
Preface

The fast development of genome sequencing has accumulated a huge amount of information concerning the nature and binding properties of the proteins coded by the genetic material of many different organisms. Thus, a high-throughput X-ray absorption spectroscopy (HT-XAS) metalloproteomic study performed on 3,879 proteins produced by the New York SGX Research Center for Structural Genomics indicated that about 9 % of them contained transition metals such as Zn, Cu, Ni, Co, Fe, or Mn. These metalloproteins, which are the subject of this book, are involved in many key biological processes such as gas transport and metabolism, photosynthesis, cell respiration, the Krebs cycle, and many other vital redox reactions.

One very important characteristic of many of the transition metal-containing enzymes is the sensitivity of their catalytic and redox centers to oxygen attack. This feature complicates their study because it often requires strict anaerobic conditions, as any exposure to O₂ may result deleterious. This book addresses the multiple aspects of metalloenzyme research: after an introductory chapter by Professor Perry A. Frey the following four chapters deal with the production and purification of metalloproteins using both standard recombinant techniques and a cell-free system. The possibility of not using standard equipment, such as a glove box, is addressed.

Once homogenous intact metalloproteins have been obtained they can be subjected to their functional characterization. One of the best techniques to explore the redox changes and catalytic properties of a great number of metalloenzymes is electrochemistry. This technique is discussed in Chapter 6. The electrochemical analysis of metalloproteins can be effectively coupled to infrared (IR) absorption spectroscopy as explained in Chapter 7. IR techniques can also be applied to monitor changes in the protein structure by using a technique called surface-enhanced infrared absorption or SEIRA. The use of resonance Raman (RR) spectroscopy to investigate the nature of short-lived intermediates formed during reactions of metalloproteins is addressed in Chapter 8. This technique is generally well suited to study enzymes carrying chromophoric prosthetic groups. Similar to IR and RR spectroscopies is a technique called nuclear resonance vibrational spectroscopy or NRVS. As discussed in Chapter 9 NRVS requires access to a synchrotron X-ray source. Data collection strategies are similar to those used in extended X-ray absorption fine structure (EXAFS) spectroscopy.

Many metalloproteins display paramagnetic states of their catalytic or redox centers when poised at relevant potentials. Such paramagnets are well suited to be studied by electron paramagnetic resonance (EPR) spectroscopy, which is described in Chapter 10. A particular advantage of this technique is that molecules with fully filled electron shells, including the solvent and the protein backbone, are EPR silent. Chapter 11 is dedicated to Mössbauer spectroscopy. This technique, specific to iron, has the advantage of being able to detect all forms of this metal ion, and their coordination sphere, found in a protein. Like in the case of NRVS, the sample should be enriched in ⁵⁷Fe.

Although paramagnetism is indeed a very convenient feature of transition metals some of them, such as the common Zn²⁺, are diamagnetic and consequently spectroscopically silent in their biologically relevant redox state. In this case, the best-adapted technique to

study the local electronic and physical structure around the metal site in a protein is X-ray absorption spectroscopy. Of course, XAS, which comprises X-ray absorption near-edge structure (XANES) and EXAFS, can also be used to study paramagnetic centers and provides very accurate stereochemical parameters in general. Like NRVs this technique requires access to a synchrotron X-ray source, and beam time should be requested accordingly. Because the overall signal for a given atom will include those from other metals present in the sample XAS is not well suited for the study of bulk cell preparations or cell lysates. Chapter 12 shows how to overcome this problem. It describes the use of XAS for the characterization of protein bands after electrophoretic separation and Western blotting.

The wealth of data provided by spectroscopic analyses can be ideally coupled to the structural data that can be obtained using X-ray crystallography (although nuclear magnetic resonance (NMR) is also a powerful technique for protein structure determination it is not well suited for the study of paramagnetic species). Chapter 13 discusses the use of X-rays not only to determine the three-dimensional structure of a crystallized metalloprotein but also to establish the nature of the metal(s) that it contains. Phasing using the multi-wavelength anomalous dispersion (MAD) method is described in some detail. Metalloprotein crystals can also be analyzed by X-ray fluorescence (to identify metals) or UV/visible spectroscopy (to monitor redox changes). Like in the other cases where tunable X-rays are required, beam time should be requested at a suitable synchrotron facility.

The last two chapters of this book deal with the theoretical interpretation of metalloenzyme catalytic and redox processes. Chapter 14 reviews the main procedures and computational methods that use quantum mechanics (QM) to study the reactivity and electronic properties of transition metal-containing enzymes. The continuous increase in computing power has recently allowed scientists to apply QM methods to the study of relatively large systems such as the active site of enzymes, including complex metal components. Finally, Chapter 13 describes in detail a particular domain of density functional theory (DFT) called broken symmetry (BS). DFT-BS is a very powerful albeit complex technique used to compute the energetic and spectroscopic properties of bioinorganic clusters (especially those containing transition metal ions). It is especially well suited to calculate coupling constants and g tensors as well as isomer shifts and quadrupolar tensors to be respectively compared to those obtained from EPR and Mössbauer spectroscopic experiments.

In summary, this book, which collects the contributions from the laboratories of renowned scientists, covers most of the relevant aspects of the metalloprotein field. It is the hope of the editors that the practical experience acquired in those laboratories and now contained in this compilation will be useful for both scientists who have already worked in the field for many years as well as those that are just joining in.

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Metalloproteins

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