FEBS Letters 580 (2006) 2534–2539

Hypothesis

Ubiquinone (coenzyme Q_{10}) binding sites: Low dielectric constant of the gate prevents the escape of the semiquinone

V.P. Shinkarev*

Department of Biochemistry, University of Illinois at Urbana-Champaign, 156 Davenport Hall, 607 South Mathews Avenue, Urbana, IL 61801, USA

Received 25 March 2006; accepted 6 April 2006

Available online 21 April 2006

Edited by Peter Brzezinski

Abstract The photosynthetic reaction center (RC) from purple bacteria is frequently used as a model for the interaction of ubiquinones (coenzyme Q) with membrane proteins. Single-turnover flash activation of RC leads to formation of the semiquinone (SQ) of the secondary acceptor quinone after odd flashes and quinol after even flashes. The ubiquinol escapes the binding site in $\leqslant$1 ms, while the SQ does not leave the binding site for at least 5 min. Observed difference between these times suggests a large energetic barrier for the SQ. However, high apparent dielectric constant in the vicinity of the quinone ring ($\geqslant$ 25) results in a relatively small electrostatic energy of SQ stabilization. To resolve this apparent contradiction I suggest that a significant part of the kinetic stabilization of the SQ is achieved by the special topology of the binding site in which quinone can exit the binding site only by moving its headgroup toward the center of the membrane. The large energetic penalty of transferring the charged headgroup to the membrane dielectric can explain the observed kinetic stability of the SQ.

Keywords: Coenzyme Q_{10}; Semiquinone; Ubiquinone; Lipid membrane; Electron transfer; Born energy

1. Introduction

1.1. Ubiquinone function

The ubiquinone (coenzyme Q_{10}) is the main intrinsic lipid-soluble component of the energy-transducing membranes in mitochondria and in some bacteria. It is responsible for the exchange of reducing equivalents between different electron-transfer complexes and plays the role of lipid-soluble antioxidant (reviewed in [1–3]). Recently, interest in the ubiquinone function has increased significantly due to its role in longevity, adjunctive therapy in cardiovascular diseases, and partial prevention of age-related diseases [3–6].

*Fax: +1 217 244 6615.
E-mail address: vshinkar@uiuc.edu (V.P. Shinkarev).

Abbreviations: Cyt, cytochrome; E_h, redox potential of the medium; P870, primary electron donor, bacteriochlorophyll dimer in the RC; Q_A and Q_B, the primary and secondary quinone-type electron acceptors in the RC; Q, coenzyme Q (ubiquinone); Q_{10} (Q_{10}), coenzyme Q with 10 isoprene units; QH_2, dihydroquinone (quinol); RC, photosynthetic reaction center; *Rb.*, *Rhodobacter*; SQ, semiquinone

1.2. Quinone binding sites of membrane complexes

Quinones of energy-transducing membranes usually belong to one of two main populations. One population is represented by pool quinones that are freely diffusible in the hydrophobic core of phospholipid membrane. The other population is represented by quinones bound to proteins. In turn, protein-bound quinones form two large subpopulations. Type A quinones play the role of one-electron prosthetic group; they stay bound to the protein during their function. Typical representatives of this group are one-electron acceptor quinones in photosynthetic reaction centers (RCs) of purple bacteria and Photosystem II, and K_A and K_B in Photosystem I. Type B quinones function as two-electron carriers and are responsible for exchange of reducing equivalents via quinone pool between different electron-transport complexes (reviewed in [1–3,7–10]). The quinones of this type leave the protein as part of their functional turnover.

1.3. Quinones in reaction centers of purple bacteria

Quinones in RCs of purple bacteria provide examples of type A and type B quinones (reviewed in [9,11,12]). The RCs from purple bacteria, such as *Rhodobacter sphaeroides*, have two ubiquinone-binding sites. Ubiquinone molecules bound at these binding sites have different properties, determined by their interaction with protein. The primary acceptor ubiquinone (Q_A) functions as one-electron carrier, while the secondary acceptor ubiquinone (Q_B) functions as a two-electron carrier. The reduction of the secondary acceptor quinone (Q_B) by the primary acceptor quinone (Q_A) occurs stepwise: $Q_A^- Q_B \rightarrow Q_A Q_B^-$, $Q_A^- Q_B^- \rightarrow Q_A Q_B H_2$ and needs two light activations. Such stepwise mechanism suggests that the semiquinone (SQ) should be kinetically stabilized at the binding site for a long time, so the next light activation of the RC will enable the transfer of the second electron to Q_B^- to form ubiquinol, which, in turn, can be replaced by quinone from the membrane pool (see e.g. [13–16]). As a result, after two RC turnovers the acceptor quinone complex restores its initial state with both Q_A and Q_B in the oxidized state. The behavior of the type B quinones in RCs is currently considered a good model for the operation of quinone-reducing sites in other membrane complexes, including PSII, bc_1/b_6f complex, succinate dehydrogenase and NADH dehydrogenase.

1.4. Fast exchange of neutral quinones

There are many indications that both quinone (Q_B) and quinol ($Q_B H_2$) can exchange rapidly with quinones from the

0014-5793/$32.00
doi:10.1016/j.febslet.2006.04.022

membrane pool. Indeed, the turnover time of isolated RCs from *Rb. sphaeroides* under light-saturating conditions is $\leqslant$1 ms [17–20]. This turnover time includes all processes leading to the quinol release and to cytochrome (Cyt) *c* oxidation. Therefore, both quinone binding and quinol release in detergent-solubilized RCs occur faster than ~1 ms. This time correlates well with the time of quinol release from RC measured via the lag phase of the reduction of cytochrome b_H in chromatophores at high redox potentials [21–25]. Similarly, turnover rates above 1000 per second have been reported for bovine bc_1 complex [26]. Such fast turnover indicates that quinone/quinol binding and release, needed for the operation of Q_o and Q_i centers of the bc_1 complex, are also faster than 1 ms.

Thus, data for both RCs and bc_1 complex indicate that exchange of neutral forms (Q, QH_2) of ubiquinone is faster than 1 ms. One can expect that similar rates of exchange are valid for other membrane complexes involved in the oxidation or reduction of pool quinones, including succinate and NADH dehydrogenases.

1.5. Semiquinone lifetime

In contrast to neutral forms of quinones (Q, QH_2), the lifetime of SQ anion at the Q_B binding site is significantly longer, especially in chromatophores. By measuring the amplitude of the absorbance changes at 450 nm corresponding to SQ, it has been determined that SQ can be stabilized at the binding site for at least 5 min [14,16,27,28]. Thus, the escape of the SQ from Q_B binding site is at least six orders of magnitude slower than the escape of neutral quinone or quinol from RC. Different mobility of the SQ and neutral forms of quinone points to the electrostatic nature of the anionic SQ stabilization at the binding site.

1.6. Kinetic and thermodynamic stabilization of the semiquinone

The stoichiometry of flash-induced SQ at the Q_B binding site is close to 1 per RC at neutral pH. This is significantly higher than the amount of thermodynamically stable SQ, which can be formed via redox equilibrium with environment in chromatophores [29]. Thus, practically all flash-induced SQ at neutral conditions is stabilized kinetically, not thermodynamically. Moreover, equilibration of the flash-induced semiquinone with redox mediators leads to the disappearance of the semiquinone [16].

In most cases the main pathway of SQ disappearance is its oxidation by exogenous (redox mediators) or endogenous (oxidized primary donor) acceptors [14,16,30].

1.7. The potential barrier for SQ stabilization

One can estimate the relative potential barrier for SQ stabilization by using the ratio of the escape times for the SQ and QH_2:

$$\Delta E \approx 60\log(\tau_{SQ}/\tau_{QH_2}) \geqslant 60\log(10^6) = 360\ \text{meV}. \quad (1)$$

Thus, to stabilize SQ at the Q_B binding site for the observed time, the potential barrier for the SQ should be higher than that for QH_2 by at least 0.36 eV. The negatively-charged SQ could be stabilized at the binding site via electrostatic interactions, including proton uptake by multiple protonatable groups, anion release/cation uptake, the reorientation of dipoles of amino-acid residues and internal water molecules, and by forming hydrogen bonds (see e.g. [31–38]).

1.8. Electrostatic energy of the semiquinone at the binding site

The high value of the apparent dielectric constant at Q_B binding site (25–50, see e.g. [12,39–41]) indicates that the electrostatic energy of SQ stabilization arising from its interaction with surroundings is relatively small. Indeed, the electrostatic energy (in eV) of the SQ interaction with all other charged groups can be estimated by assuming that it is a sphere with radius *r* (=2.5 Å) in infinite dielectric with dielectric constant $\varepsilon = 30$:

$$\Delta W_{\text{sphere}} = \frac{1}{2}\frac{e^2}{4\pi\varepsilon_0\varepsilon r} \approx \frac{7.15}{r\varepsilon} \sim 0.1\ \text{eV}, \quad (2)$$

where radius *r* in angstroms.

Thus, the electrostatic energy of interaction of SQ at the binding site, where $\varepsilon \sim 30$, is relatively small and consistent with small changes with pH in the equilibrium constant of electron transfer between Q_A and Q_B [12]. It appears that there is not enough electrostatic energy to trap the SQ at the binding site.

1.9. Hydrogen bonds

Hydrogen bonds play important roles for binding and stabilization of neutral and semiquinone forms of Q_B. Many putative hydrogen-bonding partner(s) to Q_B and Q_B^- have been identified in different RC crystal structures [42–49].

The Q_B semiquinone is usually found at the "proximal" site where C_4 carbonyl forms putative hydrogen bond with His-L190, while C_1 carbonyl forms putative hydrogen bonds with the peptide NH groups of Ile-L224, Gly-L225 and the hydroxyl group of Ser-L223 [42,48].

Several different positions of neutral Q_B have been found in RC crystal structures [42–49]. Time-resolved crystallographic experiments indicate predominant binding of Q_B in the proximal position in both the neutral and charge-separated states and no large motion associated with Q_B was observed after the flash on the timescale of secondary electron transfer [50,51]. Similarly, isotope-edited FTIR difference spectroscopy did not reveal significant changes in carbonyl vibrations expected from the different hydrogen bonding in the distal and proximal positions [52]. The study of the Ser-L223 → Ala mutant showed that there is no significant net change in the interaction of the carbonyl oxygen atoms of Q_B and Q_B^- with the protein upon removal of the Ser-L223 hydroxyl group [53].

While hydrogen bonds definitely contribute to stabilizing SQ with neighboring amino acid residues, there are some indications that such stabilization is not strong enough to provide the observed kinetic stability of the SQ. Indeed, the binding of Q_B in the proximal position in both the neutral and charge-separated states [50,51] points to a relatively small role of hydrogen bonding in *differentiation* of binding between quinone and semiquinone. In addition, the energy for breaking hydrogen bonds between Q_B and individual amino acid residues, including His-190 and Ser-L223, does not exceed 110 meV [54,55]. Thus, there should be additional mechanisms for SQ stabilization at the binding site.

2. Hypothesis

To resolve the apparent deficiency of energy for SQ stabilization I suggest that a significant part of this stabilization is achieved by a special construction of the binding site with exit near the center of the membrane. As a result, quinone can

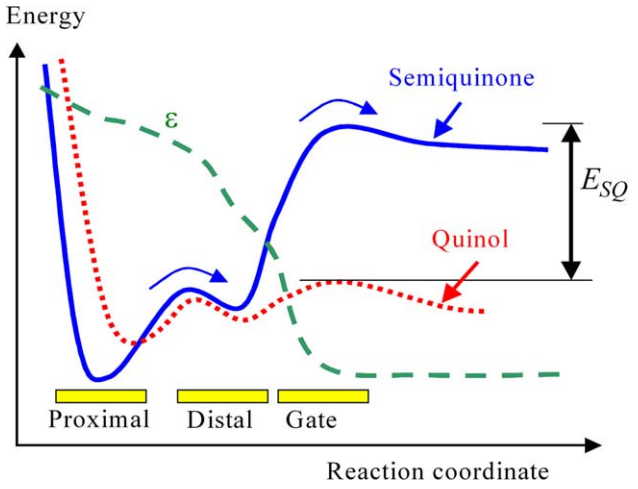

Fig. 1. Schematic representation of potential energy profiles for quinol and semiquinone (SQ) at the binding site in reaction center protein. Main components of SQ stabilization at the binding site, in comparison to the neutral form of quinone, include: (i) electrostatic interactions of SQ at the binding site, (ii) possible hydrogen bonding of the SQ, and (iii) a large potential barrier created by low dielectric gate (Born energy) (E_{SQ}). Dashed line indicates apparent dielectric constant (ε).

leave the binding site only by moving its head down, in the direction of the center of the membrane. Such construction of the binding site will provide a significant potential barrier to SQ anion radical movement into the membrane dielectric and could be responsible for kinetic stabilization of the SQ (Fig. 1). The presence of a low-dielectric gate explains why SQ can be stabilized for minutes, in contrast to quinone and quinol, which can leave the binding site in less than 1 ms. The kinetic stability of the SQ has a topological origin and is determined by the electrostatic interactions of charged amino acid groups at the binding site, and by their absence in the gate.

3. Discussion

3.1. Born energy may be the main contributing factor to the semiquinone stabilization

The Born energy (in eV) for transferring an ion with charge q and radius a from region of dielectric constant ε_2 to one of dielectric constant ε_1 is given by the following equation [56,57]:

$$\Delta W_{\text{Born}} = \frac{1}{2}\frac{q^2}{4\pi\varepsilon_0 a}\left(\frac{1}{\varepsilon_1}-\frac{1}{\varepsilon_2}\right) \approx \frac{7.15}{a}\left(\frac{1}{\varepsilon_1}-\frac{1}{\varepsilon_2}\right), \quad (3)$$

where $q = e^-$ and radius a is in angstroms.

Assuming that radius of SQ is 2.5 Å, one can estimate that the energy, ΔW_{Born}, needed for moving SQ from $\varepsilon_1 = 30$ to $\varepsilon_2 = 2$ is ~1.3 eV. Thus, the estimated Born energy is large enough to provide the kinetic stabilization of the semiquinone at the binding site.

3.2. Ubiquinone location in the membrane

The localization, orientation and movement of ubiquinone within membranes have been the subject of many studies (reviewed in [58,59]). According to current consensus, coenzyme Q mostly occupies a domain in the hydrophobic core at the center of the lipid bilayer matrix parallel to the membrane plane.

Molecular dynamic simulations of coenzyme Q with 10 isoprene units (Q10) inside a lipid bilayer indicate that the diffusion of Q10 in the midplane position is faster than the diffusion of the lipids, while it is comparable with that of the lipids when the Q10 head is close to the membrane surface [60]. The faster diffusion of Q10 in the midplane is explained by lower density and viscosity near the membrane midplane [61]. Such fast lateral diffusion could explain the importance of ubiquinone for effective transfer of reducing and oxidizing equivalents between different quinone-utilizing electron transfer complexes.

Thus, the localization and movement of native long-tailed quinones within membranes correspond well to the entrance to quinone binding site near the center of the membrane, as revealed by structures of many quinone-binding membrane proteins.

3.3. Ubiquinone location in the proteins

The location of quinones in quinone-binding proteins, including bacterial RCs [44], Photosystem I [62], Photosystem II [63], and bc_1 complex [64], has been determined in many crystal structures. In all known structures, the quinone headgroup is oriented towards the membrane surface, while the tail is exposed to the membrane dielectric (Fig. 2). Thus, the position of quinones in known structures agrees with the preferable position of pool quinones in the midplane.

Fisher and Rich [65] explored protein sequences responsible for quinone binding. They found several weak sequence motifs that include histidine, but were unable to find a strong, universal sequence motif for quinone-binding sites. The model for the semiquinone stabilization considered here emphasizes the importance of the topology of the quinone-binding site in the membrane. Indeed, the binding site should be exposed to the membrane interface, needed for quinone protonation/deprotonation after its reduction/oxidation, and, at the same time, it should be exposed to the membrane dielectric, needed for effective exchange with pool quinones. The combination of suggested sequence motifs [65] and these topology limitations may improve the search for putative quinone-binding sites.

3.4. Alternative mechanism for the semiquinone stabilization

Recently, Madeo and Gunner [67] studied the binding of tailless quinones to the Q_A binding site of *Rb. sphaeroides* reaction centers and emphasized the role of the protein in creating the kinetic barriers for binding and release of anionic quinones. These authors suggested that protein rearrangement is the main source of slow binding/unbinding kinetics for anionic quinones. This mechanism is different from the kinetic

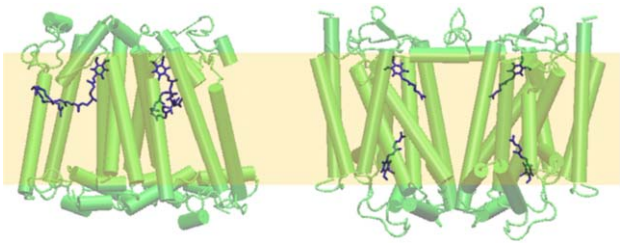

Fig. 2. The quinones in the reaction center (left) and bc_1 complex (right). The quinone headgroup is located near membrane interface, while the tail is exposed to the membrane dielectric. The figure was prepared by VMD software [66] using files 1AIG.pdb and 1NTZ.pdb.

stabilization suggested here. Although these two hypotheses do not completely exclude each other, the difference in mechanisms could originate from the specifics of binding sites for type A and type B quinones. Indeed, B-type quinone-binding sites are designed to facilitate fast ($k_{off} \sim 10^3\ s^{-1}$) quinol/quinone exchange during RC turnover, needed for fast delivery of reducing and oxidative equivalents between different quinone-utilizing complexes. As a result, there are no significant potential barriers to prevent such fast exchange of neutral quinones. This situation is significantly different from the A-type quinone-binding sites in which the quinone remains bound to the protein during its redox cycle. The presence of a significant barrier for binding/unbinding of the quinone agrees well with relatively slow exchange ($k_{off} \sim 1\ s^{-1}$) of neutral forms of tailless quinones with Q_A binding site observed by the authors [67].

3.5. The role of the tail

Natural quinones have extremely hydrophobic tails, ensuring their presence in the membrane. Different organisms produce quinones with different types of headgroup and with different numbers of isoprene units in the tail and this has been used for taxonomic purposes [68]. Extensive studies of the role of tail lengths on different characteristics of electron transfer in RCs showed, surprisingly, that the tail is not very important for many reactions. The kinetics of electron transfer from Q_A to Q_B is practically independent of the quinone tail length [69]. Similarly, the binding of quinone at the Q_B site is also almost quinone tail length independent [70], although positioning of the quinone head group at the binding site is different for short- and long-tailed quinones in crystals [71]. All this indicates that the tail is more important for quinone behavior in the membrane than in the quinone-binding site. It appears that the main function of the tail is to keep quinone molecule closer to the membrane midplane, where the lateral diffusion is faster [60] due to lower density and viscosity [61]. In addition, the tail could facilitate entering the quinone-binding site near the center of the membrane.

3.6. Simple strategy to prevent competitive interference of phospholipids

The mechanism by which quinone-binding sites prevent competitive interference of phospholipids has been explored by Warncke et al. [70], who suggested that the tail–protein interactions are important to deter phospholipids from entering the binding site. The topological construction of a quinone binding site, in which the entrance is located near the center of hydrophobic core of membrane, could provide an effective mechanism for deterring amphiphilic molecules present in native membrane from entering the binding site. Indeed, the large energetic penalty of transferring the charged head of phospholipids to the center of membrane prevents them from entering the quinone-binding site. At the same time, unlike phospholipids, neutral quinones with long tail are mostly located near membrane midplane and can easily enter the binding site.

4. Conclusions

It has been known for over 25 years that semiquinone can be kinetically stabilized in the structure of the RC protein for unexpectedly long times [28]. Such stabilization contrasts sharply with the fast release of neutral forms of the quinone from the RC. The observed difference between release of the SQ and quinol suggests that the energetic barrier for the SQ has electrostatic origin and should be significantly higher than that for quinol. However, high apparent dielectric constant in the vicinity of the quinone headgroup results in relatively small electrostatic energy of the SQ stabilization, which is not enough to provide observed difference between escape rates for SQ and quinol. To resolve this apparent contradiction I suggest here that a significant part of kinetic stabilization of the SQ is achieved by special construction of the binding site in which quinone can exit the binding site only by moving its headgroup in the direction of the center of the membrane. Such construction of the binding site does not significantly limit the mobility of neutral forms of quinones (Q, QH_2) needed for effective exchange of reducing and oxidizing equivalents between different enzymes in membrane, but provides a significant barrier for moving charged headgroup of the SQ to the membrane dielectric. It is suggested that a similar mechanism of semiquinone stabilization is valid for quinone-reducing sites in other membrane complexes, including PSII, bc_1/b_6f complex, succinate dehydrogenase and NADH dehydrogenase.

Acknowledgments: Author is grateful to Eiji Takahashi and Colin Wraight for useful comments. This work was supported by NIH Grant GM 53508.

References

[1] Skulachev, V.P. (1988) Membrane Bioenergetics, Springer, Berlin.
[2] Cramer, W.A. and Knaff, D.B. (1990) Energy Transduction in Biological Membranes, Springer, New York.
[3] Kagan, V.E. and Quinn, P.J., Eds., (2000). Coenzyme Q: Molecular Mechanisms in Health and Disease, CRC Press, Boca Raton, FL.
[4] Beal, M.F. (2003) Mitochondria, oxidative damage, and inflammation in Parkinson's disease. Ann. N. Y. Acad. Sci. 991, 120–131.
[5] Aguilaniu, H., Durieux, J. and Dillin, A. (2005) Metabolism, ubiquinone synthesis, and longevity. Genes Dev. 19, 2399–2406.
[6] Littarru, G.P. and Tiano, L. (2005) Clinical aspects of coenzyme Q10: an update. Curr. Opin. Clin. Nutr. Metab. Care 8, 641–646.
[7] Rich, P.R. (1984) Electron and proton transfers through quinones and cytochrome *bc* complexes. Biochim. Biophys. Acta 768, 53–79.
[8] Shinkarev, V.P. (1990) Function of quinones in bacterial photosynthesis (in Russian), Advances in Science and Technology, Biophysics Series, vol. 33, VINITI, Moscow, pp. 1–206.
[9] Ke, B. (2001) Photosynthesis: Photobiochemistry and Photobiophysics, Kluwer Academic Publishers, Dordrecht, The Netherlands.
[10] Dutton, P.L., Ohnishi, T., Darrouzet, E., Leonard, M.A., Sharp, R.E., Gibney, B.R., Daldal, F. and Moser, C.C. (2000) Coenzyme Q oxidation–reduction reactions in mitochondrial electron transport in: Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V.E. and Quinn, P.J., Eds.), pp. 65–82, CRC Press, Boca Raton, FL.
[11] Okamura, M.Y., Paddock, M.L., Graige, M.S. and Feher, G. (2000) Proton and electron transfer in bacterial reaction centers. Biochim. Biophys. Acta 1458, 148–163.
[12] Shinkarev, V.P. and Wraight, C.A. (1993) Electron and proton transfer in the acceptor quinone complex of reaction centers of phototrophic bacteria (Deisenhofer, J. and Norris, J., Eds.), The Photosynthetic Reaction Center, Vol. 1, pp. 193–255, Academic Press, New York.
[13] Wraight, C.A. (1979) Electron acceptors of bacterial photosynthetic reaction centers. II. H^+ binding coupled to secondary

electron transfer in the quinone acceptor complex. Biochim. Biophys. Acta 548, 309–327.
[14] Shinkarev, V.P., Verkhovsky, M.I., Kaurov, B.S. and Rubin, A.B. (1981) The kinetic model of two-electron gate in photosynthetic RCs of purple bacteria. Mol. Biol. (Moscow) 15, 1069–1082.
[15] Shopes, R.J. and Wraight, C.A. (1985) The acceptor quinone complex of *Rhodopseudomonas viridis* reaction centers. Biochim. Biophys. Acta 806, 348–356.
[16] Mulkidjanian, A.Y., Shinkarev, V.P., Verkhovsky, M.I. and Kaurov, B.S. (1986) A study of the kinetic properties of the stable semiquinone of the reaction center secondary acceptor in chromatophores of non-sulfur purple bacteria. Biochim. Biophys. Acta 849, 150–161.
[17] Rongey, S.H., Paddock, M.L., Feher, G. and Okamura, M.Y. (1993) Pathway of proton transfer in bacterial reaction centers: second-site mutation Asn-M44 → Asp restores electron and proton transfer in reaction centers from the photosynthetically deficient Asp-L213 → Asn mutant of *Rhodobacter sphaeroides*. Proc. Natl. Acad. Sci. USA 90, 1325–1329.
[18] Osvath, S. and Maroti, P. (1997) Coupling of cytochrome and quinone turnovers in the photocycle of reaction centers from the photosynthetic bacterium *Rhodobacter sphaeroides*. Biophys. J. 73, 972–982.
[19] Gerencser, L., Laczko, G. and Maroti, P. (1999) Unbinding of oxidized cytochrome *c* from photosynthetic reaction center of *Rhodobacter sphaeroides* is the bottleneck of fast turnover. Biochemistry 38, 16866–16875.
[20] Larson, J.W. (2000) Cytochrome *c* Oxidation by the Bacterial Photosynthetic Reaction Center from *Rhodobacter sphaeroides*. Ph.D. thesis, University of Illinois at Urbana-Champaign, Urbana.
[21] Venturoli, G., Fernandezvelasco, J.G., Crofts, A.R. and Melandri, B.A. (1986) Demonstration of a collisional interaction of ubiquinol with the ubiquinol-cytochrome c_2 oxidoreductase complex in chromatophores from *Rhodobacter sphaeroides*. Biochim. Biophys. Acta 851, 340–352.
[22] Shinkarev, V.P., Crofts, A.R. and Wraight, C.A. (2005) Spectral analysis of the bc_1 complex components in situ: beyond the traditional difference approach. Biochim. Biophys. Acta 1757, 67–77.
[23] Crofts, A.R., Meinhardt, S.W., Jones, K.R. and Snozzi, M. (1983) The role of the quinone pool in the cyclic electron-transfer chain on *Rhodopseudomonas sphaeroides* – a modified Q-cycle mechanism. Biochim. Biophys. Acta 723, 202–218.
[24] Crofts, A.R. and Wang, Z.G. (1989) How rapid are the Internal reactions of the ubiquinol-cytochrome-c_2 oxidoreductase. Photosynth. Res. 22, 69–87.
[25] Drachev, L.A., Kaurov, B.S., Mamedov, M.D., Mulkidjanian, A.J., Semenov, A.J., Shinkarev, V.P., Skulachev, V.P. and Verkhovsky, M.I. (1989) Flash-induced electrogenic events in the photosynthetic reaction center and *bc* complex of *Rhodobacter sphaeroides* chromatophores. Biochim. Biophys. Acta 973, 189–197.
[26] Ljungdahl, O., Pennoyer, J.D., Robertson, D.E. and Trumpower, B.L. (1987) Purification of highly-active cytochrome bc_1 complexes from phylogenetically diverse species by a single chromatographic procedure. Biochim. Biophys. Acta 891, 227–241.
[27] Diner, B.A., Schenck, C.C. and De Vitry, C. (1984) Effect of inhibitors, redox state and isoprenoid chain length on the affinity of ubiquinone for the secondary acceptor binding site in the reaction centers of photosynthetic bacteria. Biochim. Biophys. Acta 766, 9–20.
[28] Vermeglio, A. (1977) Secondary electron transfer in reaction centers of *Rhodopseudomonas sphaeroides*. Out-of-phase periodicity of two for the formation of ubisemiquinone and fully reduced ubiquinone. Biochim. Biophys. Acta 459, 516–524.
[29] Rutherford, A.W. and Evans, M.C.W. (1980) Direct measurement of the redox potential of the primary and secondary quinone electron acceptors in *Rhodopseudomonas sphaeroides* (wild-type) by EPR spectrometry. FEBS Lett. 110, 257–261.
[30] Shinkarev, V.P. (1998) The general kinetic model of electron transfer in photosynthetic reaction centers activated by multiple flashes. Photochem. Photobiol. 67, 683–699.
[31] Lancaster, C.R.D., Michel, H., Honig, B. and Gunner, M.R. (1996) Calculated coupling of electron and proton transfer in the photosynthetic reaction center of *Rhodopseudomonas viridis*. Biophys. J. 70, 2469–2492.
[32] Alexov, E.G. and Gunner, M.R. (1999) Calculated protein and proton motions coupled to electron transfer: electron transfer from Q_A^- to Q_B in bacterial photosynthetic reaction centers. Biochemistry 38, 8253–8270.
[33] Lancaster, C.R.D. (2003) The role of electrostatics in proton-conducting membrane protein complexes. FEBS Lett. 545, 52–60.
[34] Beroza, P., Fredkin, D.R., Okamura, M.Y. and Feher, G. (1995) Electrostatic calculations of amino acid titration and electron transfer, $Q_A^-Q_B \rightarrow Q_AQ_B^-$, in the reaction center. Biophys. J. 68, 2233–2250.
[35] Sham, Y.Y., Muegge, I. and Warshel, A. (1999) Simulating proton translocations in proteins: probing proton transfer pathways in the *Rhodobacter sphaeroides* reaction center. Proteins 36, 484–500.
[36] Rabenstein, B., Ullmann, G.M. and Knapp, E.W. (1998) Energetics of electron-transfer and protonation reactions of the quinones in the photosynthetic reaction center of *Rhodopseudomonas viridis*. Biochemistry 37, 2488–2495.
[37] Rabenstein, B., Ullmann, G.M. and Knapp, E.W. (2000) Electron transfer between the quinones in the photosynthetic reaction center and its coupling to conformational changes. Biochemistry 39, 10487–10496.
[38] Zhu, Z. and Gunner, M.R. (2005) Energetics of quinone dependent electron and proton transfers in *Rhodobacter sphaeroides* photosynthetic reaction centers. Biochemistry 44, 82–96.
[39] Paddock, M.L., Feher, G. and Okamura, M.Y. (1997) Proton and electron transfer to the secondary quinone (Q_B) in bacterial reaction centers: the effect of changing the electrostatics in the vicinity of Q_B by interchanging Asp and Glu at the L212 and L213 sites. Biochemistry 36, 14238–14249.
[40] Cherepanov, D.A., Bibikov, S.I., Bibikova, M.V., Bloch, D.A., Drachev, L.A., Gopta, O.A., Oesterhelt, D., Semenov, A.Y. and Mulkidjanian, A.Y. (2000) Reduction and protonation of the secondary quinone acceptor of *Rhodobacter sphaeroides* photosynthetic reaction center: kinetic model based on a comparison of wild-type chromatophores with mutants carrying Arg → Ile substitution at sites 207 and 217 in the L-subunit. Biochim. Biophys. Acta 1459, 10–34.
[41] Baciou, L., Sinning, I. and Sebban, P. (1991) Study of Q_B-stabilization in herbicide-resistant mutants from the purple bacterium *Rhodopseudomonas viridis*. Biochemistry 30, 9110–9116.
[42] Fritzsch, G., Koepke, J., Diem, R., Kuglstatter, A. and Baciou, L. (2002) Charge separation induces conformational changes in the photosynthetic reaction center of purple bacteria. Acta Cryst. D 58, 1661–1663.
[43] Pokkuluri, P.R., Laible, P.D., Deng, Y.-L., Wong, T.N., Hanson, D.K. and Schiffer, M. (2002) The structure of a mutant photosynthetic reaction center shows unexpected changes in main chain orientations and quinone position. Biochemistry 41, 5998–6007.
[44] Lancaster, C.R.D. and Michel, H. (2001) Photosynthetic reaction centers of purple bacteria in: Handbook of Metalloproteins (Messerschmidt, A., Huber, R., Poulos, T. and Wieghardt, K., Eds.), pp. 119–135, Wiley, Chichester.
[45] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Structure of the reaction center from *Rhodobacter sphaeroides* R-26: The cofactors. Proc. Natl. Acad. Sci. USA 84, 5730–5734.
[46] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Structure of the protein subunits in the photosynthetic reaction center from *Rhodopseudomonas viridis* at 3 Å resolution. Nature 318, 618–624.
[47] Ermler, U., Fritzsch, G., Buchanan, S.K. and Michel, H. (1994) Structure of the photosynthetic reaction centre from *Rhodobacter sphaeroides* at 2.65 Å resolution: cofactors and protein–cofactor interactions. Structure 2, 925–936.
[48] Stowell, M.H.B., McPhillips, T.M., Rees, D.C., Soltis, S.M., Abresh, E. and Feher, G. (1997) Light induced structural changes in photosynthetic reaction center: implications for mechanism of electron-proton transfer. Science 276, 812–816.

[49] Lancaster, C.R.D. (1998) Ubiquinone reduction and protonation in photosynthetic reaction centres from *Rhodopseudomonas viridis* X-ray structures and their functional implications. Biochim. Biophys. Acta 1365, 143–150.
[50] Baxter, R.H.G., Ponomarenko, N., Srajer, V., Pahl, R., Moffat, K. and Norris, J.R. (2004) Time-resolved crystallographic studies of light-induced structural changes in the photosynthetic reaction center. Proc. Natl. Acad. Sci. USA 101, 5982–5987.
[51] Baxter, R.H.G., Seagle, B.L., Ponomarenko, N. and Norris, J.R. (2005) Cryogenic structure of the photosynthetic reaction center of *Blastochloris viridis* in the light and dark. Acta Cryst. D 61, 605–612.
[52] Breton, J. (2004) Absence of large-scale displacement of quinone Q_B in bacterial photosynthetic reaction centers. Biochemistry 43, 3318–3326.
[53] Nabedryk, E., Paddock, M.L., Okamura, M.Y. and Breton, J. (2005) An isotope-edited FTIR investigation of the role of Ser-L223 in binding quinone (Q_B) and semiquinone (Q_B^-) in the reaction center from *Rhodobacter sphaeroides*. Biochemistry 44, 14519–14527.
[54] Rahaman, A. and Wheeler, R.A. (2004) Potential energy landscape for conformationally gated secondary ubiquinone binding in the photosynthetic reaction center of *Rhodobacter sphaeroides*. ChemPhysChem 5, 249–252.
[55] Ishikita, H. and Knapp, E.W. (2004) Variation of Ser-L223 hydrogen bonding with the Q_B redox state in reaction centers from *Rhodobacter sphaeroides*. J. Am. Chem. Soc. 126, 8059–8063.
[56] Bockris, J.O.M. and Reddy, A.K.N. (1970) Modern Electrochemistry, Plenum Press, New York.
[57] Israelachvili, J.N. (1992) Intermolecular and Surface Forces, Academic Press, San Diego, CA.
[58] Lenaz, G. (2000) Mobility of Coenzyme Q in membranes in: Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V.E. and Quinn, P.J., Eds.), pp. 5–27, CRC Press, Boca Raton, FL.
[59] Quinn, P.J. (2000) Topography of coenzyme Q in membranes in: Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V.E. and Quinn, P.J., Eds.), pp. 29–42, CRC Press, Boca Raton, FL.
[60] Solderhall, J.A. and Laaksonen, A. (2001) Molecular dynamics simulations of ubiquinone inside a lipid bilayer. J. Phys. Chem. B 105, 9308–9315.
[61] Tieleman, D.P., Marrink, S.J. and Berendsen, H.J.C. (1997) A computer perspective of membranes: molecular dynamics studies of lipid bilayer systems. Biochim. Biophys. Acta 1331, 235–270.
[62] Jordan, P., Fromme, P., Witt, H.T., Klukas, O., Saenger, W. and Kraus, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 angstrom resolution. Nature 411, 909–917.
[63] Ferreira, K., Iverson, T., Maghlaoui, K., Barber, J. and Iwata, S. (2004) Architecture of the photosynthetic oxygen-evolving center. Science 303, 1831–1838.
[64] Berry, E.A., Guergova-Kuras, M., Huang, L.S. and Crofts, A.R. (2000) Structure and function of cytochrome *bc* complexes. Annu. Rev. Biochem. 69, 1005–1075.
[65] Fisher, N. and Rich, P.R. (2000) A motif for quinone binding sites in respiratory and photosynthetic systems. J. Mol. Biol. 296, 1153–1162.
[66] Humphrey, W., Dalke, A. and Schulten, K. (1996) VMD – visual molecular dynamics. J. Mol. Graphics 14.1, 33–38.
[67] Madeo, J. and Gunner, M.R. (2005) Modeling binding kinetics at the Q_A site in bacterial reaction centers. Biochemistry 44, 10994–11004.
[68] Collins, M.D. and Jones, D. (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol. Rev. 45, 316–354.
[69] McComb, J.C., Stein, R.R. and Wraight, C.A. (1990) Investigation on the influence of headgroup substitution and isoprene side-chain length in the function of primary and secondary quinones of bacterial reaction centers. Biochim. Biophys. Acta 1015, 156–171.
[70] Warncke, K., Gunner, M.R., Braun, B.S., Gu, L., Yu, C.A., Bruce, J.M. and Dutton, P.L. (1994) Influence of hydrocarbon tail structure on quinone binding and electron-transfer performance at the Q_A and Q_B sites of the photosynthetic reaction center protein. Biochemistry 33, 7830–7841.
[71] Lancaster, C.R.D. and Michel, H. (1997) The coupling of light induced electron transfer and proton uptake as derived from crystal structures from reaction centers of *Rhodopseudomonas viridis* modified at the binding site of the secondary quinone Q_B. Structure 5, 1339–1359.