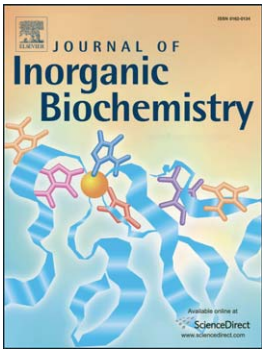

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Nitrite coordination in myoglobin

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Abstract: The coordination of nitrite in myoglobin (Mb) has been characterized by resonance Raman spectroscopy and the frequencies of the nitrite bound to the heme Fe as well to the 2-vinyl have been computed by Density Functional Theory (DFT) calculations. The DFT Natural Bond Orbital (NBO) analysis and the extensive isotope-labeling in the resonance Raman experiments indicate that NO_2^- (O1-N=O2) is bound to the heme Fe via O1. Based on the vibrational characterization of the reversible transition between low and high spin Fe-O-N=O/2-nitrovinyl species, we suggest that the key step that triggers the spin-change is the increase of the proximal Fe-N_{His93} bond length. The frequencies of the O and N sensitive bands of the Fe-O-N=O/2-nitrovinyl species remained largely unchanged in the low-to high-spin transition. Therefore the "greening" process in the reaction of ferric Mb with NO_2^- proceeds through the Fe-O-N=O/2-nitrovinyl species, which can exist in either the high or low-spin state.

Keywords: Density functional theory calculations, heme proteins, nitrite, Raman spectroscopy

1. Introduction

Nitrite (NO_2^-) at micromolar levels in tissues and nanomolar levels in blood can be reduced to bioactive nitric oxide (NO) during hypoxia and also mediate physiological signaling [1-4]. In oxygenated hemoproteins, the reaction with NO leads to the formation of peroxynitrite at the heme site and to nitrosative protein damage [5]. Nitrite is derived from dietary sources such as cured meats and also from the presence of NO_3^- found in green leafy vegetables, which is reduced to NO_2^- in the body by bacteria in the gastrointestinal tract [6]. Nitrite is reduced by a series of nitrite reductase proteins in hypoxia including myoglobin (Mb) and hemoglobin (Hb), molybdenum containing enzymes, nitric oxide synthase (NOS) and cytochrome P450 [1]. The reduction of NO_2^- can also produce nitrosating (N_2O_3) and nitrating ($^{\cdot}NO_2$) species which can modify proteins and lipids to form nitrated fatty acids (FA-NO_2), Fe-NO_2, S-nitrosothiols (RSNO) and nitroamines (RN-NO_2) [7-10]. These species affect the signaling which induces modulation of mitochondria function, vasodilation, decrease inflammation, modulation in glucose metabolism and host defense [9-10]. The Mb-dependent reduction of NO_2^- to NO results in the inhibition of cytochrome c oxidase in the heart, and the nitrite dependent inhibition of cytochrome *c* oxidase regulates responses to physiological hypoxia, such as that found in the muscle during exercise [9-10]. Although the field of nitrite biophysics has advanced in the last few years, fundamental issues have remained unexplored.

The crystallographic data of the reaction of metmyoglobin (metMb) with NO_2^- have revealed that the nitrite ligand adopts the O-binding (*nitrito*,-O1-N=O2) mode, whereas those based on EPR spectroscopy demonstrated that nitrite can in fact bind to the iron via its nitrogen atom forming a low-spin (LS) heme Fe-NO_2^- (*nitro*, -NO_2) species [7, 11-16]. The green pigment of nitrite-cured meat is the result of nitration at the 2-vinyl position. The crystallographic data have found support from quantum chemical analysis supporting the O-binding to form the low-spin ferric *nitrito* (-O1-N=O2) species [15]. Recently, the Mb heme Fe-O-N=O and heme Fe-O-N=O/2-nitrovinyl species were characterized by resonance Raman spectroscopy [17].

In the current work, we have employed resonance Raman (RR) spectroscopy and Density Functional Theory (DFT) calculations to interrogate the interactions between metMb and nitrite, building on our previously reported work [17]. The optimized molecular structures of the myoglobin heme Fe-O-N=O (Mb-NO_2^-) and heme Fe-O-N=O/2-nitrovinyl (NMb-NO_2^-) complexes computed at the B3LYP/lacvp** level of theory are shown in **Figure 1**. A full elucidation of the structure and the electronic configuration of all the forms of nitrite binding are important for understanding the mechanism of ligand binding to the heme Fe and the proton substitution/ligand binding to the 2-vinyl. Herein, we report the RR characterization of the vibrational modes of the Fe-O-N=O/2-nitrovinyl (NMb-NO_2^-) species (green pigment) and DFT calculations for all the Mb-nitrite complexes. To our knowledge, detailed vibrational analysis has not been performed for the Mb-nitrite complexes. Theoretical studies on the molecular structure and vibrational spectra of Mb-nitrite complexes contribute to the in-depth understanding of their properties. A H_2O/D_2O exchange was also probed, but has negligible effects to the NO_2^- vibrations. Our understanding of the ligand binding properties of NO_2^- to Mb is relatively premature compared with the CO, NO and O_2 binding and photodissociation, mostly because of the lack of structural information on transient reaction intermediates by RR spectroscopy or DFT calculations. Theoretical studies on the molecular structure and vibrational spectra of Mb-nitrite complexes provide insight into the structural, energetics, and electronic structure of key nitrimyoglobin species (green pigment) occasionally present in nitrite-cured meat.