Earlier data has shown that the E122, E125, E198 and E267 are essential for activity, but not assembly, whereas the E202 is not essential for activity. We present single turn-over data (on fully reduced NOR and oxygen) on alanine mutants of all five conserved glutamates. Our data show that, except the E202, they are all crucial for the oxidative phase of the reaction which is limited by proton uptake to the active site. Together with a model of the NorB, we propose that the E122 and E125 sit at the entrance of the proton pathway which also contains E267 and E198, but not the E202.

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S11.32 Single-turnover of *ba*$_3$ oxidase from *Thermus thermophilus*

Ilya Belevich[a], Sergey A. Siletsky[b], Audrius Jasaitis[c], Alexander A. Konstantinov[b], Mårten Wikström[a], Tewfik Soulimane[d], Michael I. Verkhovsky[a]
[a]*HBG, Institute of Biotechnology, University of Helsinki, Helsinki, Finland*
[b]*Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia*
[c]*European Laboratory for Non-Linear Spectroscopy, University of Florence, Italy*
[d]*Materials and Surface Science Institute, University of Limerick, Ireland*
E-mail: ilya.belevich@helsinki.fi

Cytochrome *ba*$_3$ from *Thermus thermophilus* belongs to the large family of structurally related heme-copper terminal oxidases. It catalyses the process of oxygen reduction to water and couples it with creation of an electrochemical transmembrane gradient of protons, which is subsequently used for ATP synthesis. The kinetics of the oxidation of fully-reduced *ba*$_3$ oxidase by oxygen were followed by time-resolved optical spectroscopy and electrometry. Four catalytic intermediates were resolved during this reaction. The chemical nature and the spectral properties of three intermediates (A, P, O) reproduce the general features of *aa*$_3$-type oxidases. However the F intermediate in *ba*$_3$ oxidase has a spectrum identical to the P state. This indicates that the proton taken up during the P→F transition does not reside in the binuclear site but is rather transferred to the covalently cross-linked tyrosine near that site. The total charge translocation associated with the F→O transition in *ba*$_3$ oxidase is close to that observed during the F→O transition in the *aa*$_3$ oxidases. However, P_R→F is characterized by significantly lower charge translocation, which probably reflects the overall lower measured pumping efficiency during multiple turnovers.

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S11.33 Characterization of the membrane-bound tri-heme *c* quinol peroxidase functionally connected to the respiratory chain

Eizo Takashima, Hiroyuki Yamada, Konishi Kiyoshi
Department of Microbiology, School of Life Dentistry at Tokyo, Nippon Dental University, Japan
E-mail: eizo.takashima@gmail.com

Recently, we discovered quinol peroxidase (QPO) activity, the reduction of hydrogen peroxide by ubiquinol-1 as an electron donor, from the membrane fraction of the bacteria *Aggregatibacter actinomycetemcomitans* that is closely related to *Haemophilus* and has been associated with localized aggressive periodontitis. The aim of this study is to biochemically characterize QPO. QPO was purified to >90% purity from the membrane fraction. Using the N-terminal amino acid sequence of the QPO, we identified the *qpo* gene. The amino acid sequence of QPO shared 46~54% sequence identity with gene homologues in *Escherichia coli*, *Bacteroides fragilis*, etc. QPO also has a high sequence homology to bacterial di-heme cytochrome *c* peroxidase (BCCP), but QPO did not catalyze peroxidation in the presence of horse heart cytochrome *c*. MALDI-TOF MS analysis showed that QPO is a 53.6-kDa protein that contains 3 heme *c* molecules. The Km value for ubiquinol-1 was 107 μM and the optimum pH was 7.5. The Kcat value was 582 s^{-1}, comparable to that of *Paracoccus pantotrophus* BCCP. Moreover, the membrane fraction of *A. actinomycetemcomitans* had an apparent QPO-dependent peroxidase activity in the presence of NADH and succinate. Based on these findings, we present a new mechanism for the scavenging of reactive oxygen species in which quinol in the respiratory chain is consumed.

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S11.34 The semiquinone at the Q_H site of the cytochrome *bo*$_3$ from *Escherichia coli*

Lai Lai Yap[a], Rimma I. Samoilova[d], Myat T. Lin[b], Robert B. Gennis[a], Sergei A. Dikanov[c]
[a]*Department of Biochemistry, University of Illinois, USA*
[b]*Department of Biophysics, University of Illinois, USA*
[c]*Department of Veterinary Clinical Medicine, University of Illinois, USA*
[d]*Institute of Chemical Kinetics and Combustion RAS, Novosibirk, Russia*
E-mail: dikanov@uiuc.edu

The aim of this study was advanced pulsed EPR characterization of the semiquinone (SQ) in the high-affinity Q_H-site of the cytochrome *bo*$_3$ ubiquinol oxidase. Our studies have shown that a SQ at the Q_H site is a *neutral* species in the wild-type protein, with two strong H-bonds to Asp-75 and either Arg-71 or Gln-101. Selective ^{15}N labeling of the side chain nitrogens was performed to distinguish between these two residues. Pulsed EPR studies have been extended to two mutants at the Q_H site. The D75E mutation has little influence on the catalytic activity, and the pattern of H-bonding is similar to the wild type. In contrast, the D75H mutant is virtually inactive. Pulsed EPR revealed significant structural changes in this mutant. The H-bond to Arg-71 or Gln-101 that is present in both the wild type and D75E mutant oxidases is missing in the D75H mutant. Instead, the D75H has a single, strong H-bond to a histidine, likely His-75. The D75H mutant stabilizes an *anionic* semiquinone as a result of the altered H-bond network. Either the redistribution of charge density in the semiquinone species, or the altered H-bonding network may be responsible for the loss of catalytic function.

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S11.35 Resolution of a novel catalytic intermediate in cytochrome *bd* terminal oxidase in real time: A true peroxy species?

Vitaliy B. Borisov[a], Ilya Belevich[b], Michael I. Verkhovsky[b]
[a]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia*
[b]*Helsinki Bioenergetics Group, Institute of Biotechnology, University of Helsinki, Helsinki, Finland*
E-mail: bor@genebee.msu.su

Cytochrome *bd* is a terminal quinol oxidase of bacterial respiratory chains containing three hemes: b_{558}, b_{595} and *d*. Transient formation of catalytic intermediates in reaction of cytochrome *bd* terminal oxidases from *Escherichia coli* and *Azotobacter vinelandii* with oxygen was monitored by microsecond-resolved absorption spectroscopy and electrometry. Initial binding of O_2 by three-electron-reduced enzyme is followed by conversion of oxy-complex (A) to previously unresolved oxygen species,