remains a subject of intense debate. We have performed structure-based calculations of the activation barriers for the initial coupled ET/PT steps in CcO [1]. The calculations have, for the first time, reproduced the barriers that account for the directionality and sequence of events in the primary PT in CcO. We have also addressed the effect of the conformational change of Glu286 and the role of bridging water molecules. (2) Nitric oxide reductase (NOR) of denitrifying bacteria belongs to the superfamily of heme-copper oxidases and, due to high structural similarities to CcO, is generally believed to be the evolutionary ancestor of cytochrome oxidases. However, in contrast to CcO, there were no crystal structures of NOR available until recently, and previous analyses of PT in NOR were based on a homology-built model [5]. We will report preliminary results of our simulations of PT in NOR, which are based on the recently solved crystal structure [6]. This includes both the large-scale MD simulations that were performed to identify specific PT pathways leading to the active site and explicit EVB calculations of the barriers for individual PT steps.

References

[1] A.V. Pisliakov, Proc. Natl. Acad. Sci. USA 105 (2008) 7726–7731.
[2] M.H.M. Olsson, A. Warshel, Proc. Natl. Acad. Sci. USA 103 (2006) 6500.
[3] P. Brzezinski, R.B. Gennis, J. Bioenerg. Biomembr. 40 (2008) 521.
[4] V.R. Kaila, M.I. Verkhovsky, G. Hummer, M. Wikström, Proc. Natl. Acad. Sci. USA 105 (2008) 6255.
[5] Flock U et al. (2008) J. Biol. Chem. 283: 3839 To be published.

doi:10.1016/j.bbabio.2010.04.300

11P.20 Cytochrome *c* oxidase activity in mitochondria is regulated by the ATP/ADP ratio and by the phosphorylation pattern of the enzyme

Rabia Ramzan, Bernhard Kadenbach, Sebastian Vogt
Biomedical Research Center, Phillips-University, D-35032 Marburg, Germany
E-mail: rabiaramzan23@yahoo.com

The conditions for measuring the allosteric ATP-inhibition of cytochrome *c* oxidase (CcO) in isolated mitochondria were investigated. The oxygen consumption of mitochondria in the presence of 1% Tween-20 was recorded polarographically with ascorbate as substrate at increasing concentrations of cytochrome *c* in the presence of ADP and of ATP. Only by increasing the ATP/ADP ratio with the ATP-regenerating system phosphoenolpyruvate and pyruvate kinase to high values full ATP-inhibition of CcO could be seen. The extent of allosteric ATP-inhibition was found to vary between different preparations of mitochondria from heart, liver and kidney of rat and bovine. The phosphorylation pattern of CcO was determined by isolating the enzyme complex by Blue Native PAGE and subsequent Western blots with antibodies against phosphoserine, phosphothreonine and phosphotyrosine. The correlations between kinetics of allosteric ATP-inhibition and the phosphorylation patterns are discussed.

doi:10.1016/j.bbabio.2010.04.301

11P.21 NO reduction by heme-copper oxidases

Joachim Reimann, Pia Adelroth
Stockholm University, Department of Biochemistry and Biophysics, Stockholm, Sweden
E-mail: jreimann@dbb.su.se

The mechanism by which certain heme-copper oxidases (HCuO) reduce nitric oxide to nitrous oxide is far from being understood despite increasing efforts. It is not the chemistry alone that is intriguing but the accompanying proton transfer that deserves attention. The nitric oxide reductases (NORs) are predicted to be structurally similar to traditional members of the superfamily of heme-copper oxidases but their proton chemistry is fundamentally different. Early work on NOR from *Paracoccus denitrificans* revealed its non-electrogenic character for both substrates O_2 and NO. Later, it was shown that this is due to the uptake of electrons and protons from the same side of the membrane and the lack of proton pumping. We are trying to understand why the reduction of NO, a reaction as exergonic as the reduction of O_2, is not coupled to endergonic vectorial proton transfer in a system that seems to possess a proton pumping machinery, using proton-pumping members of the HCuO family capable of NO reduction. Our recent studies showed that in the cbb_3-type oxidase from *Rhodobacter sphaeroides*, typical proton pumping was only observed for the reaction with O_2, whereas the reaction with NO resulted in a small membrane potential not big enough to account for proton pumping. Studying the ba_3 oxidase from *Thermus thermophilus* extends our investigation into the B-family with well-described chemical intermediates and a high-resolution protein structure. We are currently characterizing the reaction between the fully reduced ba_3 oxidase and NO, results from such optical flow-flash experiments will be presented.

doi:10.1016/j.bbabio.2010.04.302

11P.22 Potential generation during CO photodissociation from the fully reduced cytochrome *c* oxidase from *Paracoccus denitrificans*

Marko Rintanen, Ilya Belevich, Michael I. Verkhovsky
University of Helsinki, Institute of Biotechnology, Helsinki, Finland
E-mail: marko.rintanen@helsinki.fi

Cytochrome *c* oxidase (CcO) is the terminal oxidase of the electron transfer chain. CcO uses electrons donated by cytochrome *c* from the P-side and protons from the N-side of the inner mitochondrial membrane to reduce molecular oxygen to water and associates the released energy to pumping of four protons per one O_2 reduced. The aim of this study was to elucidate the nature of the 1.5 μs phase of potential generation upon the photodissociation of CO from the fully reduced CcO from *Paracoccus denitrificans*. The 1.5 μs phase is absent in the two electron reduced CO-bound enzyme and emerges only upon additional reduction of two other redox centers: heme *a* and Cu_A. It was found that the amplitude of this phase depends only on enzyme concentration and can be used as an internal ruler for the calibration of the electrogenic events in the enzyme. The obtained data shows that the fast phase is followed with an additional phase which is growing and slowing down with increase of pH. This additional phase emerges when the fully reduced CO bound enzyme is first oxidized by an oxygen pulse and then immediately re-reduced with sodium dithionite. The amplitude of the slow phase is not stable and it fades away with a time constant of about 15–20 min. Comparison of results from mutant enzymes suggests that these events are linked to the proton conducting channel K of CcO.

doi:10.1016/j.bbabio.2010.04.303

11P.23 Interaction of acidic cytochrome *c* with wild and mutant B-type cytochrome oxidases from thermophilic Bacillus

Junshi Sakamoto, Jun-ichi Kishikawa[1], Satoko Hidaka, Hiroshi Okada, Yoshiki Kabashima[2]
Kyushu Institute of Technology, Department of Bioscience and Bioinformatics, Fukuoka, Japan
E-mail: sakamoto@bio.kyutech.ac.jp

Mitochondrial cytochrome *c* oxidases and many bacterial ones are structurally related and grouped as A-type, while many oxidases in extremophiles compose another subgroup and are classified as B-type. We have found a B-type oxidase, cytochrome bo_3, in a transformable Gram-positive thermophile *Geobacillus thermodenitrificans* K1041 and have studied its functional properties using basic cytochrome *c*-551 (pI = 8.46) from a close relative Bacillus PS3 as the substrate [1–3]. Recent whole genome project on *G. thermodenitrificans* revealed that its cytochrome *c*-551 is really quite similar to the PS3 counterpart in amino acid sequence, however, a few residues are substituted, which surprisingly result in a significant shift of the calculated pI value to 4.99. Since most cytochromes *c* ever studied are basic and electrostatic bonds are crucial in their docking to A-type oxidases, it is interesting to see how an acidic substrate interacts with a B-type partner oxidase. Here, we investigated the interaction between this acidic cytochrome *c*-551 and cytochrome bo_3-type oxidase, either the wild-type enzyme or site-directed mutant ones. The gene for the acidic cytochrome *c* was cloned using genomic DNA from *G. thermodenitrificans* as PCR template, ligated to pSTE12 a shuttle vector between thermophilic Bacillus and *E. coli*, and over-expressed in the authentic host cells. The oxidase activity of the wild type enzyme increased as the salt concentration increased and approached maximum around 200 mM. The optimal pH was 5.0, which is much lower than that obtained with using the basic substrate, 6.7. The substrate-binding site of bo_3-type oxidase might be on the hydrophilic CuA-cupredoxin domain, which contains several acidic amino acid residues and the calculated pI value for which is 4.82. Therefore, the total charge of the acidic cytochrome *c* and the CuA-domain are both neutral at their optimal pH, while the basic substrate is positively charged and the CuA-domain is charged to the opposite direction with a similar extent at their own optimum. Several mutants are constructed for bo_3-type oxidase, in which acidic residues are substituted to neutral ones, and the study using them is underway.

References

[1] J. Sakamoto, J. Biochem. 122 (1997) 764–771.
[2] K. Nikaido, et al., Biochim. Biophys. Acta 1456 (2000) 35–44.
[3] Y. Kabashima, et al., J. Biosci. Bioeng. 109 (2010) 325–330.

[1]Present address: Kyoto Sangyo University, Faculty of Life Science, Kyoto, Japan.
[2]Present address: Kyorin University School of Medicine, Department of Chemistry, Tokyo, Japan.

doi:10.1016/j.bbabio.2010.04.304

11P.24 Assessment of Michaelis–Menten parameters by analysis of single time courses of enzyme-catalyzed reactions

Michael Schleeger[1], Joachim Heberle[1], Sergej Kakorin[2]
[1]*Experimental Molecular Biophysics, Department of Physics, Free University of Berlin, Arnimallee 14, D-14195 Berlin, Germany*
[2]*Biophysical Chemistry, Department of Chemistry, Bielefeld University, Universitaetsstrasse 25, D-33615 Bielefeld, Germany*
E-mail: michael.schleeger@fuerlin.de

Michaelis–Menten K_M and turnover k_{cat} constants are usually determined by applying the Michaelis–Menten rate equation to a set of initial reaction rates v_0 of product formation at different starting concentrations of substrate. Our theoretical analysis of the quasi-steady state approximation of the Michaelis–Menten model shows that the values of v_0 are inherently underestimated at their experimental determination. We propose a new method for assessment of K_M and k_{cat} without measuring of v_0. The method is based on an analysis of a single time course of product formation or substrate decay by non-linear regression. The non-linear regression procedure uses an explicit solution of the Michaelis–Menten rate equation in terms of the Lambert-W function [1, 2] with transformed variables. Previous attempts to use the integrated Michaelis–Menten equation to assess K_M and k_{cat} have so far met with very little success, because of bad convergence. We significantly improve the convergence by transformation of coordinate system used for the non-linear regression. Test of the new method in experimental conditions of enzyme kinetics of cytochrome *c* oxidase from *Rhodobacter sphaeroides*, as well as tests with computer-simulated data yield that our single curve method is more precise in assessment of K_M and k_{cat} than conventional linear and nonlinear methods using v_0. We infer from our study, that the single trace method can replace usual time- and sample-consuming assays of enzymatic activity, especially where a rapid and reliable control is required.

References

[1] S. Kakorin, Ber. Bunsenges. Phys. Chem. 102 (1998) 670–675.
[2] S. Schell, et al., J. Theor. Biol. 187 (1997) 207–212.

doi:10.1016/j.bbabio.2010.04.305

11P.25 Heme-copper oxygen reductases superfamily revisited

Filipa L. Sousa[1], Renato J. Alves[2], Miguel Teixeira[1], José B. Pereira-Leal[2], Manuela M. Pereira[1]
[1]*Instituto de Tecnologia Química e Biológica, (EAN), Oeiras, Portugal*
[2]*Instituto Gulbenkian de Ciência, Oeiras, Portugal*
E-mail: fsousa@itqb.unl.pt

Heme-Copper Oxygen reductases (HCOs) are the principal enzymes responsible for reduction of dioxygen in respiratory chains being present in Bacteria, Archaea and mitochondria. Besides catalysing the last reaction of respiratory chains, the reduction of dioxygen to water, HCOs directly contribute to energy conservation first by charge separation (protons and electrons needed for the reaction come from opposite sides of the membrane), and second by proton translocation, part of the energy released during the chemical reaction is used to promote unfavourable proton translocation across the membrane. A primary structural analysis of several HCOs including examples from organisms of different taxonomic groups leads to the identification of distinct patterns for the proton channels. These became the basis for the classification of those enzymes into families, A, B and C, the first, further divided in two, A1 and A2 [1]. Recently, and also based on sequences comparisons, an extension of this classification (family D to H) was proposed, [2]. With the increasing number of available sequences and the new high-throughput methods of sequencing, the number and the taxonomic distribution of known HCO sequences expanded considerable. With this more comprehensive dataset, a re-evaluation of the accepted classification was a natural requirement. We calculated phylogenetic profiles for each of the sub-types of HCOs, and will discuss several evolutionary considerations.

References

[1] M.M. Pereira, M. Santana, M. Teixeira, Biochim Biophys Acta 1505 (2001) 185–208.
[2] J. Hemp, R.B. Gennis, Res. Probl. Cell. Differ. 45 (2008) 1–31.

doi:10.1016/j.bbabio.2010.04.306