S12-005

Resolution of proton and electron transfer events in the photosynthetic reaction center and the cytochrome-bc_1 complex of phototrophic bacteria

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Keywords: proton transfer, electron transfer, bc_1 complex, *Rhodobacter capsulatus.*

1. Introduction.

Flashes of light on chromatophore vesicles from phototrophic bacteria drive transmembrane charge separation events in the photochemical reaction center (RC) and lead to the reduction of a bound ubiquinone Q_B to a quinol Q_BH_2. The quinol oxidation by the cytochrome-bc_1 complex (bc_1) causes the further increase in the membrane potential and the acidification of the chromatophore interior. As a result, protons are driven out of chromatophores through the F_OF_1-ATP-synthase. Here we report about our efforts to deconvolute this proton and electron circulation into partial steps.

2. Proton transfer steps in the RC of *Rb. sphaeroides*.

X-ray studies showed that the Q_B ring relocates in its binding pocket during the turnover (Ermler et al., 1994; Stowell et al., 1997). This finding, together with diverse spectroscopic data and the results of the electrometric tracing of proton transfer through the RC of *Rb. sphaeroides* (Gopta et al., 1997; Cherepanov et al., 2000), allowed to suggest a scheme where the electron and proton transfer steps were attributed to the structural changes in the Q_B^- binding pocket (see Fig. 1 and (Mulkidjanian, 1999; Cherepanov et al., 2000)). The key feature of the scheme is the alternating interaction of the Q_B ring with Glu-212 of the L subunit:

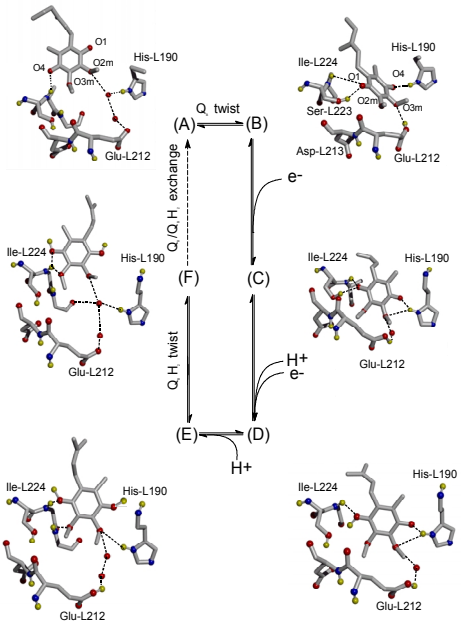


Fig. 1. Scheme of Q_B turnover

1) Q_B is seen in two isoenergetic positions in its binding pocket (Stowell et al., 1997). In the distal state **A** Q_B is away from Glu-L212. The latter is involved as a hydrogen bond acceptor in a water bridge to His-L190. The pK_{212} of Glu-L212 in this state was estimated as ≤ 6.0. In the proximal state **B,** a hydrogen bond between Q_B and Glu-L212 seems to account for the high, experimentally established pK_{212} of ~ 10.0.

2) Formation of $Q_B^{\bullet-}$ brings all quinone molecules into the proximal semiquinone position **C**. The joint transfer of the second electron and the first proton to $Q_B^{\bullet-}$ (**C** → **D** transition) yields a proximally bound Q_BH^-.

3) On the transfer of the second proton, Q_BH_2 detaches to move into the distal, quinol position (**D** → **E**). This detachment is the rate limiting step of the overall turnover. Glu-L212 looses the proton and its pK_{212} switches back to ≤ 6.0, as the water molecules wedge in and restore the bridge between Glu-L212 and His-L190 (**E** → **F**).

In a separate study we showed that the electrogenic proton transfer from the surface-located acids into the Q_B site followed the reduction of Q_B without measurable delay (100 μs at pH 6.2), whereas the re-protonation of these acids from the bulk was distinctly retarded (400 μs). The slowness of proton donation by neutral water (pK ~ 15) accounted for the retardation. Both hydronium ions and pH-buffers were not competent proton donors due to the constrained diffusion in the surface water layer (see ref. (Gopta et al., 1999) for details).

3. Proton transfer steps in the cytochrome-bc_1 complex of *Rb. capsulatus*.

According to the *Q*-cycle mechanism (Mitchell, 1976), quinol is oxidized by bc_1 in center *P* at the interface between the iron-sulfur protein (FeS) and the di-heme cytochrome *b* (Crofts et al., 1999). The first electron is removed from quinol by FeS. To deliver the electron to the cytochrome c_1, the mobile headpiece of FeS has to move by 70°, from its "cytochrome *b*" state FeS_b into the "cytochrome c_1" position FeS_c. The second electron is accepted by the low-potential heme b_l and then transferred across the membrane, via the high-potential heme b_h, to another quinone-binding center *N.* Here a quinone molecule is reduced to a semiquinone anion $Q_N^{\bullet-}$. Antimycin A blocks center *N,* whereas myxothiazol inhibits center *P*.

Both $\Delta\psi$ generation and proton release into the chromatophore lumen seem to retard relative to the anticipated rate of quinol oxidation in center *P* (Mulkidjanian and Junge, 1994). In the absence of antimycin A, however, cytochrome *b* is oxidized

faster than it is reduced, so that any kinetic comparison with the rate of $\Delta\psi$ generation is ambiguous.

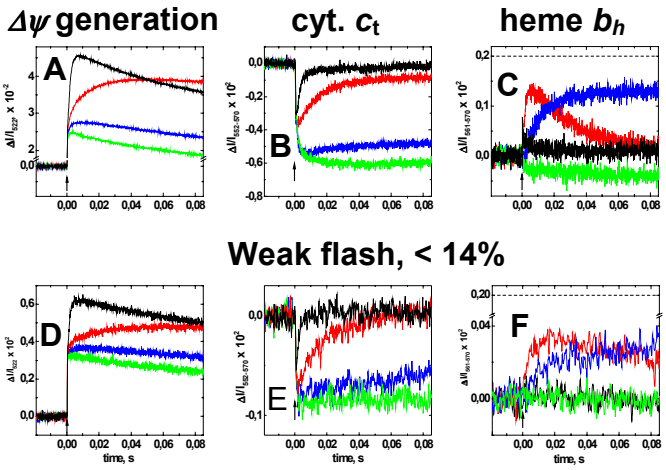


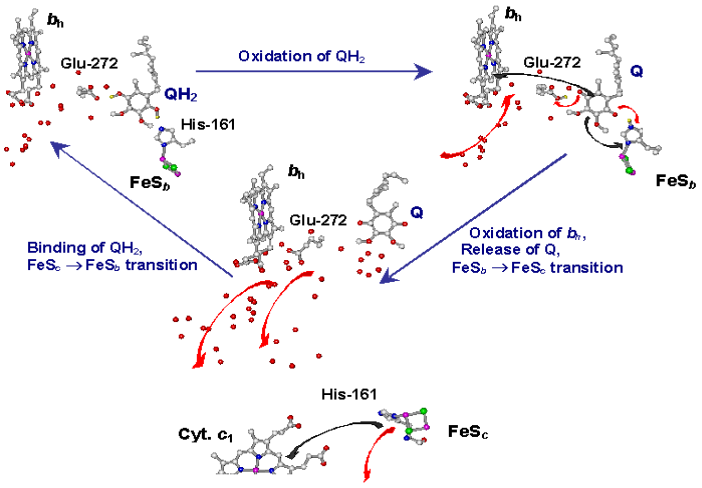
Fig. 2. Impact of Zn^{2+} on the bc_1 turnover in *Rb. capsulatus* chromatophores. Colour code: black, control traces, red, +50 µM $ZnSO_4$, blue, +50 µM $ZnSO_4$, +5 µM antimycin A, green, +50 µM $ZnSO_4$, +5 µM antimycin A, +5 µM myxothiazol. Incubation medium: 25 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 5 µM TMPD, 8 µM methylene blue, 2 mM KCN, 2 mM potassium succinate, 100 µM potassium fumarate. The dashed lines in C and F show the total amount of heme b_h (as the quinone pool was half-reduced, b_h was partially reduced in the dark in the presence of Antimycin A).

We tried to tackle the flash-induced bc_1 turnover in chromatophores of *Rb. capsulatus* by applying Zn^{2+}, an inhibitor of the mitochondrial bc_1 (Link and von Jagow, 1995). The upper row in Fig. 2 shows the impact of 50 µM Zn^{2+} on $\Delta\psi$ generation by bc_1 (**A,** electrochromic carotenoid bandshift at 522 nm), on the redox behavior of the "total" cytochrome *c* (**B**, cytochrome c_1 + c_2, 551 –570 nm) and on the redox changes of heme b_h (**C**, 561-570 nm). The panels **D** – **F** show similar traces as in the upper row but measured with a seven-fold attenuated light flash, so that only some bc_1-complexes turned over. We found out that Zn^{2+} retarded the oxidation of the cytochrome *b* and allowed thus to follow its flash-induced redox changes.

Concurrently, the $\Delta\psi$ generation and the reduction of the flash oxidized cytochromes *c* retarded as well. The heme b_h reduction was consistently faster that the cytochrome *c* reduction and the onset of $\Delta\psi$ generation. This kinetic discrepancy could not be attributed to multiple turnovers involved in $\Delta\psi$ generation and cytochrome c_1 reduction (as compared to the heme b_h reduction after the first turnover) because the heme b_h reduction was faster even under the single turnover conditions (see panels **D** – **F**). Instead, the rates of cytochrome *c* reduction and of $\Delta\psi$ generation correlated roughly with the kinetics of heme b_h oxidation (that became exposed in the presence of Zn^{2+}). From our opinion, the presented data provide a conclusive evidence in favor of our earlier suggestions on (1) the non-electrogenicity of the heme b_h reduction and (2) the electrogenicity of heme b_h oxidation and its coupling with the reduction of cytochrome *c* (Mulkidjanian et al., 1991; Mulkidjanian and Junge, 1994; Gopta et al., 1998).

We rationalized the data obtained by a hypothetical scheme (see Fig. 3) based on the structural analysis from (Crofts et al., 1999; Hunte et al., 2000). As depicted on panel **A**, quinol is bound most probably by His-161 of FeS and Glu-272 of cytochrome *b* (beef numeration, (Crofts et al., 1999)). The slow reduction of cytochrome *c* under the single-turnover conditions indicates that the FeS_b-cytochrome *b* complex does not dissociate immediately after quinol is oxidized to quinone (**A** → **B**). The absence of a fast $\Delta\psi$ generation implies that both protons released at quinol oxidation stayed in center *P* (seemingly, at His-161 and Glu-272). They, apparently, help to "hold together" the FeS_b-cytochrome *b* complex as shown on panel **B**. The reduction of b_l, in turn, is likely to attract protons from the lumen via the water chain that is seen in the X-ray structure ((Hunte et al., 2000), shown in Fig. 3). Such an

electrogenic proton binding, favorable under coupled conditions, could make the electron transfer from center *P* to heme b_h electrically silent.



The observed kinetic coupling between heme b_h oxidation, cytochrome *c* reduction and $\Delta\psi$ generation could be rationalized by a *single* assumption that the formation of a $Q_N^{\bullet-}$ semiquinone promotes the $FeS_b \rightarrow FeS_c$ transition. This suggestion finds support in the recently observed shift of the $FeS_b \leftrightarrow FeS_c$ equilibrium to the right in response to the binding of antimycin A, a semiquinone analogue, in the center *N* (Valkova-Valchanova et al., 2000).

Then the oxidation of heme b_h leads to the following events (**B** → **C**): (i) the FeS domain moves towards cytochrome c_1, reduces the latter and looses its proton; (ii) the "liberated" Glu-272 rotates towards the "output" water chain and releases its proton; (iii) the proton(s), that were electrostatically compensating the electron at the cytochrome *b* heme(s), are released as well. Thus, several different electrogenic proton transfer reactions, *all* of them kinetically coupled with the oxidation of heme b_h, are suggested to contribute to the $\Delta\psi$ generation by the cytochrome-bc_1 complex. Two Zn^{2+}-binding sites have been revealed in the X-ray structure of mitochondrial bc_1, both close to center *P* (Berry et al., 2000). The suggested conformational coupling allows, in addition, to understand how the binding of Zn^{2+} close to center *P* could retard the oxidation of heme b_h in center *N*.

One possible reason why the proton release from center *P* is retarded might be a strong proton backpressure under coupling conditions. We found out that the local pH at the inner chromatophore surface decreases by 1-2 pH units already after a single flash (Feniouk et al., 2000). This decrease is apparently due to the diffusion barrier for the proton exchange between the surface and the bulk ((Gopta et al., 1999), see above). It might be that the driving force for the quinol oxidation in center *P* is not sufficient to push out protons into the lumen under coupled conditions, so that the free energy of the quinone reduction by heme b_h in center *N* is to be invoked, via the conformational coupling, to bring the bc_1 turnover to completion.

4. Outlook

The example of Glu-L212 in the RC shows (see section 2) that an enzyme can go between isoenergetic conformations differing dramatically in the pK value of the key, catalytic residues. Such a conformationally controlled pK switching allows enzymes to generate strong intrinsic acids or bases when needed (Mulkidjanian, 1999). It is attractive to speculate that the two conformations of Glu-272, as seen in the X-ray structures of bc_1, might represent such a pK-switch. The suggested binding of quinone by Glu-272 in the FeS_b conformation (state **B** in Fig. 3) implies $pK_{272} > 9.0$. The suggested ability of Glu-272 to "pump out" a proton (Crofts et al., 1999) implies a

sharp decrease in the pK_{272} value during the **B** → **C** transition (see Fig. 3). We believe that the further studies of the Zn^{2+}-treated chromatophores, that are currently in progress, could help to understand the mechanism of the bc_1 turnover.

Acknowledgments

Very helpful discussions with Profs. A.R. Crofts, F. Daldal, and V. Shinkarev are appreciated. We thank Prof. W. Junge for his generous support. This work has been supported by grants from the *Deutsche Forschungsgemeinschaft* (Mu-1285/1, Ju-97/13, 436-RUS-113/210).

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