Biochimie 140 (2017) 82–92

Contents lists available at ScienceDirect

Biochimie

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

Research paper

Gaseous ligand selectivity of the H-NOX sensor protein from *Shewanella oneidensis* and comparison to those of other bacterial H-NOXs and soluble guanylyl cyclase

Gang Wu [a, *], Wen Liu [b], Vladimir Berka [a], Ah-lim Tsai [a, **]

[a] Division of Hematology, Department of Internal Medicine, The University of Texas – McGovern Medical School, 6431 Fannin Street, Houston, TX, 77030, USA

[b] Center for Infectious and Inflammatory Diseases, Texas A&M University - Health Science Center, 2121 West Holcombe Blvd, Houston, TX, 77030, USA

ARTICLE INFO

Article history:
Received 3 April 2017
Accepted 23 June 2017
Available online 26 June 2017

Keywords:
H-NOX
Gaseous ligand selectivity
Soluble guanylyl cyclase
Sliding scale rule

ABSTRACT

To delineate the commonalities and differences in gaseous ligand discrimination among the heme-based sensors with Heme Nitric oxide/OXygen binding protein (H-NOX) scaffold, the binding kinetic parameters for gaseous ligands NO, CO, and O_2, including K_D, k_{on}, and k_{off}, of *Shewanella oneidensis* H-NOX (*So* H-NOX) were characterized in detail in this study and compared to those of previously characterized H-NOXs from *Clostridium botulinum* (*Cb* H-NOX), *Nostoc* sp. (*Ns* H-NOX), *Thermoanaerobacter tengcongensis* (*Tt* H-NOX), *Vibrio cholera* (*Vc* H-NOX), and human soluble guanylyl cyclase (sGC), an H-NOX analogue. The K_D(NO) and K_D(CO) of each bacterial H-NOX or sGC follow the "sliding scale rule"; the affinities of the bacterial H-NOXs for NO and CO vary in a small range but stronger than those of sGC by at least two orders of magnitude. On the other hand, each bacterial H-NOX exhibits different characters in the stability of its 6c NO complex, reactivity with secondary NO, stability of oxyferrous heme and autoxidation to ferric heme. A facile access channel for gaseous ligands is also identified, implying that ligand access has only minimal effect on gaseous ligand selectivity of H-NOXs or sGC. This comparative study of the binding parameters of the bacterial H-NOXs and sGC provides a basis to guide future new structural and functional studies of each specific heme sensor with the H-NOX protein fold.

1. Introduction

Diatomic gases nitric oxide (NO), carbon monoxide (CO), and O_2 play messenger roles under many physiological and pathological conditions [1,2]. Living organisms have evolved different systems to respond to the environmental changes of these gaseous ligands [1,3]. Heme sensor proteins are the most important components of these gaseous messenger-sensing systems [1,4]. Heme Nitric oxide/OXygen binding proteins (H-NOXs) are one of the six major groups of heme sensor proteins whose bindings with the gaseous messengers cause changes in the downstream effector proteins, evoking various responses [1,5].

All the bacterial H-NOXs show strong affinities for NO and CO [6], but only some H-NOXs exhibit strong affinities for O_2. Whether an H-NOX binds O_2 under atmospheric pressure correlates with its biological origin from either a facultative or obligate anaerobe [1,4]. H-NOXs from facultative anaerobes are encoded in the bacterial genomes as stand-alone proteins and in the same operons with either putative histidine kinases or diguanylate cyclases [4]. These H-NOXs usually do not show any affinity for O_2 under atmospheric pressure, exemplified by the ones found in proteobacteria *Vibrio cholera* (*Vc* H-NOX) and cyanobacteria *Nostoc* sp. PCC7120 (*Ns* H-NOX) [7–10]. On the other hand, H-NOXs found in obligate anaerobes are domains of methyl-accepting chemotaxis proteins (MCP) and often bind O_2 to form an oxyferrous complex, such as the ones

Abbreviations: H-NOX, heme nitric oxide and oxygen binding protein; *Cb* H-NOX, H-NOX from *Clostridium botulinum*; Lb, leghemoglobin; *Ns* H-NOX, H-NOX from *Nostoc* sp. PCC7120; *So* H-NOX, H-NOX from *Shewanella oneidensis*; *Tt* H-NOX, H-NOX from *Thermoanaerobacter tengcongensis*; *Vc* H-NOX, H-NOX from *Vibrio cholera*; sGC, human soluble guanylyl cyclase; 5c NO-heme, five coordinate NO complex with NO ligand on the distal side of heme; 5c heme-NO, five coordinate NO complex with NO ligand on the proximal side of heme.

* Corresponding author.
** Corresponding author.
E-mail addresses: gang.wu@uth.tmc.edu (G. Wu), ah-lim.tsai@uth.tmc.edu (A.-l. Tsai).

http://dx.doi.org/10.1016/j.biochi.2017.06.014
0300-9084/

from firmicutes *Clostridium botulinum* (*Cb* H-NOX) and thermophilic *Thermoanaerobacter tengcongensis* (*Tt* H-NOX, renamed *Caldanaerobacter subterraneus* H-NOX recently) [7,11–13].

In animals, the heme domain in the β subunit [β1(1–194)] of soluble guanylyl cyclase (sGC), a heterodimer that consists of α and β subunits, shows significant sequence homology with the bacterial H-NOXs (Fig. S1). The fact that the heme proximal ligand His105, heme interacting triad Tyr135/Ser137/Arg139, Pro118 that buttresses the heme, and four glycines, Gly3/Gly18/Gly71/Gly148 (all numbers are based on sGC sequence) are 100% conserved in the six heme sensors indicates that they are structural homologs. The bacterial H-NOXs have therefore been used as the model systems to understand the gaseous ligand selectivity of sGC [4,14–16]. On the other hand, the overall sequence identity among these six heme sensors is less than 35%, which may reflect their different biological functions. Whether the primary sequence divergences contribute to the gaseous ligand selectivity of H-NOXs and sGC has not been systematically evaluated.

sGC is the only known NO receptor in animals and its enzymatic activity that converts GTP to cGMP increases several hundred-fold above basal level upon binding to NO, a critical mediator for many physiological processes [15–17]. Although an analogue of the bacterial H-NOXs, sGC exhibits significantly weaker affinities for CO and NO than the formers, nonetheless, it is capable of selectively binding NO with complete exclusion of O_2 under atmospheric pressure [6,15–17].

In previous studies, we characterized the binding kinetics for the gaseous ligands of sGC, *Cb*, *Ns*, *Tt*, and *Vc* H-NOXs [6,9–11,18]. These studies bring attention to the similarities but more importantly the differences between the gaseous ligand selectivity of the bacterial H-NOXs and sGC. To better reveal the similarities and differences of the heme sensor proteins with H-NOX fold in bacteria and animals, in this study, we first characterized the binding kinetics of another bacterial H-NOX isolated from facultative anaerobe *Shewanella oneidensis* (*So* H-NOX), with NO, CO, and O_2, and then compared in detail the binding parameters of sGC, *Cb*, *Ns*, *So*, *Tt,* and *Vc* H-NOXs. This comprehensive comparative study reveals that the gaseous ligand bindings of each of these heme sensor proteins obeys the "sliding scale rule"; on the other hand, each heme sensor protein exhibits its own characteristic gaseous ligand selectivity, including the extent of oxygen binding/autoxidation and the efficiency of multiple-step NO-binding.

2. Material and methods

2.1. Materials

CO and NO gases were from Matheson-TriGas Inc. (Houston, TX) and NO was pre-purified by passing through a NaOH trap [9]. Sodium hydrosulfite ($Na_2S_2O_4$), ferricyanide, imidazole, heme, δ-aminolevulinic acid, isopropyl-1-thio-β-D-galactopyranoside (IPTG), ampicillin, chloramphenicol, and egg lysozyme were from Sigma (St. Louis, MO). Restriction enzymes were from New England BioLabs (Beverly, MA). Vector pET43.1a and *E. coli* strain Rosetta 2(DE3)pLysS were from Novagen (Madison, WI). TALON Co^{2+} affinity resin was purchased from BD Biosciences Clontech (Palo Alto, CA). The 10DG desalting column and DC protein assay kit were from Bio-Rad Laboratories (Hercules, CA).

2.2. Expression and purification of So H-NOX

The gene encoding *So* H-NOX was synthesized with codon optimization for *E. coli* expression and codons for a six-histidine tag were inserted upstream of the stop codon. The cDNA was first cloned into pBSK vector (Epoch LifeScience, Houston, TX) followed by *Nde*I and *Xho*I digestion and subcloning into pET43.1a vector. The integrity of the resulting plasmid was then confirmed at Lone Star Labs (Houston, TX).

So H-NOX was expressed and purified following the procedure described previously [9,11]. Briefly, Rosetta2(DE3)pLysS strain of *E. coli* was transformed with the expression plasmid and grown overnight at 37 °C in TB medium with chloramphenicol (45 μg/mL) and ampicillin (150 μg/mL). TB medium with ampicillin was inoculated with the overnight culture and shaken at 37 °C until A_{610} reached ~0.8. The temperature was then lowered to 20 °C, and the expression was induced with 1 mM IPTG in the presence of 2 μM heme and 0.2 mM δ-aminolevulinic acid. The cells were harvested 48 h after induction.

The cells were lyzed in 100 mM potassium phosphate (pH 7.5) containing 100 mM NaCl, 10% glycerol, and 1.2 mg/mL egg lysozyme. The supernatant after centrifugation was loaded onto Co^{2+} affinity resin, and *So* H-NOX was eluted with 250 mM imidazole, which was subsequently removed using a 10DG column. The amount of protein was determined with a DC protein assay kit using bovine serum albumin as the standard. The Soret extinction coefficient of *So* H-NOX was determined by the pyridine hemochrome assay [19]. Ferric *So* H-NOX was prepared by oxidizing the resting protein with ferricyanide followed by a cleanup with a 10DG column.

2.3. UV–Vis and magnetic circular dichroism (MCD) spectroscopy

The UV–Vis and MCD spectra of *So* H-NOX were recorded with a Hewlett-Packard 8452A diode-array spectrophotometer (Agilent Technologies, Santa Clara, CA) and a Jasco J-815 CD spectropolarimeter (Tokyo, Japan) with an Olis permanent magnet (Bogart, GA), respectively. The field strength of the magnet was calibrated as described before [9]. MCD expressed in molar delta absorption coefficient, ΔA, in units of $M^{-1}cm^{-1}tesla^{-1}$, was obtained with a bandwidth of 5 nm, averaged from 4 repetitive scans, and calculated using the spectral analysis software coming with the instrument [9]. The CO complex of *So* H-NOX was prepared by flushing ferrous *So* H-NOX with CO gas in an anaerobic cuvette sealed with an air-tight septum. The Soret wavelength of the CO complex observed with spectrophotometer and resolved from rapid-scan data exhibited a difference of 2 nm, which was due to the different spectral resolutions of these two instruments and the spectral data deconvolution process.

2.4. Stopped-flow measurements

The binding kinetics of NO, CO, and O_2 to ferrous *So* H-NOX were studied at room temperature under anaerobic conditions with SX-18MV stopped-flow apparatus (Applied Photophysics, Leatherhead, UK) as described previously [9,11]. The dead time of the instrument is 1.5 ms. To make sure that *So* H-NOX started with a homogenous 5c ferrous heme in the kinetic characterizations of gaseous ligand binding, purified *So* H-NOX was first oxidized to its ferric state to release any bound ligand, cleaned up using a 10DG column, and subsequently reduced to its ferrous state by $Na_2S_2O_4$ titration. Only residual amount of unreacted $Na_2S_2O_4$ was present in the final samples [9]. NO binds to *So* H-NOX at a rate faster by several orders of magnitude than it reacts with $Na_2S_2O_4$ [20], any residual $Na_2S_2O_4$ left in the sample did not affect the kinetic measurement of NO binding to *So* H-NOX. The rapid-scan spectral changes were monitored with a diode-array accessory, and the data were analyzed using Pro-Kineticist global analysis package (Applied Photophysics). The time courses were followed with a monochromator set at different wavelengths, and the observed rates, k_{obs}, were obtained by fitting to standard exponential function [11].