Cytochrome c oxidase (CcO) is the terminal enzyme in the electron transfer chain in both mitochondria and bacteria. CcO reduces oxygen to water and harnesses the energy to pump protons across the inner membrane of mitochondria in eukaryotic cells or the plasma membrane in aerobic bacteria. Despite extensive studies, the mechanism by which the redox reaction is coupled to proton translocation remains unresolved owing to the difficulty of visualizing proton movement within the massive protein matrix. Three pathways, H-, D-, or K-channels have been identified in the enzyme. There is strong evidence that in the bacterial isoforms of CcO, the proton translocation occurs via the D-channel, which terminates near the heme α3 binuclear center. In contrast, studies on mammalian CcO support a different proton pumping pathway, the H-channel, which passes by heme α, instead of the binuclear center [1].

Recently, we reported that the vibrational modes of each of the propionate groups of heme α and heme α3 can be resolved in the resonant Raman spectrum and are solvent isotope sensitive [2]. Thus, they serve as indicators of hydrogen/deuterium (H/D) exchange in the H-bonding network and offer a new method to follow proton migration through the enzyme. The application of this H/D exchange Raman spectroscopic method to studies of the proton pumping mechanism in bovine CcO revealed a redox-controlled proton gate near the heme α moiety in the middle of the H-channel, where protons can be stored prior to being pumped out to the p-side of the membrane. When heme α is reduced, the gate closes; upon oxidation of heme α, the gate opens facilitating proton uptake from the negative side of the membrane. Based on these novel findings, a new model for the molecular basis of proton translocation along the H-channel has been formulated by which the electron transfer from heme α to heme α3, associated with the oxygen reduction chemistry, is coupled to unidirectional proton translocation.

References

doi:10.1016/j.bbabio.2012.06.384

20P5

The Paramagnetic and Redox Properties of the Hemes of E. coli Nitrate Reductase

Justin G. Fedor, Richard A. Rothery, Joel H. Weiner
Department of Biochemistry, Faculty of Medicine & Dentistry, University of Alberta, 4-043 Katz Group – Rexall, T6G 2H7, Edmonton, Canada E-mail: jgfedor@ualberta.ca

Escherichia coli nitrate reductase A (NarGHI) is an excellent model for studying electron transfer through membrane-bound b-type cytochromes. NarGHI serves to scaffold an electron transfer relay composed of a bis-molybdopterin cofactor, five Fe/S clusters and two b-type hemes, where quinol oxidation is coupled to nitrate reduction [1]. NarGH is anchored to the cytoplasmic face of the membrane by NarI, which coordinates two distinct EPR visible b-type hemes and is the site for quinol oxidation. Traditionally, multiple components and non-ideal electron stoichiometry have been used to fit EPR redox titration spectra of the NarGHI hemes and the Narl-proximal [3Fe-4S] cluster (FS4) of NarH. The alternative, and less arbitrary interpretation, is the existence of anti-cooperative interactions between the two hemes and FS4 of NarGHI.

Application of a three-center (FS4, heme bo, heme bp) redox model to this sub-system gives satisfactory fits for quinone-site mutants and quinone-like inhibitor bound states, but the three-center model does not fully account for redox titration line-shape of the wild-type enzyme. However, by extending the model to include the quinone redox transitions, the fit is improved considerably. This follows as Narl is a quinone binding protein with multiple redox centers. The inevitable consequence of multiple redox active centers being close in proximity and in midpoint potential is anti-cooperative redox interactions, which is what our analysis confirms. By considering multiple cofactors simultaneously and in the context of an interacting system, midpoint potentials differ from previously reported values result, as is indeed the case for NarGHI. Moreover, the magnitude of the interaction potentials suggests a non-inverse squared distance relationship, which implies non-electrostatic effects (oxidation state-dependant conformational changes) may also be present in the system, as has been suggested for a number of proteins. Conformational coupling of the two hemes, for instance, is quite likely due to the fact that they are < 6 Å from each other and coordinated axially by two common α-helices [1]. The research presented discusses the redox characteristics of the hemes and [3Fe-4S] cluster of NarGHI in the context of redox cooperativity, and the paramagnetic properties of the two hemes.

Reference

doi:10.1016/j.bbabio.2012.06.385

20P6

Dehydration affects the stability of primary charge separation in bacterial reaction centers: Studies by optical and differential FTIR spectroscopy

F. Francia1, A. Mezzetti2,3, M. Malferri1, G. Venturioli1
1Dipartimento di Biologia, Università di Bologna, 40126 Bologna, Italy
2Service de Bioénergétique, Biologie Structurale et Mécanismes, URA CNRS 2096, IBITeC-S, DSV, CEA-Saclay, 91191 Gif-sur-Yvette cedex, France
3LASIR UMR 8516, Université Lille 1, Cité Scientifique, 59655 Villeneuve d’Ascq, France
E-mail: francesco.francia@unibo.it

The photosynthetic reaction center (RC) of Rb. sphaeroides catalyzes light-induced electron transfer events which are connected to the conformational dynamics of the protein. The light-induced charge separation between the primary donor (P) and the quinone acceptor (Qa) is stabilized by solvent/protein conformational rearrangements. After a laser pulse P+Qa recombination, which occurs with a lifetime t ~100 ms in room temperature solutions, is accelerated (t ~20 ms) at cryogenic temperatures [1] and in dehydrated glassy matrices at room temperature [2]. After prolonged photoexcitation, a slow phase of recombination (t ~250 s) is observed, attributed to additional conformational changes [3]. Differential FTIR bands of water associated with the Qa/Qr transition have been observed upon continuous illumination, leading to propose that weakly bound water molecules plays a role in P+Qa stabilization [4]. By controlling the hydration level of RC-detergent films, through equilibration at given relative humidities (r), a strong inhibition of the P+Qa conformational stabilization has been observed at low hydration [5]. We
compared FTIR light-minus-dark (P\textsuperscript{-}Q\textsubscript{A}/PQ\textsubscript{A}) differential spectra in hydrated (r = 76\%) and dehydrated (r = 11\%) RC films over the 4000-1000 cm\textsuperscript{-1} range. The spectra differ significantly in the 3750-3550 cm\textsuperscript{-1} range, the band attributed to weakly hydrogen bonded water molecules [5] being strongly reduced in the dried film. Dehydration also affects the 1800-1200 cm\textsuperscript{-1} range, which includes contributions from P, the quinones and the peptide. Optical absorption measurements performed under the same photoexcitation regime reveal a slow (t \approx 5 s) kinetic component of P\textsuperscript{-}Q\textsubscript{A} recombination which disappears in the dehydrated sample, indicating at low r a destabilization of the charge separated state. As a whole the data suggest a correlation between the hydration shell dynamics and the conformational RC dynamics which stabilize the charge separated state.

References


doi:10.1016/j.bbabio.2012.06.386

20P7

Molecular basis for semiquinone stabilization in respiratory enzymes: a pulsed EPR study of the menasemiquinone binding mode in E. coli nitrate reductase A

Stephane Grimaldi, Rodrigo Arias-Cartin, Sevdalina Lyubenova, Pascal Lanciano, Burkhard Endeward, Thomas Prisner, Axel Magalon, Bruno Guigliarelli

Institut de Microbiologie de la Méditerranée, Unité de Bioénergétique et Ingénierie des Protéines (UMR 7281) & Laboratoire de Chimie Bactérienne (UMR7283), CNRS, 13009 Marseille, France; Aix-Marseille Univ, 13009 Marseille, France; Institut fuer Physikalische und Theoretische Chemie, JW Goethe Universitaet, 60438 Frankfurt, Germany

E-mail: grimaldi@imm.cnrs.fr

Quinone binding sites (or Q sites) in respiratory complexes are the primary places for the production of reactive oxygen species that occurs as side reactions during the catalytic cycle and leads to cellular oxidative stress. This is mainly due to the transient formation of the highly reactive semiquinone species at these sites during electron transfer processes. Indeed, semiquinone stabilization is an obligate step during these processes since quinols/quinones are two-electron redox components while the proximal metal centers within respiratory complexes are one-electron transfer systems. The stabilization degree of a protein-bound semiquinone can differ by several orders of magnitude depending on the enzyme, and its importance for the function of the enzyme remains to be established. Moreover, the molecular determinants that drive this stability remain to be elucidated.

Due to the very high stability of the menasemiquinone bound to its quinol oxidation site (QD), E. coli nitrate reductase A (NarGH) is a prime model for investigating the relationship between semiquinone binding mode and stabilization. Indeed, we have previously shown that this radical exhibits the highest stability measured so far for a quinone-utilizing respiratory enzyme [1]. Taking advantage from this peculiar property, the radical was used as a magnetic probe of its immediate environment. The detection of weak magnetic couplings between the unpaired electron and neighboring nuclei provided unprecedented information on the menasemiquinone binding mode [2, 3]. Combining multifrequency high-resolution pulsed EPR methods and H\textsubscript{2}O/D\textsubscript{2}O exchange experiments, several hydrogen atoms were unambiguously detected in the vicinity of the radical. They were assigned to specific chemical groups from either the quinone itself or from a single H-bond having unusual characteristics. Taken together, these results indicate a peculiar binding mode of the menasemiquinone at the NarGH QD site which we consider to strongly contribute to its unusual redox properties.

References


doi:10.1016/j.bbabio.2012.06.387

20P8

Computational investigation of the electronic structure of the Cu\textsubscript{A} site in bovine cytochrome c oxidases: the functional role of the axial methionine residue

J. Kang, M. Tateno
Graduate School of Life Science, University of Hyogo, 3-2-1 Kouto, Kamigohri, Akoh, Hyogo 678-1297, Japan
E-mail: jiyoungkang01@gmail.com

Cytochrome c oxidase (CcO), the terminal enzyme of the electron transport system, reduces an oxygen molecule, and thereby generates the gradient of the proton concentration between the matrix and the intermembrane space of mitochondria or the periplasmic space of bacteria. In the Cu\textsubscript{A} site of CcO, two Cu ions form a covalent bond, and receive electrons from cytochrome c, thereby providing the electrons with heme a. The previous experiments revealed that the substitutions of the axial Met ligand, which coordinates to a Cu ion, with leucine largely induced the changes of the redox potentials in various species. For example, for R. sphaeroides CcO, the redox potential of the M207L mutant increases by 118 mV (large) from that of wild type. In contrast, for T. thermophilus ba\textsubscript{3} oxidase, the redox potential of the mutant increases by 53 mV (middle), and for the engineered azurin, which is a reconstructed azurin possessing a similar Cu-coordination in the Cu\textsubscript{A} site, the redox potential of the mutant increases by 16 mV (small). Thus, since the effects of the Met residue on the mutants are likely to be different among the species, the functional roles of the Met residue are still ambiguous.

In this study, to explain this diversity, we theoretically investigated the electronic structures of the Cu\textsubscript{A} site by employing hybrid ab initio quantum mechanics / molecular mechanics calculation. As a result of the analysis, we revealed that the Met residue creates the characteristic feature in the electronic structure of the Cu\textsubscript{A} site, without the significant rearrangements. Furthermore, we calculated the inner-sphere reorganization energy of the Cu\textsubscript{A} site with respect to the wild type and the mutants, and found that the effects of the Met residue are not significant. Accordingly, we concluded that the Met residue may act as the “fine-modulator” of the properties relevant to the various reactions occurring in CcO. Moreover, we investigated the