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# Coupling of helix E-F motion with the O-nitrito and 2-nitrovinyl coordination in myoglobin

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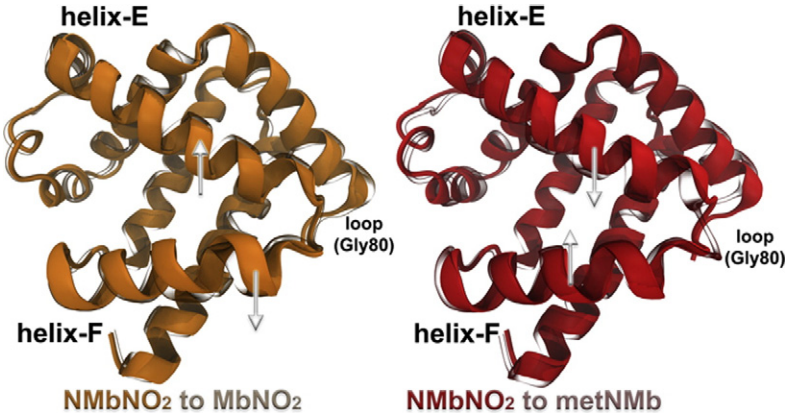
## HIGHLIGHTS

- Molecular Dynamics (MD) simulations for the Mb-nitrite complexes
- Formation of the 2-nitrovinyl species in Mb is associated with motion of helix F.
- Coordination of $NO_2^-$ to heme iron is mainly associated with the motion of helix E.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Myoglobin (Mb) is known to react slowly with nitirite to form the green pigment by $NO_2^-$ cordination to the heme Fe in the O-binding nitrito (O1—N═O2) mode and to the heme 2-vinyl position. Nitrite is a powerful oxidizing agent and a biological reservoir for NO that has been implicated in a variety of aerobic biological systems. Accordingly, it is important to elucidate the nature and variety of $NO_2^-$ reaction mechanisms with Mb. We have performed principal component analysis (PCA, or essential dynamics) on Molecular Dynamics trajectories of all Mb—$NO_2$ coordination states to resolve the most important motions in the protein at 298 K. We show that the coordination or removal of $NO_2^-$ to/from the heme iron is associated mainly with a motion of helix E and the coordination of $NO_2^-$ to the 2-vinyl is associated with a motion of helix F and a correlated motion of helices E-F. This latter correlated motion can be attributed to the interaction of Val68 and Ile107 with the 2-nitrovinyl moiety. The resonance Raman results show that coordination of $NO_2^-$ to the 2-vinyl is increased at pH 6.0 demonstrating that the amide protons in the F helix are not protected from access of solvent water and the helix F motion allows solvent access to the 2-vinyl group, without affecting the coordination to the heme Fe.



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## 1. Introduction

Nitrite is a powerful oxidizing agent and a biological reservoir for NO that has been implicated in a variety of aerobic biological systems [1–5]. Nitration reactions in proteins can lead to structural and functional changes, some of which can contribute to changes in protein activity [1,2]. The electron withdrawing characteristics of the $-NO_2$ substituent perturb the structural and electronic features of the proteins that can lead to protein gain-or loss-of-function [1–3]. There are many proposed reaction-pathways mechanisms of protein nitration reported to explain the effect of changes in protein structure and function at the molecular level [1–10]. Among those are the mechanisms involved in the formation of the green pigment of nitrite-cured meat which is the result of the modification at the heme 2-vinyl position of myoglobin [7,8] and that of the tyrosine nitration which is a post-translational modification for a number of proteins [1–3,10]. The nitration process that involves tyrosyl radical intermediates is critical, because it occurs under basal physiological conditions and in cases that is associated with biomembranes is linked to lipid peroxidation process that involves lipid peroxyl radicals (LOO) [1,2]. The mitochondrial proteins cytochrome *c* and manganese superoxide dismutase MnSOD have shown structural effects of specific tyrosine nitration and subsequent impact in the protein function [1–3]. Of interest is the formation of ferryl-Mb and $NO_2$ that are both produced during the reaction of peroxynitrite with met myoglobin [6]. The freely-diffusing $NO_2$ that is liberated from the radical pair nitrates Tyr103 or escapes to Xe4 site (Fig. 1) [6]. In cytochrome *c*, nitration of the solvent exposed Tyr74 induces Tyr deprotonation, which in return, causes gain of peroxidase activity at physiological pH [3]. In the crystal structure of the green pigment of Mb, nitration of Tyr146 and Tyr103 was not detected unlike that proposed for nitriheme formation from the metMb/nitrite/$H_2O_2$ reaction [8,10].

The Mb-nitrite complexes have been characterized by X-ray crystallography and the unusual O-binding of nitrite to the heme Fe was proposed to be modulated by the distal His64, whereas the replacement of a H by $NO_2^-$ at the heme 2-vinyl group was attributed to steric effects at the heme active site that play a critical role in directing the observed 2-nitrovinyl [8,11–13]. The observed region specific 2-vinyl nitration in Mb is different compared to that observed in legHb and horseradish peroxidase where 4-vinyl is preferred [8,14,15]. The detailed mechanism of this process has not yet been fully elucidated, but is clear from the complexity and importance of the nitration of heme proteins continues to develop. Recent results have indicated that the formation of the nitrito-heme Fe—O—N═O/2-nitrovinyl species is pH-dependent [16]. The conditions under which the nitrito heme Fe—O—N═O/2-nitrovinyl species is formed strongly suggested that this form corresponds to an acid induced transformation. It was proposed that the movement of helices E and F at low pH results in the protonation of nitrito heme Fe—O—N═O by His64 Nε-H (E) to form the nitrous heme Fe—O(H)—N═O species [16]. In addition, it was reported that the high spin heme Fe—O—N═O species is converted to a low-spin heme Fe—O—N═O/2-nitrovinyl species that can be reversibly switch between a low- and a high-spin state without removing the bound nitrite [17]. On the other hand, the heme Fe—O—N═O species is fully reversible to metMb after removal of the excess $NO_2^-$ [17]. In all of the abovementioned events, the role of the protein in controlling the dynamics of ligand binding and in determining the ligand association with the 2-vinyl in Mb, but not with the 4-vinyl as it has been reported to occur in HRP and soybean legHb [14,15], have not been adequately explored. Neither the experiments nor the calculations are at present sufficient to address these issues. Accurate simulations to complement structure sensitive detailed experiments are necessary for understanding the structure-function relationship. Here, we report the computational simulations that provide this missing information and supplement the experimental picture with additional data to give further insight into the role of protein conformational changes on the protein activity.

The Mb—$NO_2$ structure with the $NO_2^-$ binding sites is shown in Fig. 1. A full elucidation of the $NO_2^-$ binding sites is important for understanding in addition to the mechanism of ligand binding to the heme Fe and at the 2-vinyl site the role of helices E and F in the ligand binding discrimination sites. In this work, we report the resonance Raman (RR) characterization of an acidic transition for the heme Fe—O1—N═O2/2-nitrovinyl species and Molecular Dynamics (MD) simulations for the Mb-nitrite complexes, which demonstrate that the removal or coordination of $NO_2^-$ to the 2-vinyl is associated with the motion of helix F and a correlated motion of helices E-F, whereas the removal or coordination of $NO_2^-$ to heme iron is mainly associated with the motion of helix E. This motion of the E-F helices can be due to the interaction of Val68 and Ile107 with the 2-vinyl/2-nitrovinyl moiety upon $NO_2^-$ coordination changes.

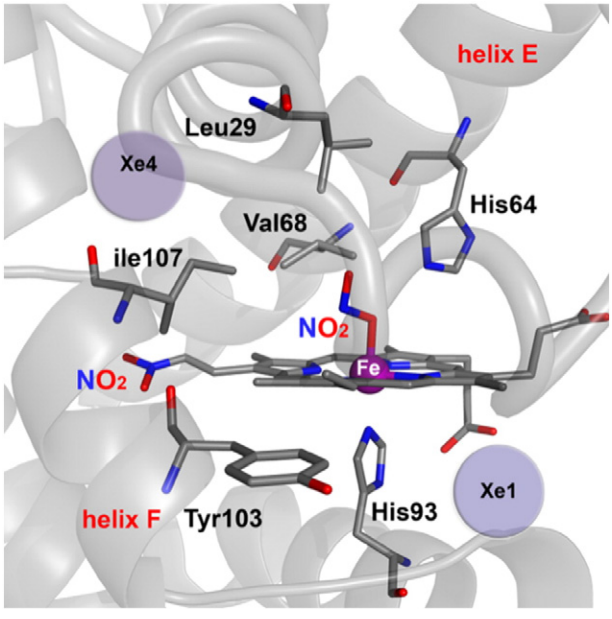


**Fig. 1.** The Mb active site cavity depicting the interactions of 2-nitrovinyl species with two residues Val68 and Ile107 that could also affect the helix E/F motion.

## 2. Materials and methods

### 2.1. Materials and experimental methods

Myoglobin from equine skeletal muscle (lyophilized powder, 95–100%) and all chemicals were purchased from Sigma Aldrich. The stock solutions of metMb were prepared by dissolving lyophilized Mb in 100 mM of sodium phosphate, pH 7.5 and pH 6.0. The Mb-nitrite adducts were prepared by mixing metMb with sodium nitrite to final concentrations of 50 μM for Mb and 25 mM for nitrite. The isotopically labeled $Na^{15}NO_2$ (98% $^{15}N$, 95% CP) and $Na^{15}N^{18}O_2$ (98% $^{15}N$, 90% $^{18}O$, 95% CP) were also obtained from Sigma Aldrich.

Resonance Raman spectra were acquired at room temperature using a 640 mm focal length Czerny-Turner spectrograph (Horiba, T64000 system operated in single stage), equipped with 1800 g/mm holographic grating and a Horiba Symphony BIUV1024 × 256 CCD detector. Samples were placed in a quartz spinning tube to minimize local heating, and scattering was collected in a 90° geometry. The slit width was set to 100 μm. An Ondax SureLock LM-405 laser with an integrated CleanLine ASE filter was used to provide the excitation wavelength at 405 nm, and a Semrock StopLine 405 nm single-notch filter was used to reject Rayleigh scattering. The power incident on the sample was 4 mW and total accumulation time for each spectrum was 20–40 min. The Raman shifts were calibrated using toluene. Origin software was used for spectra processing and analysis. Optical absorption spectra