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The superfamily of heme–copper oxygen reductases: Types and evolutionary considerations ☆

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ABSTRACT

Heme–copper oxygen reductases (HCO) reduce O_2 to water being the last enzymatic complexes of most aerobic respiratory chains. These enzymes promote energy conservation coupling the catalytic reaction to charge separation and charge translocation across the prokaryotic cytoplasmatic or mitochondrial membrane. In this way they contribute to the establishment and maintenance of the transmembrane difference of electrochemical potential, which is vital for solute/nutrient cell import, synthesis of ATP and motility. The HCO enzymes most probably share with the nitric oxide reductases, NORs, a common ancestor. We have proposed the classification of HCOs into three different types, A, B and C; based on the constituents of their proton channels (Pereira, Santana and Teixeira (2001) Biochim Biophys Acta, 1505, 185–208). This classification was recently challenged by the suggestion of other different types of HCOs. Using an enlarged sampling we performed an exhaustive bioinformatic reanalysis of HCOs family. Our results strengthened our previously proposed classification and showed no need for the existence of more divisions. Now, we analyze the taxonomic distribution of HCOs and NORs and the congruence of their sequence trees with the 16S rRNA tree. We observed that HCOs are widely distributed in the two prokaryotic domains and that the different types of enzymes are not confined to a specific taxonomic group or environmental niche. This article is part of a Special Issue entitled: Respiratory Oxidases.

1. Introduction

Heme–copper oxygen reductases (HCOs) are transmembrane enzymes characterized by the presence, in subunit I, of a low-spin heme and a binuclear center, which is the catalytic site. This center is formed by a high-spin heme and a copper ion (Cu_B), and a tyrosine residue (catalytic tyrosine Tyr-I), covalently linked to one of the histidine residues binding the copper ion. Only subunit I is common to all enzymes. A second subunit, generally named subunit II, can be present, which may have no cofactor (as in quinol oxidases) or a binuclear copper average valence center (Cu_A) (Fig. 1). In addition, the latter may also contain a C-terminal extension with one or two type C heme binding domains. The *cbb*$_3$ enzymes contain also extra subunits, having one and two C type hemes, respectively (Fig. 1C). Interestingly, these cytochrome domains are related to that present at the C-terminal part of Cu_A containing enzymes [1,2]. HCOs catalyze the four electron reduction of dioxygen to water in a process coupled to proton translocation across the membrane. Because electrons and protons needed for the reaction come from opposite sides of the membrane, the enzymes also promote the formation of a difference of electrochemical potential by charge separation. Thus, since protons are chemical and pumped substrates for all these enzymes, intramolecular proton conducting pathways should be present in subunit I. Based on amino acid sequence alignments, site directed mutations and on crystallographic structures, two proton channels (D- and K-channels) were identified for mitochondrial and mitochondrial-like enzymes, and named according to key residues of those channels. However, a detailed analysis of the amino acid sequences and structural information for other HCOs revealed important differences, in particular in the proton channels [3]: not only the residues lining those channels are not conserved but, most importantly, some enzymes do not have ionizable residues in between the cytoplasmatic surface of the enzyme and the binuclear site [4,5]. These observations imply an important role of water molecules in proton conduction as well as recommend a special care when proposing general catalytic mechanisms for proton pumping and its coupling to the chemical reaction. Based on the fingerprint of these proton conducting channels we proposed a division of HCOs into three types: type A (further divided into A1 and A2), B and C HCOs [3].

Nitric oxide reductases (NORs) catalyze the two electron reduction of NO to water and N_2O, do not pump protons across the membrane and do not promote charge separation (protons and electrons

Abbreviations: HCO, heme–copper oxygen reductase; NOR, nitric oxide reductase; LTG, lateral gene transfer; Gya, giga years ago

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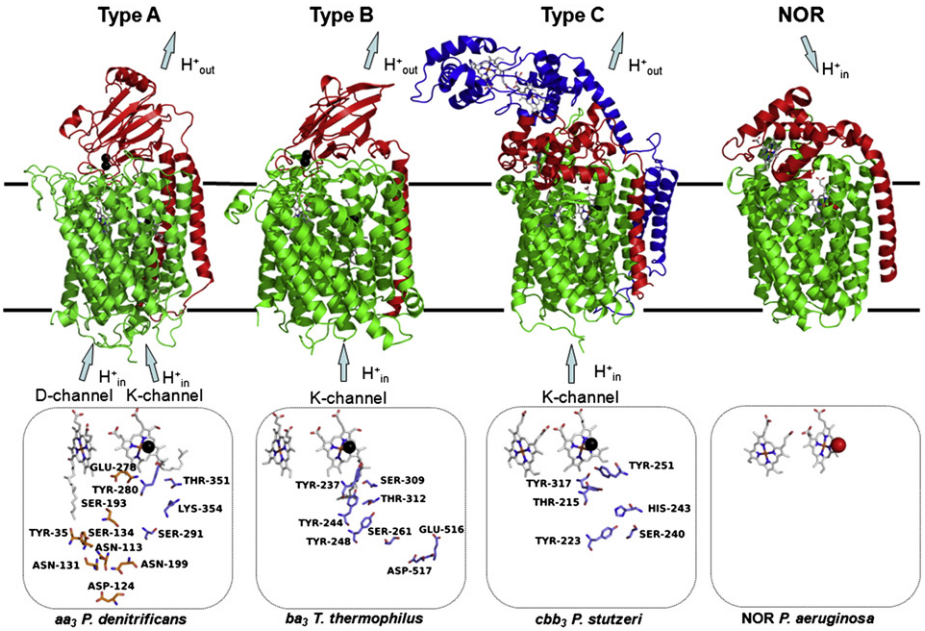

Fig. 1. Top—Crystallographic structures of heme–copper oxygen reductases and nitric oxide reductase. A representative structure is presented for each type of HCO. The catalytic subunit is shown in green, additional subunits are presented in red and blue. Copper ions are represented as black spheres and hemes are shown as sticks (orange). NORs are structurally similar to HCOs, but contain an iron atom (red sphere) in the catalytic subunit instead of a copper, and do not couple NO reduction to proton pumping. Bottom—Representation of the prosthetic groups and of the amino acid residues lining the proton channels from the different types of HCO and NORs. Structures were obtained in www.pdb.org.

come from the same side of the membrane); therefore they do not perform energy conservation [6,7]. Despite the functional and structural differences, NORs have been proposed to be evolutionary related to HCOs [7–10]. The catalytic subunit of NORs shares with that of HCOs the same general structural core, the presence of the low-spin heme, and of a binuclear center formed by a high-spin heme and an iron ion, instead of Cu_B [7]. The catalytic tyrosine residue is absent in NORs what may be considered to be a distinctive marker between HCOs and NORs. Also, no proton channels are observed in the cytoplasmatic side of the enzyme, in contrast to HCOs [7].

In this article we first review our recent expanded reanalysis of the HCO classification [9]. This comprehensive bioinformatics study strengthened our previously proposed classification [3,9]. Furthermore we analyze the taxonomic distribution of HCOs and NORs and the congruence of their sequence trees with the 16S rRNA tree. We observed that HCOs are widely distributed in the two prokaryotic domains and that the different types of enzymes are not confined to a specific taxonomic group or environmental niche. This classification of enzymes is important to establish in a systematic manner not only the natural diversity of HCOs but, most importantly, to probe for the general relevance of the so far proposed catalytic mechanisms. Moreover, the underlying analysis may allow identifying a higher number of "natural variants" which will be optimal candidates for future functional and structural studies.

2. Materials and methods

2.1. Taxonomic profile

We determined the phylogenetic profile of amino acid sequences from subunit I using completely sequenced genomes from 361 bacterial and 15 archaeal species present in the Superfamily database [9,11], and the HCO classification tool described before [9]. All retrieved sequences were mapped on NCBI Taxonomy using the BioSQL package from April 2009 available for download at ftp://ftp.ncbi.nih.gov/. The representativeness of a specific enzyme within a taxonomic group was determined based on a normalization in which the number of species with completely sequenced genomes having HCO or NOR sequences was divided by the total number of species of the same order with a completely sequenced genome available at Superfamily [12].

2.2. Phylogenetic analysis

Amino acid sequences from the HCO database (see [9] for details) were grouped according to each type of enzyme, A, B or C [9]. This analysis does not require completely sequenced genomes and the HCO database allows the most comprehensive taxonomic distribution. Multiple amino acid sequence alignments were performed using ClustalX 1.83 [13]. Default parameters were used, as no significant differences were observed with different parameter combinations. Protein weight matrix Gonnet, with Gap Opening 10 and Gap Extension 0.2 was used for multiple alignments that were manually refined in GeneDoc v.2.7.0 [14]. This manual adjustment is essential to assure an alignment that takes into consideration functional key amino acid residues, such as the metal ligands and the catalytic tyrosine residues, as well as parts of the proton channels already identified in the different enzymes. Neighbor joining (NJ) trees of each HCO type were constructed using the manually adjusted alignments with the following parameters: 10000 bootstraps, 1000 seeds and correction for multiple substitutions. One sequence of a different type of HCO or of a NOR enzyme was included to root each tree. Maximum likelihood (ML) trees were constructed using the PHYLIP package [15] with the following parameters: Jones–Taylor–Thornton probability model, 100 bootstraps and 13 seeds. In each tree, sequences of one HCO type or NOR were selected in order to have at least 3 representatives from each taxonomic class and one sequence of a different HCO or NOR type. The trees were visualized with Dendroscope v2.4 [16]. Groups were defined taking into account the first divergence of the rooted NJ tree.

3. Results

3.1. Classification

Based on the observation that all HCOs reduce oxygen to water and that those so far studied, pump protons and have the same general structural fold of the catalytic subunit (12–14 transmembrane