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Review

The role of X-ray spectroscopy in understanding the geometric and electronic structure of nitrogenase ☆

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ABSTRACT

X-ray absorption (XAS) and X-ray emission spectroscopy (XES) provide element specific probes of the geometric and electronic structures of metalloprotein active sites. As such, these methods have played an integral role in nitrogenase research beginning with the first EXAFS studies on nitrogenase in the late 1970s. Herein, we briefly explain the information that can be extracted from XAS and XES. We then highlight the recent applications of these methods in nitrogenase research. The influence of X-ray spectroscopy on our current understanding of the atomic structure and electronic structure of iron molybdenum cofactor (FeMoco) is emphasized. Contributions of X-ray spectroscopy to understanding substrate interactions and cluster biosynthesis are also discussed. This article is part of a Special Issue entitled: Fe/S proteins: Analysis, structure, function, biogenesis and diseases.

1. Introduction

Nearly 80% of the earth's atmosphere is comprised of inert dinitrogen (N_2), which must be converted into a bioavailable form for incorporation into both amino and nucleic acids, the building blocks of proteins and DNA. Industrially, the conversion of dinitrogen to ammonia is achieved using heterogeneous catalysts in the well-known Haber–Bosch process, which operates at high temperatures and pressures. The Haber–Bosch process is utilized to produce fertilizers and as such is often credited for the dramatic increase in the global population, which followed its discovery. Prior to the invention of the Haber–Bosch process, nitrogenase enzymes, found in bacteria and free diazotrophs, provided the dominant (~95%) source of fixed bioavailable nitrogen (with the remaining ~5% deriving from atmospheric N_2 conversion).

The nitrogenase family of enzymes includes Mo-dependent, V-dependent and Fe-dependent forms, all of which can affect the reduction of dinitrogen (N_2) to ammonia (NH_3) under ambient conditions. By far the best studied nitrogenase enzymes are the Modependent forms which utilize eight electrons, eight protons and sixteen ATP molecules in order to produce two molecules of ammonia and one molecule of hydrogen, as shown in the reaction below [1].

$$N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

Mo-dependent nitrogenases consist of two component proteins: the iron protein (which serves as a reductase) and the Mo–Fe protein. The Fe protein is an α_2 homodimer that contains a single [4Fe–4S] cluster that bridges the 2 units. This protein is known as a redox-active agent that is capable of transferring electrons to the MoFe protein. The MoFe protein is an $\alpha_2\beta_2$ heterodimer, where each $\alpha\beta$ unit contains two unique metalloclusters: the P-cluster and the FeMo cofactor (FeMoco) (shown in Fig. 1). The P-cluster is an [8Fe–7S] distorted dicubane, which can be thought of as a [4Fe–4S] cluster and a [4Fe–3S] cluster bridged by sulfides. The P-cluster mediates electron transfer from the [4Fe–4S] cluster of Fe protein to FeMoco. The FeMo cofactor (FeMoco) is a Mo:7Fe:9S:C–homocitrate cluster, which can be viewed as coupled [4Fe–3S] and [Mo–3Fe–3S] clusters. This cofactor is embedded in each α subunit and is generally agreed to be the active site, where substrates bind and are reduced. It is for this reason that particularly strong research efforts have been placed on understanding the structure of the FeMoco cluster [2,3].

X-ray spectroscopy has played an important role in our understanding of nitrogenase cofactors for more than three decades. It was in 1978 that the first Mo EXAFS derived structures of FeMoco were proposed [5,6]. The initial proposals suggested sulfur bridged clusters with either a

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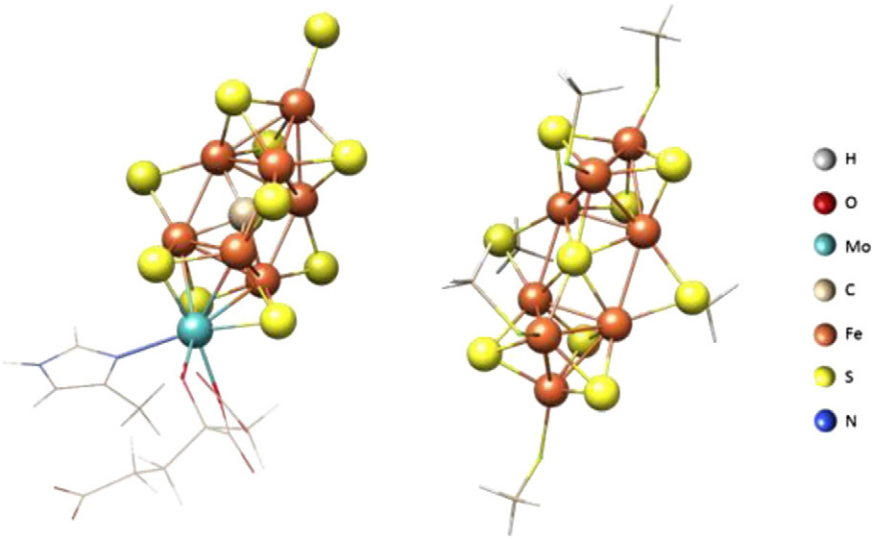

Fig. 1. Structures of the two metalloclusters of nitrogenase: FeMoco (left) and P-cluster (right) (based on PDB: 3U7Q [4]).

linear Fe–Mo–Fe unit or a $MoFe_3$ cubane-like structure. In 1982, the EXAFS proposals were somewhat modified when available Fe EXAFS data suggested that the FeMoco cluster must be larger than simply a single cubane unit [7]. While ultimately the 1992 crystal structure deviated from these initial EXAFS proposals, many of the important structural aspects had already been captured by these early X-ray spectroscopic data — namely, the ~2.35 Å Mo–S and Fe–S distances, as well as the ~2.7 Å Mo–Fe and Fe–Fe distances [5,8]. In the decades that have followed, X-ray spectroscopic methods have continued to play an important role in our understanding of nitrogenase.

In this review, we first give an overview of X-ray spectroscopic methods and the information that can be obtained. We then highlight recent applications of these methods in nitrogenase research.

2. X-ray spectroscopy.

X-ray spectroscopic methods provide element selective probes of electronic and geometric structures. X-ray absorption spectroscopy (XAS) provides information about the unoccupied levels, while X-ray emission spectroscopy (XES) probes the filled levels. Together these two experimental methods can provide a detailed experimental map of the molecular orbitals, giving insight into the oxidation state, spin state, and ligand environment around a specific absorbing atom [9]. In the case of nitrogenase, X-ray spectroscopic studies have allowed all the Fe, Mo and S atoms to be separately probed.

2.1. X-ray absorption spectroscopy (XAS)

X-ray absorption spectroscopy measures the absorption of X-rays as a function of the energy of the incident X-ray. In an XAS spectrum, a dramatic change in the absorption coefficient occurs upon excitation of a core electron. This sharp discontinuity in the spectrum is referred to as an absorption edge. An edge occurs anytime a core electron absorbs an X-ray with energy equal to or greater than its binding energy. The nomenclature of XAS edges reflects the core level from which the electron originates. For example: a K-edge corresponds to the transition from a 1s core orbital, L-edges correspond to 2s and 2p levels, and M-edges to 3s, 3p and 3d levels as shown in Fig. 2 [10]. In the case of nitrogenase research, Fe K- and Mo K-edge XAS have been most extensively applied. However, reports of Mo L- and S K-edge data have also been made [11,12,51].

The resultant XAS spectrum can be divided into two regions:

a) the X-ray absorption near edge structure (XANES) — the low-energy part below, at, and just above (20–50 eV) the edge,

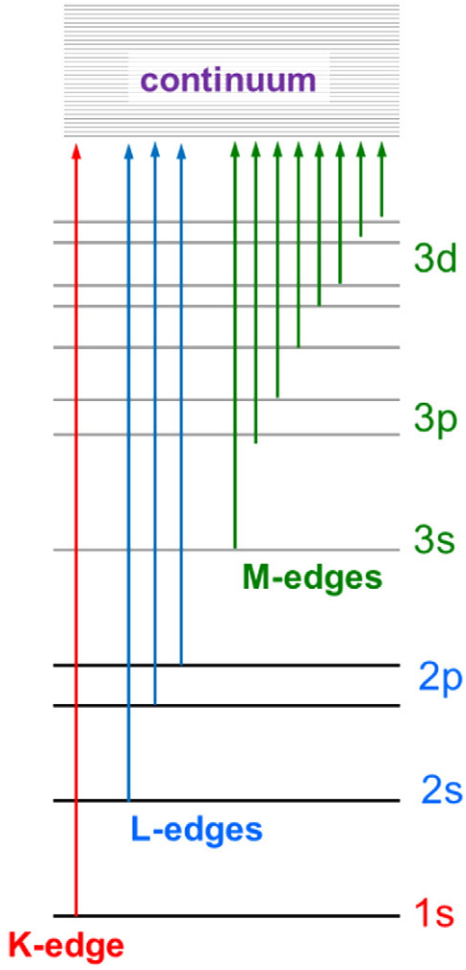

Fig. 2. Transitions resulting from the excitation of a core electron and the corresponding XAS edges.