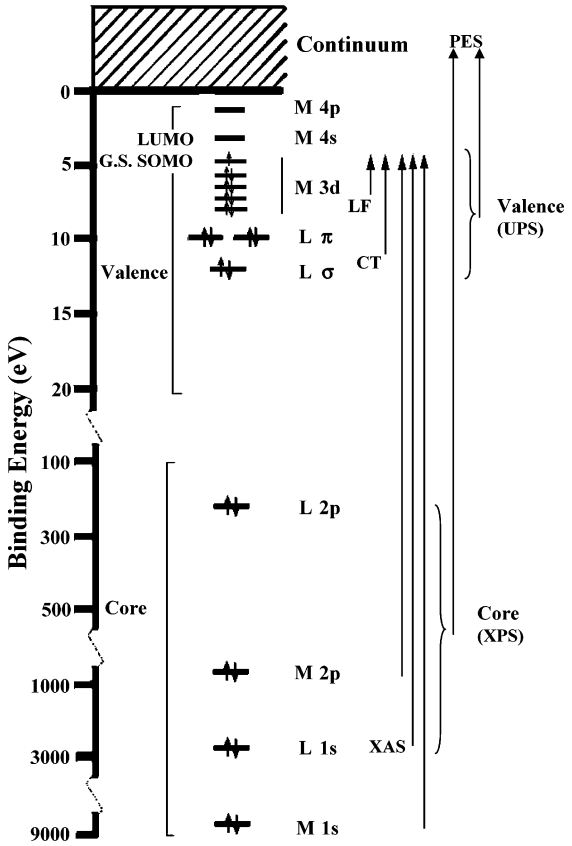

Figure 1. Energy level diagram for a transition metal complex.

However, it should also be emphasized that the metal K preedge, which involves bound-state transitions from the metal 1s orbital into the metal 3d and 4p levels, also provides geometric and electronic structural information through the energy splitting and intensity of these transitions. The 1s → 4p transition is electric-dipole-allowed, whereas the 1s → 3d is only quadrapole-allowed but gains intensity in noncentrosymmetric environments through metal 4p mixing by covalent interactions of the ligand valence orbitals with both the metal 3d and 4p levels.

The above methods involve photon detection. Photoelectron spectroscopy (PES) utilizes electron detection methods to study the ionization (excitation of electrons into the continuum) of valence and core orbitals. Because the escape depth of an electron from a sample is on the order of 10 Å, this is best studied in model systems rather than metalloproteins. This field has been revolutionized by the utilization of synchrotron radiation where one can scan the photon energy and detect its effect on the intensity of the PES peaks (the photoionization cross section). Electrons ejected from different types of orbitals (s, p, d, 2p versus 3p, etc.) have different photoionization cross sections. From the intensity dependence on photon energy, one can assign specific peaks in a PES spectrum and, of particular importance, determine how the electronic structure changes upon ionization. The latter is called electronic relaxation and can be significant in transition metal complexes because of the large magnitude of the repulsion between d electrons. This change in electronic structure with ionization (i.e., oxidation) can also have major effects on the redox properties of transition metal complexes.

All of the above methods experimentally probe the electronic structure of transition metal complexes. It is important to correlate experiment with theory to obtain maximum insight into electronic structure contributions to function. There are two extensively employed electronic structure approaches in transition metal chemistry. If the metal center is not very covalent, traditional ligand field theory (LFT) provides an accurate description of ground and ligand field excited state data and geometric structural insight into electronic structure. Highly covalent systems require molecular orbital theory (MOT). Publications in *Inorganic Chemistry* over the years have mirrored the development of MOT from extended Hückel, to semiempirical (neglect of differential overlap CNDO, INDO, etc.), Hartree−Fock including configuration interaction (MPn, CAS, etc.), and most recently density functional theory (DFT). DFT is presently the most accessible high-level approach for the description of transition metal complexes, particularly active sites in metalloproteins. These involve on the order of 100 atoms and can include more than one transition metal ion. However, it should be emphasized that there are many different types of density functionals (local and gradient-corrected, LDA, GGA, etc.) and hybrid methods (B3LYP, etc.), where, in the latter, some Hartree−Fock exchange is mixed with the density functional. The hybrid methods tend to make complexes less covalent, thus providing better agreement with data. The ideal approach is to evaluate different types of DFT calculations with experiment (i.e., geometric and electronic structural information) and to use a theoretical approach supported by the data to obtain further insight into reactivity.

This Forum has assembled contributions from six major research groups who employ a range of physical methods to obtain functional insight into many of the different classes of metalloenzymes. In the first article, Broderick et al. use rapid freeze quench (RFQ) EPR spectroscopy to define a new functional role of Fe_4S_4 clusters in the generation of catalytic radicals, and in the second article, RFQ magnetic Mössbauer is used by Krebs et al. to characterize high-valence intermediates in O_2 activation by non-heme iron enzymes. In the third contribution, Ohta and Kitagawa use resonance Raman spectroscopy to obtain structural insight into the selective sensing of small molecules by heme proteins. Of course, the dominant physical method of the inorganic community for structural determination is X-ray crystallography, and in the fourth article in this Forum, Rosenzweig et al. discuss the utility and limitations of protein crystallography in elucidating active site structure and reactivity for enzymes including ribonucleotide reductase and particulate methane monooxygenase (pMMO). Whereas paramagnetic NMR spectroscopy is widely used in elucidating geometric and electronic structures for inorganic complexes, it has thus far had more limited impact in the study of metalloproteins. In the fifth contribution, Machonkin et al. show that the limitations associated with paramagnetic relaxation broadening can be overcome by selective labeling

in 1D and 2D experiments, providing new insight into the active sites of the one and two iron sulfur proteins involved in electron transfer. Finally, redox processes are dominant in the reactions of many metalloenzymes. In the last contribution in this Forum, Vincent and Armstrong extend from cyclic voltammetry experiments on metalloproteins absorbed on electrode surfaces to use potential steps to trigger reactions and determine kinetics and mechanism in such key enzymes as [NiFe] hydrogenase. This *Inorganic Chemistry* Forum reflects the excitement of the field of bioinorganic chemistry, its relevance to fundamental and timely problems in chemistry and biology and the fact that physical−inorganic chemistry is playing a major role in its development.

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