can be coupled to energy-requiring cellular processes. The nitric oxide reductase (NOR) members of the superfamily catalyze the reduction of nitric oxide (NO) to nitrous oxide (N₂O) in microbes capable of either canonical or nitrifier denitrification.

We used comparative genomics, coupled with structural and biochemical analyses, to characterize the diversity of the hemecopper oxidoreductase superfamily. A number of previously unidentified oxygen and nitric oxide reductase families were discovered. Many of these new families have a wide environmental distribution and may play important roles in nitrogen cycling and aerobic respiration in low O₂ environments. Evolutionary analysis of the superfamily suggests that aerobic respiration originated after the evolution of oxygenic photosynthesis. In most currently known phyla early branching groups are anaerobic, implying that at the time of the superfamily suggests that aerobic respiration originated after the evolution of oxygenic photosynthesis. In most currently known phyla early branching groups are anaerobic, implying that at the time of the major diversification of microbial life aerobic respiration had not yet evolved. It is also shown that nitric oxide reductase activity evolved many times independently, from various oxygen reductase members within the superfamily, suggesting that canonical denitrification evolved after aerobic respiration. The different oxygen reductase and nitric oxide reductase families vary in their substrate (O₂ and NO) apparent binding affinities and energy conservation efficiencies (H⁺/e⁻ translocation ratio). These differences, which are due to structural variations within the proteins, have great ecological importance.

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The catalase activity of the aa₃ cytochrome c oxidase from Paracoccus denitrificans

Florian Hilbers¹, Jessica van Wonderen², Wolfram Lorenzen³, Helge B. Bode³, Fraser MacMillan², Bernd Ludwig⁴, Hartmut Michel¹

¹Molecular Membrane Biology, Max Planck Institute of Biophysics, D-60438 Frankfurt/Main, Germany
²Henry Wellcome Unit for Biological EPR, School of Chemistry, University of East Anglia, Norwich NR4 7TJ, UK
³Merck-Endowed Chair in Molecular Biotechnology, Institute of Molecular Biosciences, Johann Wolfgang Goethe-University, D-60438 Frankfurt/Main, Germany
⁴Molecular Genetics, Johann Wolfgang Goethe-University, D-60438 Frankfurt/Main, Germany
E-mail: florian.hilbers@biophysics.mpg.de

The four subunit (SU) aa₃ cytochrome c oxidase (CcO) from Paracoccus denitrificans is one of the terminal enzymes of the respiratory chain. It uses electrons from cytochrome c to reduce O₂ to H₂O. Its binuclear active center, residing in SU I, contains heme a₃ and Cu₅₅₃, the latter being ligated by three histidine residues. Apart from its oxygen reductase activity, CcO also possesses peroxidase and catalase activities.

To compare variants and the wild type (WT) protein in a more stringent way, a recombinant (rec.) WT CcO was created, carrying the gene for SU I on a low copy number plasmid. This rec. WT showed, as expected, no difference in oxygen reductase activity compared to the American Type Culture Collection (ATCC) WT CcO but, astonishingly, its catalase activity was increased by a factor of 20.

The slight overproduction of SU I due to plasmid coding and the resulting deficiency in metal inserting chaperones impairs the correct insertion of heme a₃ and Cu₅₅₃. This could lead to subtle differences in side chain orientation and changes in the water network. However, these slight changes might cause an increased accessibility of the active center for H₂O₂, resulting in an increased catalase activity. The availability of chaperones was improved by cloning the genes for the two metal inserting chaperones CtaG and SurfC on the same plasmid together with the SU I gene. This new rec. WT CcO in fact showed a reduced catalase activity. To fathom the structural difference resulting in increased catalase activity capillary DSC was used. No significant difference in thermal stability between the ATCC WT CcO and the rec. WT CcO was detected. However, upon aging, the thermal stability of the rec. WT CcO was reduced faster than that of the ATCC WT CcO pointing to a decreased structural stability of the rec. WT CcO.

Several known inhibitors were used to probe the contribution of the metal cofactors in the catalase reaction. In addition, variants in aromatic amino acid positions near the active center were constructed to assess a possible reaction mechanism of the catalase activity of CcO. Moreover, GC-coupled MS-measurements were used to analyze the oxygen produced in the catalase reaction. As a result of these experiments, a reaction cycle of the catalase activity of CcO is postulated and the structural difference between the ATCC and rec. WT CcO is outlined. The catalase activity appears to be a true catalase activity and not a “pseudocatalase” activity.

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Heterologous production and purification of the cytochrome bd ubiquinol oxidase of Aquifex aeolicus in Escherichia coli

J. Hoeser, M. Vranas, T. Friedrich
Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79104 Freiburg, Germany
E-mail: jo.hoeser@gmail.com

The cytochrome bd ubiquinol oxidase is one of the terminal oxidases of the respiratory chain of many prokaryotes. The enzyme is a heterodimeric membrane protein expressed under microaerophilic conditions and has a high affinity towards oxygen. It catalyzes the reduction of dioxygen to water while oxidizing ubiquinol to ubiquinone. The redox reaction is coupled with a vectorial translocation of four protons across the membrane, thus, contributing to a proton motive force crucial for ATP production. The cytochrome bd ubiquinol oxidase is expected to cope with oxidative stress and as it is only expressed in bacteria, including a number of human pathogens, it may be an attractive drug target.

The cytochrome bd ubiquinol oxidase from various microorganisms has been studied by secondary structure predictions and orientation of subunits. Its cofactors were characterized and the role of possible proton channels was examined [1]. However, a crystal structure of the enzyme complex at high resolution is not yet known. To fill this gap, the stable cytochrome bd ubiquinol oxidase from Aquifex aeolicus, a hyperthermophilic organism with an optimal growth temperature of 85 °C, is used as a model. The A. aeolicus cytochrome bd ubiquinol oxidase was heterologously produced in Escherichia coli, purified by chromatographic steps and the heme-cofactors were spectroscopically characterized. However, the yield of the purification is still low. To facilitate protein purification, a green fluorescent protein combined with a hexahistidine-tag is fused to the terminal oxidase. The protein is produced in an E. coli strain depleted of the cytochrome bd ubiquinol oxidase genes [2].

References