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CHAPTER TWENTY FOUR

The Role of AspL213 for Stabilizing Semiquinone Binding to the Photosynthetic Reaction Center

Eva-Maria Krammer and G. Matthias Ullmann

Abstract The binding of the semiquinone to its two position (proximal and distal) in the Q_B site of the bacterial photosynthetic reaction center of *Rhodobacter sphaeroides*, was studied using continuum electrostatics. Experimental determined populations of the semiquinone in the proximal positions could be reproduced. Residues influencing either the binding of the semiquinone or the popluation of the semiquinone in the proximal position, could be identified. Both, the population and the binding of the semiquinone to the Q_B site is coupled to the protonation state of AspL213, which is located in direct vicinity of the semiquinone. Our results show, that a protonated AspL213 stabilizes the binding of the semiquinone and it favors the proximal position.

Keywords Reaction center, continuum electrostatics, semiquinone binding, AspL213, theory and modeling

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Introduction

As the heart of photosynthesis, the bacterial photosynthetic reaction center (RC) uses the light energy to doubly reduce a coenzyme Q (CoQ) molecule. During the light-induced reaction, two electrons and two protons are transferred stepwise to a CoQ molecule bound in the Q_B site of the reaction center. The electrons originate from the special pair formed by two bacteriochlorophyll molecules. The proton entry and the proton transfer pathway organization are still under debate, but it is consensus, that the protons are transferred to GluL212 and AspL213 in the Q_B binding site and from there to Q_B (Paddock et al. 2003). Two binding positions of CoQ in the Q_B site are known (Stowell et al. 1997; Koepke et al. 2007) distal and proximal to the non-heme iron of the RC. The proximal position is considered to be the reactive position. After the transfer of two electrons and two protons, the quinol leaves the Q_B site to the quinone pool of the membrane and is replaced by a quinone. It is known, that the quinone and the quinol are weakly bound to the Q_B site, whereas the semiquinone (first reaction intermediate) is tightly bound.

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In this study, we examined the binding behavior of the semiquinone $Q^{\cdot-}$ to the two positions in the Q_B site using continuum electrostatics. We focused on possible interactions between protein residues and the binding of $Q^{\cdot-}$.

Materials and methods

Protonation and binding probabilities. The CoQ concentration of the quinone pool in the membrane is considered in the calculations of protonation and population probabilities by extending the pH dependent state energy G^n *(pH)* (Ullmann and Knapp 1999) to a pH and CoQ concentration dependent state energy $G_l^{n,q}$ *(pH, log[CoQ])*. The energy $G_l^{n,q}$ *(pH, log[CoQ])* is determined by the conformational energy C_1, the intrinsic pK value $pK_{int,l}{}^H$ of each protonatable group, the relative dissociation constant $pK_{int,l}{}^Q$ of the CoQ binding, and the interaction energy $W_{ij,l}$ between the charged forms of each possible pair of protonatable groups and CoQ:

$$G_l^{n,q}(pH,log[CoQ]) = \sum_{i=1}^{N}(x_i^n - x_i^0)RT\ln 10(pH - pK_{int,l,i}^H) + (x_q^n - x_q^0)RT\ln 10(-log[CoQ] - pK_{int,l}^Q) + \frac{1}{2}\sum_{i=1}^{N+1}\sum_{j=1}^{N+1}W_{ij,l}(x_i^n - x_i^0)(x_j^n - x_j^0) + C_l$$

where x_i^0 is the reference protonation form (1 for acidic and 0 for basic protonatable residues) and x_i^n the actual protonation form (1 for protonated and 0 for deprotonated) of the protonatable group *i*. The intrinsic p*K* is given by the experimentally determined pK_a of the protonatable group in aqueous solution and the shift of the pK_a value due to a different solvation environment inside the protein. The CoQ binding can be defined analogously, since protonation is also a binding reaction. x_q^0 is the reference binding form (unbound, 0) and x_q^n the actual binding form (0 for unbound and 1 for bound) of CoQ.

For the electrostatic calculations, the structure of the RC of *Rhodobacter sphaeroides* in the charge-separated state (2J8D) was prepared as described before (Koepke et al. 2007) except for the CoQ_B. Here, only the semiquinone $Q^{\cdot-}$ was modeled in the Q_B site. The energetic parameters $pK^H_{int,l}$, $pK^Q_{int,l}$ and $W_{ij,l}$ were calculated by solving the linearized Poisson-Boltzmann equation with the program MEAD (Bashford and Gerwert 1992) using the same parameters as before (Koepke et al. 2007). CMCT was used to perform the Monte Carlo titration calculations.

Correlation function. To analyze the stabilization of the proximal position by a certain protonation form of a given residue, we used (Koepke et al. 2007) the correlation function $c_{ic} = \langle x_i x_c \rangle - \langle x_i \rangle \cdot \langle x_c \rangle$ ($\langle x_i x_c \rangle$ and $\langle x_i \rangle$ are the probabilities, that residue *i* is protonated when CoQ populates the proximal position and that residue *i* is protonated, respectively. $\langle x_c \rangle$ is the population of CoQ in the proximal position). A similar correlation function $c_{iq} = \langle x_i x_q \rangle - \langle x_i \rangle \cdot \langle x_q \rangle$ is introduced to analyze the mutual influence between the protonation form of a given residue and the binding state of CoQ ($\langle x_i x_q \rangle$ is the probability that residue *i* is protonated when CoQ is bound; $\langle x_q \rangle$ is the probability for CoQ to be bound and $\langle x_i \rangle$ to be protonated).

Results and discusion

Population of $Q^{\cdot-}$ in the proximal position

The population of the semiquinone in the proximal position (see Fig. 1A) is pH and log[$Q^{\cdot-}$] dependent. When $Q^{\cdot-}$ is bound ($Q^{\cdot-}$ binding curve: Fig. 1B), the proximal position is highly populated up to pH 6. Afterwards the proximal population decreases until it reaches a population of 0.3 for the proximal position at pH 12. The observed proximal populations are in good agreement with the earlier measured and calculated populations (Koepke et al. 2007).

At pH 6 the proximal $Q^{\cdot-}$ population starts to decrease, because it is coupled to the protonation of AspL213, which starts to depronate at this pH value (see Fig. 1C). The coupling between the

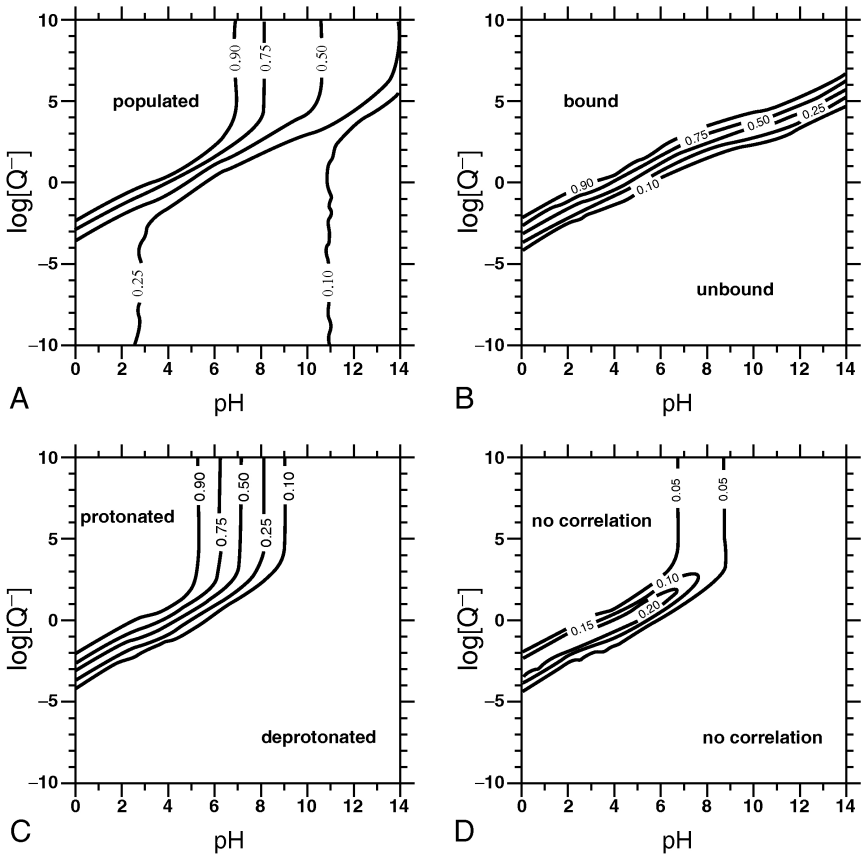

Fig. 1 (A) Population of Q^{-} in the proximal position. At low pH Q^{-} mainly populates the proximal position when Q^{-} is bound (see Fig. 1B). With increasing pH the proximal Q^{-} population decreases until it reaches 0.3 at pH 12. (B) Q^{-} Binding. The Q^{-} binding is pH and log[Q^{-}] dependent. At low pH Q^{-} binding starts at a log[Q^{-}] of around 5 whereas at high pH it starts to bind around 0. (C) Titration curve of AspL213. If Q^{-} is unbound (see Fig. 1B), AspL213 is deprotonated, whereas with bound Q^{-}, AspL213 is protonated at low pH and starts to deprotonate at pH 6. (D) Correlation c_{ic} of AspL213 and proximal population. In the pH/log[Q^{-}] region where the Asp L213 deprotonates (see Fig. 1C), a high correlation with the proximal Q^{-} population is observed, indicating that the protonation of AspL213 is coupled with the proximal Q^{-} population

proximal $Q^{\cdot -}$ population and the protonation of AspL213 is shown by a high correlation c_{ic} in the corresponding pH/log[$Q^{\cdot -}$] region (for the correlation curve see Fig. 1C). Thus, a protonated AspL213 is needed to keep the semiquinone in the reactive, proximal position.

Binding of Q^{-} to the RC

Like the population of the semiquinone in the proximal position, also $Q^{\cdot -}$ binding is pH dependent, indicating that the binding depends at least on the protonation form of one protonatable residue. Using the correlation function c_{iq}, AspL213 and GluL212 could be identified to be the major factors determining the pH dependence of the binding. Both residues are located in the Q_B binding site (see Fig. 2A) and are known to be involved in proton transfer to Q_B (Paddock et al. 2003).

At high pH (11–14) GluL212 starts to deprotonate (see Fig. 2B), leading to a correlation c_{iq} with $Q^{\cdot -}$ binding. Thus, a protonated GluL212 favors $Q^{\cdot -}$ binding, meaning that $Q^{\cdot -}$ is still bound at lower $Q^{\cdot -}$ concentrations (see Fig. 1B) in the pH range of 0–11, compared to the pH of 11–14 where GluL212 starts to depronate.

When $Q^{\cdot -}$ is bound (see Fig. 1B), AspL213 is protonated at low pH and starts to deprotonate at

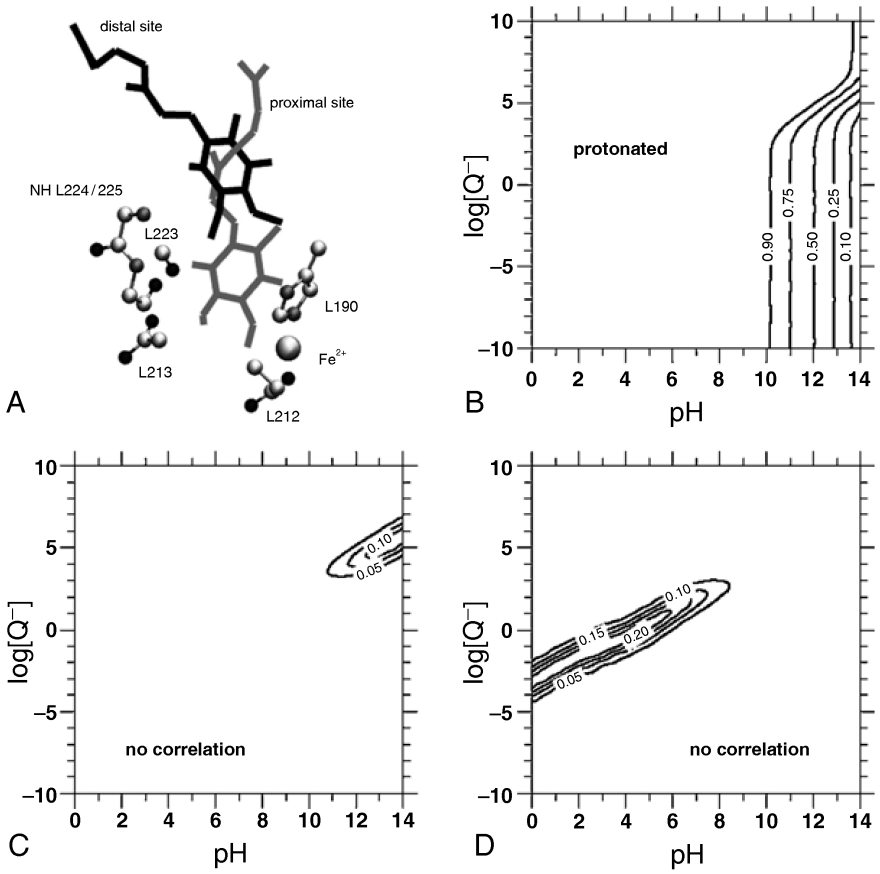

Fig. 2 (A) Position of GluL212 and AspL213 in the Q_B site. The proximal (grey) and distal (black) position of CoQ as well as possible hydrogen bond partner of CoQ in the Q_B site (HisL190, AspL213, GluL212, SerL223, and the backbone of L224 and L225) and the non-heme iron are depicted. Carbon, nitrogen, oxygen and iron atoms are shown in white, grey, black and as a white sphere, respectively. (B) Titration curve of Glu212. In absence of $Q^{\cdot}$ GluL212 starts to deprotonate at high pH. When $Q^{\cdot-}$ is bound (see Fig. 1B) GluL212 stays protonated. (C) Correlation c_{iq} of GluL212 and $Q^{\cdot-}$ binding. A correlation of the protonation of GluL212 and $Q^{\cdot-}$ binding is observed when Glu212 deprotonates (see Fig. 2B) indicating, that a protonated GluL212 stabilizes the $Q^{\cdot-}$ binding. (D) Correlation c_{iq} of AspL213 and $Q^{\cdot-}$ binding. A high correlation between the protonation of AspL213 and the $Q^{\cdot-}$ binding is seen in the pH/log[$Q^{\cdot-}$] region where AspL213 starts to deprotonate, indicating that the protonated AspL213 is coupled to $Q^{\cdot-}$ binding

pH 6 to 8, leading to high c_{iq} values. Such a high c_{iq} indicates, that a protonated AspL213 keeps the semiquione bound in the RC.

Stabilization of $Q^{\cdot-}$ in the proximal site by Aspl213

Mutational studies showed, that bacteria containing RC without AspL213 (mutated to asparagin) are not able to grow photosynthetically, even though first electron transfer rates are measurable in these mutant RCs (Rongley et al. 1993).

AspL213 has a dual role during the light induced reaction (Paddock et al. 2003): it transfers a proton to Q_B and by its negative charge, a proton is stabilized in the environment of Q_B. The data presented here suggest a third role for AspL213: a protonated AspL213 is required to keep the semiquinone bound to the proximal, the reactive, position in the Q_B site of RCs of *Rhobobacter sphaeroides*.

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