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Review

Characterization of "spectroscopically quiet" metals in biology

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Contents

Abstract

The use of X-ray absorption spectroscopy (XAS) to characterize the local environment of "spectroscopically quiet" metals in metalloproteins is reviewed, with an emphasis on studies of Cu(I) and Zn(II). Both the advantages and the weaknesses of X-ray absorption are discussed, with examples taken from the recent literature.

Keywords: Spectroscopically quiet; X-ray absorption; Cu(I); Zn (II); EXAFS; XANES; XAS

1. Aims and scope of review

1.1. Definition of "spectroscopically quiet" metals

Over the last 40 years, there have been tremendous advances in our understanding of bioinorganic chemistry. Many of these can be traced, in one way or another, to spectroscopic studies of metalloprotein active sites. Particularly informative have been studies in the UV-Vis (absorption, CD, and MCD) and microwave (EPR, ESEEM, ENDOR, etc.) spectral regions. The importance of the UV-Vis and microwave regions rests, in part, on the fact that many metals have intense, spectroscopically unique signatures in these regions. Thus, the visible spectroscopy of heme proteins and the EPR spectroscopy of Cu

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proteins were among the earliest bioinorganic investigations.

Unfortunately, metals with either filled or empty d-shells have almost no spectroscopic signature (i.e., are "spectroscopically silent") in both spectral regions. Of these metals, the most important biologically are Cu(I) and Zn(II) as trace elements and Na(I), K(I), Mg(II) and Ca(II) as more abundant elements. For such metals, synchrotron spectroscopy, in particular X-ray absorption spectroscopy (XAS), may be the only method outside of crystallography for obtaining insight into the metal-site structure. These essential metals, together with important toxins such as Cd, Hg, and Pb, and important therapeutic reagents such as Ga or Au, make up the "spectroscopically quiet" metals (quiet, rather than silent, since they are readily accessible using core-level XAS). This review is intended to provide an overview of the use of X-ray absorption to characterize the roles of spectroscopically quiet metals in biology. The emphasis is placed on Cu(I) and Zn(II) rather than Na(I), K(I), Mg(II), or Ca(II), as the former tend to form tightly-bound complexes with proteins and thus to give data that is more straightforward to interpret. Rather than an exhaustive description of all of the examples of Cu(I) and Zn(II) spectroscopic studies, an effort has been made to select examples that illustrate key features of such studies.

Another group of elements that are not accessible using conventional spectroscopies are most of the ligand elements. Recent work has shown that X-ray spectroscopy is equally useful for characterizing the local environment of ligands, especially those containing S or Cl [1–3]. This has been reviewed recently, including a contribution in the present volume [4,5], and thus is not included in the present review.

1.2. Alternatives to XAS

In addition to XAS, there are a few other spectroscopic probes that can be used to examine spectroscopically quiet metals. Mercury (^{199}Hg), cadmium (^{113}Cd), and silver (^{109}Ag) can be studied directly by NMR and, in addition, show ligand-to-metal charge transfer transitions that can be used to characterize metal–thiolate interactions [6–9]. Recently, time-differential perturbed angular correlation has been developed as a probe for investigating the local environment of Cd and Hg in biological systems [10]. This utilizes the time-correlation in the γ-ray emission from radioactive nuclei (^{111}Cd and ^{199m}Hg) to characterize the nuclear quadrupole interactions at the metal site. Unfortunately, none of these approaches are useful for studies of Zn(II) or Cu(I). Very recently, there has been exciting progress in using solid-state NMR to characterize Zn sites [11–13]. However, solid-state NMR is not, at least yet, generally applicable as a tool for understanding biological Zn environments.

Often, it is possible to substitute an active site Zn or Cu with a spectroscopically accessible probe. Cobalt, which has both EPR and UV-Vis bands, has been used as a surrogate for zinc for many years [14,15], and often can be substituted with little or no loss of reactivity. Similarly Cd(II) will often substitute for Zn(II) and Ag(I) for Cu(I) [16–19]. Although it is not necessarily the case that any of these substitutions are isostructural [20,21], they can nevertheless be very useful for obtaining some information about Cu or Zn structure. Since the present volume is focused on the applications of synchrotron radiation, ancillary techniques will be discussed only in those cases that they provide an essential complement to information available from XAS.

2. X-ray absorption spectroscopy

2.1. Physical principles of XAS

XAS is one of the premier tools for investigating the local structural environment of metal ions. It can be divided into X-ray absorption near edge structure (XANES), which provides information primarily about geometry and oxidation state, and extended X-ray absorption fine structure (EXAFS), which provides information about metal site ligation. In the present context, the three key attractions of XAS are that it is element specific, that it is always detectable, and that it can be used to study dilute non-crystalline samples. The last of these has been particularly important in the past, when protein crystal structures were relatively rare and XAS was the only way to obtain structural information for many metal sites. With the initiation of numerous structural genomics efforts worldwide, protein crystal structures are becoming less rare. One of the goals of the present review is to emphasize that even in the era of structural genomics, XAS has an important role to play in developing a complete description of metalloprotein metal sites (see Section 5).

XAS has been available as a useful structural probe for nearly 30 years, and over this time a variety of excellent reviews and monographs have been written describing the method [22–30]. The following provides a brief review of this background information.

X-rays have sufficient energy to eject one or more core electrons from an atom. Each core electron has a well-defined binding energy, and when the energy of the incident X-ray is scanned across one of these energies, there is an abrupt increase in the absorption coefficient. This is the so-called "absorption edge" of the element. The remainder of this review is concerned almost exclusively with measurements made at the K-edge (1 s initial state). For first row transition metals, the K-edge energy is in the hard X-ray region (5–10 keV, or ca. 2–1 Å). The absorption coefficient near an edge typically shows fine structure that is divided, somewhat arbitrarily, into XANES and EXAFS regions, with the former referring to structure within ca. 50 eV of the edge and the latter to structure at higher energy (see Fig. 1).

The physical basis of both EXAFS and XANES is the scattering of the X-ray excited photoelectron by the