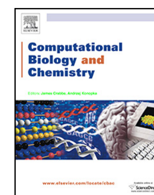
Computational Biology and Chemistry 52 (2014) 60–65

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem



Research article

Computational insight into nitration of human myoglobin

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ARTICLE INFO

Article history:
Received 10 May 2014
Received in revised form 8 September 2014
Accepted 13 September 2014
Available online 18 September 2014

Keywords:
Heme proteins
Nitration
MD simulation
Heme active site
Internal cavity

ABSTRACT

Protein nitration is an important post-translational modification regulating protein structure and function, especially for heme proteins. Myoglobin (Mb) is an ideal protein model for investigating the structure and function relationship of heme proteins. With limited structural information available for nitrated heme proteins from experiments, we herein performed a molecular dynamics study of human Mb with successive nitration of Tyr103, Tyr146, Trp7 and Trp14. We made a detailed comparison of protein motions, intramolecular contacts and internal cavities of nitrated Mbs with that of native Mb. It showed that although nitration of both Tyr103 and Tyr146 slightly alters the local conformation of heme active site, further nitration of both Trp7 and Trp14 shifts helix A apart from the rest of protein, which results in altered internal cavities and forms a water channel, representing an initial stage of Mb unfolding. The computational study provides an insight into the nitration of heme proteins at an atomic level, which is valuable for understanding the structure and function relationship of heme proteins in non-native states by nitration.

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1. Introduction

Protein nitration is one of post-translational modifications (PTMs), which is derived from the reaction of Tyr/Trp residues in proteins, including heme proteins such as myoglobin (Mb) (Bourassa et al., 2001; Witting et al., 2001; Herold et al., 2003; Herold and Shicashankar, 2003; Herold, 2004; Nicolis et al., 2006; Gómez-Mingot et al., 2013), hemoglobin (Hb) (Xiang et al., 2013), cytochrome *c* (cyt *c*) (Batthyány et al., 2005; Rodríguez-Roldán et al., 2008; García-Heredia et al., 2010), and others (Smith et al., 1992; CrySavvides et al., 2002; Quint et al., 2006; Yamakura et al., 2003; Suzuki et al., 2004), with reactive nitrogen species (RNS), such as peroxynitrite ($ONOO^-$). Compared to protein modifications by reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet -}$), less attention has been attracted to RNS (Yamakura and Ikeda, 2006). Meanwhile, RNS such as peroxynitrite can be formed by coupling of superoxide with nitric oxide radial ($NO^\bullet$), an important signaling molecule *in vivo* (Darleyusmar et al., 1992). Peroxynitrite can also be generated in cells when exposure to radiation from X-rays as presented in our daily lives, such as computed tomography (CT) scans in hospital (Brenner and Hall, 2007). Increased levels of nitrated tyrosine were observed in the mouse hippocampus within two hours of radiation exposure (8 Gy) compared to controls (Fukuda et al., 2004).

Moreover, protein nitration was found to occur in presence of nitrite (NO_2^-) and H_2O_2, especially for heme proteins (Bourassa et al., 2001; Witting et al., 2001; Herold et al., 2003; Herold and Shicashankar, 2003; Herold, 2004; Nicolis et al., 2006; Gómez-Mingot et al., 2013; Xiang et al., 2013; Batthyány et al., 2005; Rodríguez-Roldán et al., 2008; García-Heredia et al., 2010). This is of biological relevance since bacteria on human tongue surface may reduce dietary nitrate (2–3 mM daily) to nitrite (Lundberg et al., 2004), and H_2O_2 is a common ROS generated *in vivo*. Heme proteins play diverse functions in biological systems including oxygen delivery, electron transfer, catalysis and signaling, and their structure and function relationships are well-studied (Lu et al., 2003, 2009; Mayfield et al., 2011; Coelho et al., 2013; Spiro et al., 2013; Lin et al., 2013, 2014; Lin and Wang, 2013; Liu et al., 2014; Poulos, 2014). However, to the best of our knowledge, there is still no 3D structural information available for nitrated heme proteins, although nitration of Mb has been observed as early as in 2001 (Bourassa et al., 2001). Crystal structures for other nitrated proteins are also limited (Smith et al., 1992; CrySavvides et al., 2002; Quint et al., 2006). This is likely due to the difficulty in purification of nitrated proteins from unmodified ones with slight structural differences. The structure and function relationship of nitrated heme proteins remains poorly understood. Recently,

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http://dx.doi.org/10.1016/j.compbiolchem.2014.09.004
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Iniesta and co-workers (Gómez-Mingot et al., 2013) selectively nitrated horse Mb at Tyr103 using an electrochemical approach and performed spectroscopic studies on not pure nitrated Mb, which provides some undirected structural information for nitrated Mb.

As a compliment, computational approach such as molecular dynamics (MD) simulation has become a powerful tool to provide atomic level and time-dependent information for protein structure and function that otherwise is difficult to obtain from experiments (Karplus and McCammon, 2002; Daggett, 2006; Sotomayor and Schulten, 2007; Kandt and Monticelli, 2010; Lin, 2011; Lin and Liao, 2011; Lin et al., 2012a). Mb is an ideal protein model for investigating the structure and function relationship of heme proteins in both native and non-native states (Lu et al., 2003, 2009; Lin et al., 2013, 2014; Lin and Wang, 2013; Liu et al., 2014), and computer simulation plays a key role in guiding protein design and engineering based on Mb (Sigman et al., 2000; Yeung et al., 2009; Lin et al., 2012b). To provide structural insights into nitrated heme proteins, we herein performed MD simulation studies on human Mb with successive nitration of Tyr residues (Tyr103 and Tyr146) and Trp residues (Trp7 and Trp14), at 3- and 6-postions, *i.e.*, 3-nitrotyrosine and 6-nitrotryptophan, respectively (Fig. 1), which are the major nitrated products based on experimental observations (Bourassa et al., 2001; Witting et al., 2001; Herold et al., 2003; Herold and Shicashankar, 2003; Herold, 2004; Nicolis et al., 2006; Gómez-Mingot et al., 2013; Xiang et al., 2013; Batthyány et al., 2005; Rodríguez-Roldán et al., 2008; García-Heredia et al., 2010).

2. Methods

The initial structure of human Mb was taken from the X-ray structure of its double mutant, K45R/C110A Mb (PDB entry 3RGK (Hubbard et al., 1990)), since no structure of wild-type protein was available yet. Topology files for 3-nitrotyrosine (NIY) and 6-nitrotryptophan (NIW) were created according to report by Myung and Han (Myung and Han, 2010). Based on the accessibility of Tyr and Trp residues by nitration in Mb (Bourassa et al., 2001; Witting et al., 2001; Herold et al., 2003; Herold and Shicashankar, 2003; Herold, 2004; Nicolis et al., 2006; Gómez-Mingot et al., 2013), the initial structures of Mb with successive nitration of Tyr103, Tyr103/Tyr146, Tyr103/Tyr146/Trp7 and Tyr103/Tyr146/Trp7/Trp14, termed NIY_1-Mb, NIY_2-Mb, NIY_2/NIW_1-Mb and NIY_2/NIW_2-Mb, respectively, were generated with program VMD 1.9 (Visual Molecular Dynamics) (Humphrey et al., 1996). The psfgen plug-in was used to add hydrogen atoms and assign charges to nitrated Mbs according to pH 7.0. Both heme distal His64 and proximal His93 were modeled with hydrogen at the δ-position, and other histidines in Mb were protonated at the ε-nitrogen, *i.e.*, HSD and HSE in topology file, respectively. The protein was then solvated in a cubic box of TIP3 water, which extended 10 Å away from any given protein atom. Counter ions (Na^+ and Cl^-) were further added to the nitrated Mb–water systems to obtain the physiological ionic strength of 0.15 M by using the autoionize plug-in of VMD 1.9.

The resultant system was first minimized for 50,000 steps with conjugate gradient method, and subsequently equilibrated for 100,000 ps, then further minimized for 10,000 steps. The resultant configuration was subjected to MD simulation with program NAMD 2.7 (Nanoscale Molecular Dynamics) (Kalé et al., 1999) at 300 K for 20 ns by employing the classical force filed CHARMM27 (MacKerell et al., 1998). The force field parameters of 3-nitrotyrosine and 6-nitrotryptophan were developed by Myung and Han (Myung and Han, 2010). The Langevin dynamics was used for constant temperature control, with a value of the Langevin coupling coefficient setting to 5 ps. The simulations were performed with an integration time step of 1 fs. Atom-based cutoff of 14 Å with switching at 12 Å was used for non-bonded van der Waals interactions. The trajectory data was saved every 5000 steps (corresponding to 5 ps), generating 4000 structures over 20 ns of simulation for analysis. Control MD simulation was performed for native Mb under the same conditions.

Visualization and data analysis, including backbone C_α root mean square deviation (RMSD), residue RMSD and accessible surface area ($Å^2$), were done with VMD 1.9. Average structure of nitrated Mb was obtained from the last 5 ns simulation, which was further submitted to CASTp (Computed Atlas of Surface Topography of proteins) server (http://cast.engr.uic.edu) (Dundas et al., 2006) to identify internal cavities with a default probe radius of 1.4 Å. The protein pocket was visualized by program PyMOL 0.99rc6 (DeLano, 2002).

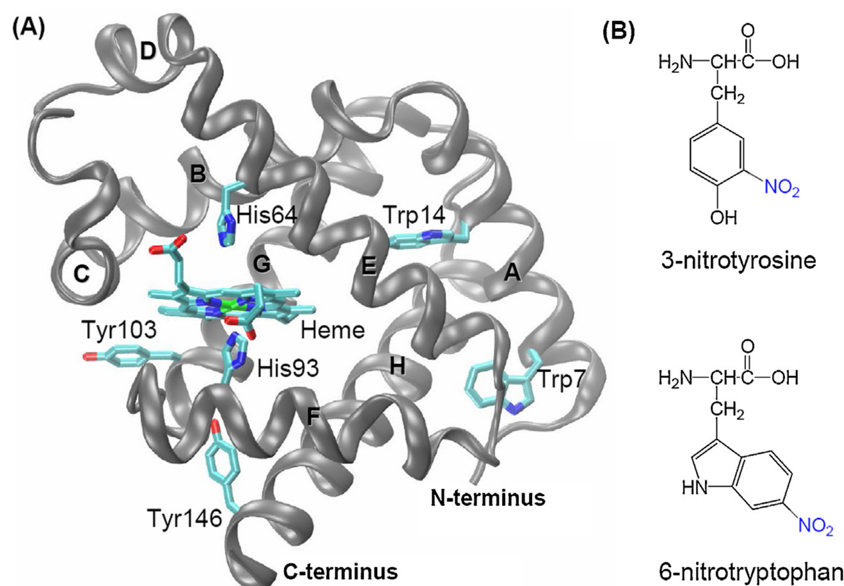

Fig. 1. (A) Crystal structure of human K45R/C110A Mb mutant (PDB entry 3RGK) showing the heme site and locations of Tyr103/Tyr146 and Trp7/Trp14. The polypeptide chain with eight α-helices is labeled as A–H; (B) chemical structure of 3-nitrotyrosine (NIY) and 6-nitrotryptophan (NIW).

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