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Proton uptake and quinone connection in the bacterial reaction centre

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Introduction

The reaction centre (RC), pigment-protein complex, is present in the inner membrane of photosynthetic bacteria. It is the minimum structural and functional unit which catalyzes electron and proton transfer processes upon light excitation. The photon absorbed by the bacteriochlorophyll dimer (P) initiates the transfer of an electron through different pigment monomers. The electron is stabilized on the tightly bound primary quinone (Q_A) or, if available, on the more loosely bound secondary quinone (Q_B). In physiological conditions, P^+ is re-reduced by an electron donor (soluble cytochrome c_2), and subsequent light excitation induces the complete turnover of the RC. Thus, the acceptor quinone complex acts as a two-electron gate. As the electron transfer is coupled to the proton uptake from the cytoplasm to form a quinol molecule (Q_BH_2), the reducing equivalents (electrons and protons) are exported in pairs from the RC. The released quinol is then replaced by a quinone from the pool resetting the system to the initial state.

Q_A^- and Q_B^- are stabilised by surrounding residues whose pK_as are shifted by the formation of the semiquinones (Maroti and Wraight, 1988; McPherson, 1988). The differential energetic stabilization between Q_A^- and Q_B^- can be probed by measuring the stoichiometries of proton uptake in the Q_A^- (H^+/Q_A^-) and the Q_B^- (H^+/Q_B^-) states, respectively. Q_A is bound to a relatively hydrophobic part of the reaction centre protein whereas Q_B is more closely surrounded by a number of ionizable residues. In particular, L213Asp and L212Glu, designated as the "Q_B cluster"(Rabenstein *et al.*, 2000; Alexov and Gunner, 1999; Alexov and Gunner, 1997), is situated respectively at 5 Å and 6 Å from Q_B, and form a strongly interacting acidic cluster which ionization state modulates the electrostatic properties of the Q_B binding pocket. Because of the strong pair-wise interactions between these residues, the cluster (which may also involve L210Asp and H173Glu) responds as a whole to the formation of either Q_B^- or Q_A^- (situated at about 18 Å from Q_B). We report here proton uptake measurements upon Q_A^- and Q_B^- formations in RC mutants from *Rhodobacter (Rb.) capsulatus* and from *Rb. sphaeroides*. In the first mutant family, we have investigated the electrostatic interactions probed by the stoichiometries of proton uptake in engineered mutants from *Rb. capsulatus* carrying the M247Ala→Tyr mutation which is the symmetry-related residues to L213 in the Q_A pocket. This single mutation is associated to a Q_B pocket where L213Asp and L212Glu are either present or changed to alanines (AA mutant)(Valerio-Lepiniec *et al.*, 1999; Hanson and Schiffer, 1998). In the second mutant family, L209Pro

situated at the border of a chain of hydrogen-bonded water molecules that connects Q_B to the cytoplasmic surface of the RC (Stowell *et al.*, 1997; Ermler *et al.*, 1994) has been changed in *Rb. sphaeroides* by site-directed mutagenesis to various residues (threonine, tryptophane, glutamate, phenylalanine and tyrosine).

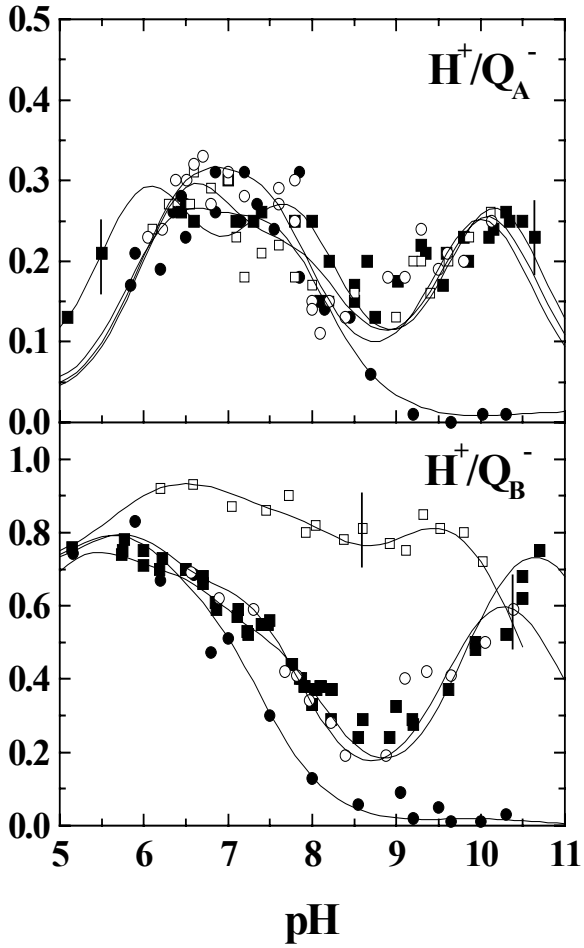
Materials and methods

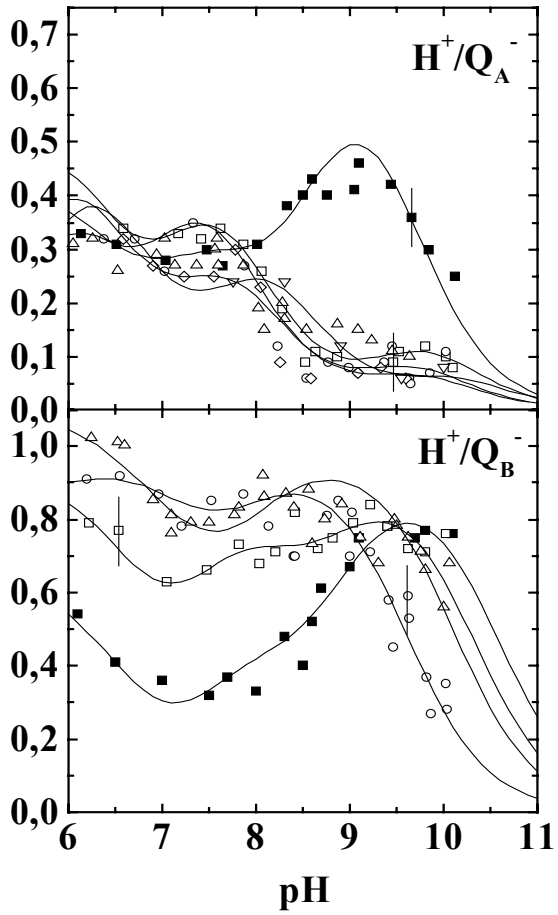
The design of the *Rb. sphaeroides* and *Rb. capsulatus* wild type or mutant strains and the reaction centre isolations were previously described (Laible, 1997; Miksovska *et al.*, 1996; Baciou and Michel, 1995). The H^+/Q_A^- and H^+/Q_B^- proton uptake stoichiometries were measured as reported earlier (Miksovska *et al.*, 1997; Miksovska *et al.*, 1996). Results obtained with pH electrodes and pH sensitive dyes were combined. Conditions: 2 μM reaction centres, 50 mM NaCl, 0.03% Triton X-100, 200 μM ferrocene, 40 μM dye (bromocresol purple, phenol red, cresol red or o-cresol phtaleine depending on the pH). The buffer concentration was kept below 10 μM by extensive dialysis. The occupancy of the Q_B site was routinely restored by the addition of 60 μM UQ_6. The H^+/Q_A^- proton uptake stoichiometries were measured in the presence of terbutryn (100 μM).

Results and discussion

The pH dependencies of the H^+/Q_A^- and H^+/Q_B^- stoichiometries in the reaction centres of the M247Tyr and the AA+M247Tyr mutants are presented in Fig. 1. The pH titrations of the stoichiometries for the WT reaction centres (Maróti *et al.*, 1995; Sebban *et al.*, 1995) and for the AA mutant reaction centres (Maróti *et al.*, 1995) have previously been described (Fig. 1). The high pH proton uptake bands (H^+/Q_A^- and H^+/Q_B) are commonly observed in the WT RCs from *Rb. sphaeroides* and *Rb. capsulatus*. These bands disappear or are shifted in all mutants reported so far in which the interactions between charges within the cluster have been modified (Brzezinski *et al.*, 1997; Miksovska *et al.*, 1997; Miksovska *et al.*, 1996; Maróti *et al.*, 1995; McPherson *et al.*, 1994). This high pH proton uptake band has been attributed to a change in the ionization state of GluL212 (Brzezinski *et al.*, 1997; Miksovska *et al.*, 1997; McPherson *et al.*, 1994). In fact, a simple mathematical model, which describes the interactions of three or four (or more) strongly interacting acidic groups, suggests that this band rather belongs to the whole cluster and arises from a cumulative effect of pair-wise pK_a shifts of the different components within the cluster (Pierre Sebban, Laura Baciou and Jérôme Lavergne, unpublished data).

PROTON UPTAKE UPON Q_A^- AND Q_B^- FORMATIONS: MODULATION OF THE HIGH PH SIGNATURE OF THE Q_B CLUSTER BY MUTATIONS LYING BETWEEN Q_B AND Q_A





Figures 1 (left) and 2 (right):

Fig. 1: Stoichiometries of proton uptake by the PQ_A^- (top) and PQ_B^- (bottom) states in *Rb. capsulatus* reaction centres isolated from the WT (■), the AA (•), the AA+M247Ala→Tyr (o) and the M247Ala→Tyr (open square) mutants. The data points presented are combined results obtained with a glass electrode and pH sensitive dyes. Conditions: : ~ 2µM RCs, 0.03 % Triton X-100, 50 mM NaCl, buffers and dyes depending on pH (see Methods); top, + 100 µM terbutryn; bottom, + 75 µM UQ_6. The lines through the points are derived from fitting the data points with a model of four protonatable interactive groups. The error bars reflect the respective experimental error of each set of measurements.

Fig. 2: pH dependence of the stoichiometries of proton uptake by the PQ_A^- (A) and PQ_B^- (B) states in RCs of the wild type (■) *Rb. sphaeroides* , the L209PE (□), the L209PT (∇), the L209PY(○), the L209PW (◊) and the L209PF (Δ) mutants. Conditions, same as Fig. 1.

As shown in Fig. 1, the addition of the compensatory mutation M247Ala→Tyr to the AA Q_B pocket restores the WT like proton uptake profiles in either the Q_A^- and Q_B^- states. In addition, the reaction centres carrying the single M247Ala→Tyr mutation displays the same H^+/Q_A^- curve as the WT reaction centres (Fig. 1, top). This shows that the M247Tyr *per se* is not responsible for the H^+/Q_A^- high pH band. However, the role of M247Tyr in conjunction

with the rest of the protein is highlighted by the observed increased H^+/Q_B^- value as compared to the WT (Fig. 1).

The second type of mutants (from *Rb. sphaeroides*) all carrying mutations at the L209 site specifically suppress the high pH proton uptake band upon Q_A^- formation (Fig. 2) but not upon Q_B^- formation (Fig. 2, bottom). Similarly to the single M247Ala→Tyr mutation, below pH 9, the proton uptake is significantly increased as compared to the WT.

Both mutant families indicate that the presence or the absence of the high pH signature attributed to the cluster has to be interpreted cautiously. Its presence as observed in the mutant carrying the AA mutation together with the single M247Ala→Tyr mutation shows that this band is not specifically associated to the Q_B acidic cluster. In the 209 mutants, the different behavior of the H^+/Q_A^- and H^+/Q_B^- curves as regard to the high pH band led us to suggest that its absence is not correlated to a strong rearrangement within the Q_B cluster. This is supported by structural data (Kuglstatter *et al.*, 2001). It is the capacity of the cluster to respond to the Q_A^- formation that has been affected in the L209 mutants.

In both families, we observed a significant increased proton uptake upon Q_B^- formation. In the L209 mutants, it is likely that the internal proton provision in the Q_B environment cannot be triggered by the Q_A^- formation due to the breakage of the connection between the two quinone pockets, probably involving structural motifs involving proline L209. In this situation, the RCs are forced to pump the proton directly from the bulk resulting in the increased H^+/Q_B^- value. This may also be the case in the M247Tyr mutant where the increased proton uptake is likely to be due to the extension of the hydrogen bond network between Q_A and Q_B. These interactions mimic those of the acidic Q_B cluster present in the WT reaction centre. In fact, the hydroxyl group of the M247Tyr points towards a cluster of water molecules (14, 16, 18, 40, 41, 42, 94, 98, 99; PDB entry code 1PCR (Ermler *et al.*, 1994)) present in the Q_A environment which has been revealed in the WT structures (Fritzsch *et al.*, 1998; Stowell *et al.*, 1997; Ermler *et al.*, 1994). Some of these waters are likely to develop hydrogen bonds with the hydroxyl group of M247Tyr. This suggests that protons are pumped by delocalized structural entities extending between Q_A and Q_B , and probably involving H-bonds networks.

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References

Alexov, E.G. and Gunner, M.R. (1997) *Biophysical Journal*. **72**: 2075-2093.
Alexov, E.G. and Gunner, M.R. (1999) *Biochemistry*. **38**: 8253-8270.
Baciou, L. and Michel, H. (1995) *Biochemistry*. **34**: 7967-7972.
Brzezinski, P., Paddock, M.L., Okamura, M.Y. and Feher, G. (1997) *Biochim. Biophys. Acta*. **1321**: 149-156.
Ermler, U., Fritzsch, G., Buchanan, S.K. and Michel, H. (1994) *Structure*. **2**: 925-936.
Fritzsch, G., Kampmann, L., Kapaun, G. and Michel, H. (1998) *Photosynthesis Research*. **55**: 127-132.
Hanson, D.K. and Schiffer, M. (1998) *Photosynth. Res.* **55**: 275-280.
Kuglstatter, A., Ermler, U., Michel, H., Baciou, L. and Fritzsch, G. (2001) *Biochemistry*. **40**: 4253-4260.

Laible, P.D., Y., Zhang, A, L. Morris, S. W., Snyder, C., Ainsworth, S., Greenfield, M. R., Wasielewski, P. Parot, B., Schoepp, M., Schiffer, D. K., Hanson & M. C. Thurnauer (1997) Spectroscopic *Photosynt. Res.* **52**: 93-103.

Maroti, P. and Wraight, C.A. (1988) *Biochim Biophys Acta*. **934**: 329-347.

Maróti, P., Hanson, D.K., Schiffer, M. and Sebban, P. (1995) *Nature Structural Biology*. **2**: 1057-1059.

McPherson, P.H., Schönfeld, M., Paddock, M.L., Okamura, M.Y. and Feher, G. (1994) *Biochemistry*. **33**: 1181-1193.

McPherson, P.H., Okamura, M.Y., and Feher, G. (1988) *Biochim. Biophys. Acta*. **934**: 348-368.

Miksovska, J., Maróti, P., Tandori, J., Schiffer, M., Hanson, D.K. and Sebban, P. (1996) *Biochemistry*. **35**: 15411-15417.

Miksovska, J., Kálmán, L., Schiffer, M., Maróti, P., Sebban, P. and Hanson, D.K. (1997) *Biochemistry*. **36**: 12216-12226.

Rabenstein, B., Ullmann, G.M. and Knapp, E.W. (2000) *Biochemistry*. **39**: 10487-10496.

Sebban, P., Maróti, P. and Hanson, D.K. (1995) *Biochimie*. **77**: 677-694.

Stowell, M.H., McPhillips, T.M., Rees, D.C., Soltis, S.M., Abresch, E. and Feher, G. (1997) *Science*. **276**: 812-816.

Valerio-Lepiniec, M., Miksovska, J., Schiffer, M., Hanson, D.K. and Sebban, P. (1999) *Biochemistry*. **38**: 390-398.