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Nitric oxide activation and reduction by heme–copper oxidoreductases and nitric oxide reductase

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Abstract

The understanding of the dynamics and conformational control involved in the interplay between structure and function of nitric oxide reductase (Nor) and heme–copper oxidoreductases in their function to convert nitric oxide (NO) to nitrous oxide (N_2O) is of fundamental importance in bioenergetics. We have applied resonance Raman spectroscopy to investigate the NO ligation/deligation reactions and the extent of communication between the metal centers at the heme a_3–Cu_B site of heme–copper oxidases and of the heme Fe–non-heme Fe in Nor. The present study provides information of the electronic and vibrational structure of intermediates, and thus, it forms the basis for an atomic-level description of the key steps in the N–N bond formation and the N–O bond cleavage mechanism. The present experiments provide evidence as to the validity of the proposed hypothesis of the common evolutionary origin of aerobic respiration and bacterial denitrification.

Keywords: Nitric oxide; Nitric oxide reductase; Heme–copper oxidoreductase

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1. Introduction

Catalytic production of laughing gas (N_2O) requires the formation of the N–N bond and the subsequent cleavage of the N–OH bond. The reaction is used by bacteria as an alternative to oxygen-based respiration [1–5]. The bacterial Nitric oxide reductase (Nor), the heme–copper *ba*$_3$ and *caa*$_3$ oxidoreductases from *Thermus thermophilus* and the *cbb*$_3$ oxidoreductase from *Pseudomonas stutzeri* catalyze the reduction of NO to N_2O [1,5,6-9]. The mechanism of the reduction of NO to N_2O by either Nor or heme–copper oxidoreductases is poorly understood because of the lack of detection of the shorted-lived intermediate species [10]. However, a six-coordinate heme Fe(III)–NO species and a five-coordinate heme Fe(II)–NO have been characterized in NOR and a six-coordinate heme Fe(II)–NO species in *ba*$_3$-oxidoreductase [11–13]. In addition, density functional theory (DFT) calculations have provided an atomic-level description of the key steps in the N–N bond formation and the N–O bond cleavage mechanism [14,15]. The optimized molecular structures of the heme Fe–Cu_B bound hyponitrite intermediate computed by the B3LYP/6-31G* theory level is shown in Fig. 1. Recently, the resonance Raman (RR) data revealed a surprising and unprecedented coordination of the formed hyponitrite species that provides insight into the catalytic mechanism: $2NO + 2e^- + 2H^+ \rightarrow N_2O + H_2O$. The initial binding of two NO molecules to the heme a_3 Fe–Cu_B binuclear center is followed by protonation of the heme a_3–NO species and concomitant formation of the N–N bond [16].

Nitric oxide reductase. The bacterial nitric oxide reductase complex (Nor) is a membrane bound cytochrome *bc* complex composed of two subunits, NorC and NorB which catalyzes the reduction of NO to N_2O [1–5]. The complex contains four known redox centers: three heme groups and one non-heme Fe atom [1–5]. Heme *c* (six-coordinate, low-spin) is bound to NorC subunit and functions as the

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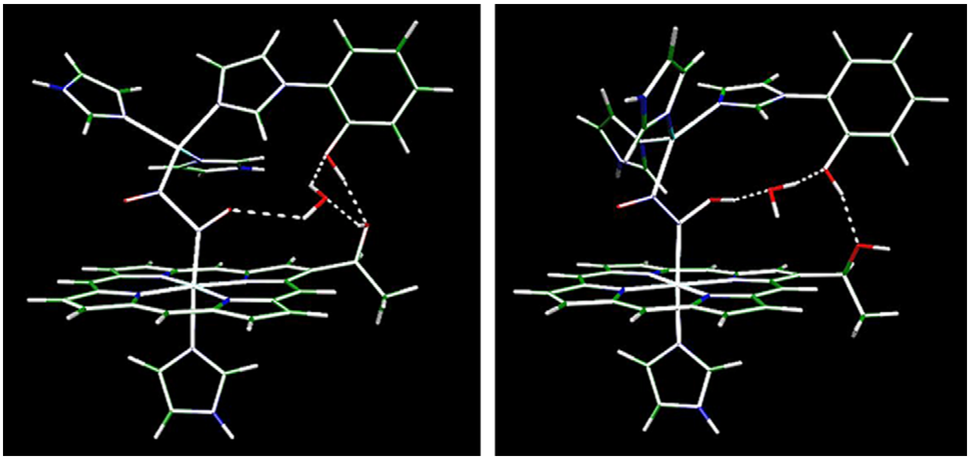

Fig. 1. Optimized molecular structures of the heme a_3 Fe–Cu_B bound-hyponitrite intermediate computed by the B3LYP/6-31G* theory level in the protonated (right panel) and deprotonated (left panel) form.

electron entry site of the enzyme. NorB contains one six-coordinate, low-spin heme *b* and a five-coordinate, high-spin heme b_3, which, together with a non-heme iron atom form the dinuclear, NO reduction site [4,5]. The sequence of NorB contains all six histidines which are the ligands of heme *a*, heme a_3 and Cu_B in cytochrome *c* oxidase [1–5]. Resonance Raman (RR) and time-resolved RR have revealed the properties of the dinuclear center of oxidized, reduced, CO- and NO-bound Nor from *Paracoccus denitrificans* [11,17,18]. The spectra of the oxidized enzyme showed two distinct ν_{as}(Fe–O–Fe) modes at 813 and 833 cm^{-1} that have been attributed to two different conformations (*open* and *closed*) of the catalytic site of the enzyme [11].

Heme–copper oxidoreductases. The thermophilic Gram-negative eubacterium *T. thermophilus* HB8 (ATCC27634) expresses cytochromes caa_3 and ba_3 that serve as terminal oxidases for reducing oxygen to water [9]. Both enzymes, in contrast to the eucaryotic cytochrome aa_3 (CcO), also catalyze the reduction of nitric oxide to nitrous oxide under anaerobic conditions [8]. These findings support the hypothesis of a common evolutionary origin of bacterial denitrification and aerobic respiration. Cytochrome ba_3 from the thermophilic eubacterium *T. thermophilus* HB8 (ATCC27634) is expressed under limited O_2 supply and is composed of three subunits. The largest subunit (I) contains the ligands to a low-spin heme *b* and a binuclear center that consists of Cu_B and heme a_3 and subunit II contains a mixed-valence [$Cu_A{}^{1.5+}$–$Cu_A{}^{1.5+}$] homodinuclear copper complex. Subunit IIa forms just one transmembrane helix. Based on the crystal structure the binuclear center of cytochrome ba_3 is similar to those of the aa_3 type oxidases from *P. denitrificans* and bovine heart [9]. In the oxidized form, heme a_3 is high-spin, five-coordinate and Cu_B is ligated with three histidines, His290, His291, and His240 (If not stated otherwise, we adopt the residue numbering of the bovine heart enzyme). Tyrosine 244 is covalently linked to His240. The A-type heme in cytochrome ba_3 contains a hydrophobic hydroxyethylgeranylgeranyl group which is straight and reaches the cytoplasmic side, without interfering with the proton pathways, instead of a hydroxyethylfarnesyl chain as seen in most bacterial and eucaryotic aa_3 oxidases. Resonance Raman and time-resolved step-scan FTIR spectroscopies have been successfully applied to study the ligand dynamics and the structural and functional relationships of ba_3 and caa_3 oxidoreductases [19–28]. In this report, the reaction of oxidized Nor and ba_3 with NO will be reviewed. Comparison of ba_3 to Nor may provide the means to identify conserved structural features, which can be assumed to be involved in basic functions common to both classes of enzymes. In contrast, dissimilarities between these enzymes are likely to be involved in the fine-tuning to specific needs demanded by differences in the local biological environments of the enzymes.

2. Results and discussion

2.1. Nitric oxide reductase (Nor)

The optical absorption spectrum of oxidized enzyme (trace A in Fig. 2) displays maxima at 411, 530 and 558 nm, which are indicative of low-spin hemes- *b* and *c*, and high spin heme b_3. The shoulder at 595 nm is typical of the porphyrin-to-ferric charge transfer (CT) transition characteristic of ferric high-spin heme b_3. The spectrum of the NO-bound oxidized enzyme (trace B in Fig. 2) was obtained by the direct addition of gaseous NO to the resting enzyme and shows a Soret maximum at 416 nm and the visible transition at 562 nm along with the disappearance of the 595 nm band. For the reduced enzyme (trace C in Fig. 2) the Soret is at 420 nm and the visible transitions are at 521, 551, and 558 nm.

The high-frequency RR spectra are used to assess the spin and ligation states of the hemes via the porphyrin marker band frequencies, and for the resting, deoxy, NO-bound and the photoproduct form of heme b_3, the status of the proximal and distal environments. The modes of the oxidized enzyme (Fig. 3 trace A) at 1373 (ν_4) 1492