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[b]*Mitochondriale physiopathologyie laboratory, INSERM U688, France*
[c]*LABRI, 351, cours de la Libération, F 33405 Talence, France*
E-mail: jpm@u-bordeaux2.fr

A stochastic approach based on Gillespie algorithm is particularly well adapted to describe the time course of the redox reactions that occur inside the respiratory chain complexes because they involve the motion of single electrons between individual unique redox centers of a given complex and not populations of electrons and redox centers as usually considered in ordinary differential equations. In this way we approach the molecular functioning of the bc_1 complex based on its known crystallographic structure and the rate constants of electron tunnelling derived from the Moser and Dutton phenomenological equation. The main features of our simulations are the dominant and robust emergence of a Q-cycle mechanism and the near absence of short-circuits in the normal functioning of the bc_1 complex. Thus, in our paper, the Mitchell Q-cycle no longer appears as an *a priori* hypothesis but arises out of the bc_1 complex structure and of the kinetic laws of redox reactions.

doi:10.1016/j.bbabio.2008.05.402

(S15) *bc*₁ complexes symposium abstracts (poster and raised abstracts)

S15.5 Construction of a bacterial *bc*₁ complex hetero dimer

Thomas Kleinschroth, Oliver Anderka, Bernd Ludwig
Institute of Biochemistry, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany
E-mail: T.Kleinschroth@em.uni-frankfurt.de

The bc_1 complex is a homodimer. When the first structures were published it was an unexpected result: both monomers are intertwined by their ISP subunits: The head domain of the ISP anchored in monomer A belongs to the Qo center of monomer B, and *vice versa*. These findings raise the question whether the two monomers are also functionally interacting, as indicated by recent results. To answer this experimentally two operons were constructed to express two different monomers: one is wild type, the other carries an inactivating mutation in the cytochrome *b*. Statistically, assembly will result in 50% heterodimeric complexes. These are detected and purified by different tags. We assume that the activity of such a complex is 50% of wild type if there is no cooperativity between monomers. In case of an interaction, a lower activity should result from kinetics. We chose two different tags, Strep-tag II and His-tag, which were cloned and tested in different positions in the *b* subunit of the complex. These were checked for activity, spectral properties, resin binding and assembly. We were able to introduce an inactivating mutation in the cytochrome *b* that does not disturb subunit assembly or spectral properties. Both operons were stably introduced in a *P. denitrificans* bc_1 complex deletion strain and co-expression of both complexes were verified by their respective tags.

doi:10.1016/j.bbabio.2008.05.403

S15.6 The unprecedented peroxidase-like activity by nitrophorin-2, the no carrying heme protein from *Rhodnius prolixus*

Rahul Singh[a], Hongjun Zhang[b], Robert E. Berry[b], Ann Walker[b], Anabella Ivancich[a]
[a]*NRS URA 2096 and iBiTec-S, Service de Bioénérgétique (SB2SM), CEA Saclay, 91191, Gif-sur-Yvette, France*
[b]*University of Arizona, Department of Chemistry, Tucson, Arizona, 85721-0041, USA*
E-mail: rahul.singh@cea.fr

We have characterized the NO carrier protein Nitrophorin-2 (NP2) and its variants from *Rhodnius prolixus* for their reaction with H_2O_2 and peroxyacetic acid (PAA). The enzyme demonstrated substantial peroxidase activity with a pH optimum of 6.8 using ABTS and o-dianisidine. The K_m for ABTS (500 μM) is comparable to that reported for some of the catalase-peroxidases (KatGs). The K_m for H_2O_2 (1.1 mM) was much higher than K_m for PAA (32 μM) but comparable to the values reported for some KatGs. Tyr38Ala variant showed lower peroxidase activity but with very high K_m values for both H_2O_2 and PAA. The stopped flow analysis of the wild type and the variants was consistent with the formation of Compound I ([Fe(IV)=O Por.$^+$]) but with different rates. The 9 GHz-EPR spectra showed the formation of two different [Fe(IV)=O Por.$^+$] species, one weakly ferromagnetically coupled signal (typical of peroxidases) at basic pH, and a novel strongly ferromagnetically coupled signal at neutral pH, exclusively observed in model heme complexes. Moreover, we also identified an [FeIV= O Tyr.] species formed by intra-molecular electron transfer. Characterization of NP2 variants indicated Tyr85 being the site for the protein radical. Our results suggest that NP2 can perform not only the "heme-edge" oxidation but can also use alternative protein-based radical intermediates as shown in the case of KatGs.

doi:10.1016/j.bbabio.2008.05.404

S15.7 Estimation of the lifetime of the complex between cytochrome *c* and cytochrome *bc*₁ using electron paramagnetic resonance

Marcin Sarewicz[a], Arkadiusz Borek[a], Fevzi Daldal[b], Wojciech Froncisz[a], Artur Osyczka[a,*]
[a]*Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland*
[b]*Department of Biology, Plant Science Institute, University of Pennsylvania, Philadelphia, USA*
E-mail: osyczkaa@biotka.mol.uj.edu.pl

Electron transfer (ET) between cytochrome *c* and cytochrome bc_1 is an integral part of several energy-conserving systems, including mitochondrial respiration. We investigate the molecular association of those two proteins using ET-independent electron paramagnetic resonance techniques (EPR). We employed site-directed spin labeling to modify bacterial and mitochondrial cytochromes *c* at several surface-exposed positions. Continuous wave EPR spectra and saturation recoveries of those forms recorded in the absence and presence of cytochrome bc_1 demonstrated that EPR detects a binding of cytochrome *c* to cytochrome bc_1. The bound cytochrome *c* fraction successively decreases as the ionic strength increases with a limit of approximately 120 mM NaCl above which essentially no bound cytochrome *c* can be detected by EPR. This dynamic equilibrium between bound and free cytochrome *c* exposed by EPR allowed us to estimate that the average lifetime of the tightly-bound complex decreases from over 100 μs at low ionic strength to less than 400 ns at the physiological ionic strength. This strongly supports an early idea of diffusion-coupled reactions that link the soluble electron carriers with the membranous complexes, which, we believe, provide robust means to regulate electron flow through these complexes. *AO is The Wellcome Trust International Senior Research Fellow.

doi:10.1016/j.bbabio.2008.05.405