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11P.2 Oxygenated heme *d* in cytochrome *bd* from *Escherichia coli*

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Cytochrome *bd* is typically expressed under low oxygen tension and has high affinity for O_2. The enzyme as isolated is a mixture of two stable or metastable forms: (i) ferrous heme *d* bound to molecular oxygen ("state **A**"), and (ii) ferryl oxene heme *d* ("state **F**"), with the latter form contribution of *ca* 20–50%. Both forms can be reversibly destroyed by (a) anaerobic (at $E_h > +380$ mV *vs NHE*, pH 7, 0.1% SML) or aerobic ($>+620$ mV) oxidation or (b) depletion of oxygen at redox potentials where the enzyme mostly remains in one-electron-reduced state; in both cases state "**O**" is formed. Binding of O_2 to heme *d* as a function of E_h was studied using the quasi-equilibrium OTTLE spectro-electrochemistry, where oxygen at varying concentration was allowed to equilibrate with the enzyme at a given redox potential. Under the anaerobic conditions heme *d* has the apparent midpoint potential E_m^{app} about +260 mV with both hemes *b* mostly oxidized [1]; at 1.2 mM O_2, the E_m^{app} value becomes $+495 \pm 5$ mV. The $[O_2]$-dependence of E_m^{app} is essentially linear at the concentrations above 10 μM with the slope -60 mV/pO_2 and the effective dissociation constant for the reduced heme *d*, $K_D^{(eff)}$ about 150 ± 20 nM O_2. The latter value differs from the earlier data, $K_D^{(direct)} \approx 280$ nM, where the heme *d* oxygen affinity was directly measured in one-electron-reduced isolated enzyme [2]. The difference is attributed to the fact that in the presence of oxygen the enzyme slowly turns over catalyzing a steady-state flux of electrons supplied by the working electrode. The turnover numbers of the enzyme at each redox potential can be determined *in situ* as the difference between the values of the cell current (I_{WE}) in the presence and in the absence of the enzyme. Kinetic modeling proves that in the steady-state, the $K_D^{(eff)}$ of about 280 nM can be reached when the (rate-limiting) rate constants for the **O** → **A** and **A** → (**F**) → **O** transitions are equal, which is indeed true, provided that both constants are limited by the electron delivery from the working electrode, and the **A** → **F** transition is much faster. The **O** ↔ **A** and **A** ↔ **F** transitions show similar E_m^{app} values during the redox titration over the broad $[O_2]$ range. We propose that in the state **F**, heme *d* of cytochrome *bd* has an unusually low redox potential comparable to that of the state **A**-heme *d*.

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11P.3 Characterization of the semiquinone radical stabilized by the cytochrome aa_3-600 menaquinol oxidase of *Bacillus subtilis*

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Cytochrome (cyt) aa_3-600 is one of the principle respiratory oxidases from *Bacillus subtilis* and is a member of the heme-copper superfamily of oxygen reductases. This enzyme catalyzes the 2-electron oxidation of menaquinol and the 4-electron reduction of O_2 to $2H_2O$. Cyt aa_3-600 is of interest because it is a very close homologue of the cyt bo_3 ubiquinol oxidase from *E. coli*, except that it uses menaquinol instead of ubiquinol as a substrate. One question of interest is how the proteins differ in response to the differences in structure and electrochemical properties between ubiquinol and menaquinol. Cyt bo_3 has a high affinity binding site for ubiquinol that stabilizes an ubisemiquinone (SQ). This has permitted the use of pulsed EPR techniques to investigate the protein interaction with the ubiquinone. Pulsed EPR methods have revealed several salient features of the interactions between the residues at the Q_H-site of cyt bo_3 and the SQ: 1) The hydrogen bonding to the SQ is highly asymmetric, with strong hydrogen bonds to carbonyl O-1 and weaker interactions at carbonyl O-4 side; 2) There is one strong hydrogen bond between the ε-nitrogen of R71 and carbonyl O-1 of the SQ, resulting in substantial transfer of unpaired electron spin to this nitrogen; 3) There is a strong hydrogen bond between D75 and carbonyl O-1 of the SQ; 4) There is a weak interaction between H98 and carbonyl O-4 of the SQ with a small amount of spin density found on the nitrogens of H98; 5) There is a very weak interaction between carbonyl O-4 of the SQ and the side chain of Q101; 6) The SQ in cyt bo_3 is in the neutral, protonated state at pH 7.5. The current work initiates studies to characterize the equivalent site in cyt aa_3-600. Cyt aa_3-600 has been cloned and expressed in a his-tagged form in *B. subtilis*. Following isolation of the enzyme in dodecylmaltoside, it is shown that the pure enzyme contains one equivalent of menaquinone-7, and that the enzyme stabilizes a mena-semiquinone. Pulsed EPR studies have shown that there are both similarities as well as significant differences in the interactions of the mena-semiquinone with cyt aa_3-600 in comparison with the ubi-semiquinone in cyt bo_3. Our data indicate weaker hydrogen bonds of the menaquinone in cyt aa_3-600 in comparison with ubiquinone in cytochrome bo_3. In addition, the electronic structure of the SQ cyt aa_3-600 is more shifted towards the anionic form from the neutral state in cyt bo_3.

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11P.4 Circular dichroism of cytochrome *c* oxidase: Modeling spectra using classical polarizability theory

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Circular dichroism (CD) spectroscopy in the heme absorption bands provides a sensitive tool for monitoring functional state of cytochrome *c* oxidase (CcO) redox centers. Although CD spectra of mitochondrial CcO were described in the past (Tiesjema RH & Van Gelder BF, 1974, *Biochim. Biophys. Acta* **347:** *202–214;* Myer YP (1985) In *Curr Topics Bioenerg*/ Edited by C. P. Lee. — Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokyo, Academic Press, Inc.*)*, the origin of the observed optical activity was not established. In this work we compare CD and absorption spectra of bovine heart CcO in the fully reduced ($a^{2+}a_3^{2+}$), fully oxidized ($a^{3+}a_3^{3+}$) and various ligand-bound states ($a^{2+}a_3^{2+}$-CO, $a^{3+}a_3^{3+}$-CN, $a^{3+}a_3^{2+}$-CO, $a^{2+}a_3^{3+}$-CN). In addition, we