Revealing the Involvement of Extended Hydrogen Bond Networks in the Cooperative Function between Distant Sites in Bacterial Reaction Centers*

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In reaction center proteins of photosynthetic bacteria, the amplitude of proton uptake induced by the one-electron reduction of either of the two quinone electron acceptors (QA and QB) is an intrinsic observable of the electrostatic interactions associated with the redox function of the complex. We report here that, in Rhodobacter capsulatus, complete restoration of proton uptake (upon formation of QA and QB) to the level found in the wild type is observed in a mutant reaction center in which a tyrosine substitution in the QA environment (AlaM247 → Tyr) is coupled with mutations of acidic residues near QA (GluL212 → Ala/AspL213 → Ala) that initially cancel the proton uptake above pH 8. This result demonstrates that proton uptake occurs by strong cooperation between structural motifs, such as hydrogen-bonded networks, that span the 18 Å distance between the two quinone acceptors.

In photosynthetic bacteria, light energy is converted into chemical free energy by the reaction center, a transmembrane protein cofactor complex. The reaction center is composed of the L, M, and H proteins. The L and M subunits carry all the cofactors involved in the initial electron transfer chain. The first photochemical event, which initiates the rapid photo-oxidation of a dimer of bacteriochlorophylls (“P”), results in the formation of QA (H+/QA) and the QB (H+/QB) states, respectively. In the native reaction centers from Rhodobacter capsulatus and Rhodobacter sphaeroides, it has previously been reported that for both the QA and QB states, this cluster (in which a main participant is GluL212) has a signature redox behavior, with a high pH dependence of the one-electron transfer process (3–5).

We show here in R. capsulatus, a wild-type pattern of the H+/QA and H+/QB proton uptake pH dependence is fully restored in the AA double mutant when AlaM247 has been mutared to Gln or Ala (9–12), in the AspL213 → Asn mutant (9) and in the double mutant GluL212 → Ala/AspL213 → Ala (“AA”) (11, 13).

Materials and Methods

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The GluL212 → Ala/AspL213 → Ala double mutant has been described previously (14).

Proton Uptake Measurements—Reaction centers were prepared as described previously (11). The photoexcitation of the protein was triggered by one saturating (Yag) laser flash at 532 nm (12). The H+/QA and H+/QB proton uptake stoichiometries were measured in the presence of 100 μM ferrocene as electron donor to P+ and 300 μM ferrocyanide. In both types of measurements, pH electrodes and pH-sensitive dyes were combined. When using pH-sensitive dyes, the proton uptake by the RCs was measured by following the absorption changes at 585 nm (isosbestic point of P absorption changes). The calibrations were performed by additions of known amounts of HCl (1 M stock; Merck). The H+/QA proton uptake stoichiometries were measured in the presence of tertbutryn (100 μM), which prevents the binding of QA

The proton uptake by the PQAQB state (ΔHQAQB) is deduced from the measured value after one flash (ΔHobs) according to the following equation (10).

$$\Delta H'_{QAQB} = \Delta H_{obs} - (\delta + \alpha(1 - \delta))\Delta H'_{QA}$$

(ΔH', the proton uptake by the RC in the absence of QA. The fraction of RCs without QA activity (δ) and the partition coefficient between the QA and QB states (α) were determined from the P'/Q' → P'QB charge recombination kinetics monitored at 430 nm.

The conditions were as follows: 2 μM reaction centers, 50 mM NaCl, 0.05% Triton X-100, 200 μM ferrocene, 40 μM dye (bromocresol purple, phenol red, cresol red, or o-cresol phthalein depending on the pH). The buffer concentration was kept below 10 mM by extensive dialysis. The buffers (<10 μM) used were MES (Sigma) between pH 5.5 and 6.5, Bis-Tris propane (Sigma) between pH 6.3 and 9.5, and CAPS (Calbiochem) above pH 9.5.

RESULTS AND DISCUSSION

The Additional AlaM247 → Tyr Mutation Restores the WT Proton Uptake Patterns in the AA Mutant—The residues in the QA pocket that are symmetry-related to GluL212 and AspM213 are AlaM246 and AlaM247. To reverse this charge asymmetry, a photosynthetically incompetent quadruple mutant (GluL212 → AlaM246 → AlaM247 → Glu/AlaM247 → Asp) was constructed in R. capsulatus (15). A spontaneous phenotypic revertant carrying the AlaM247 → Tyr mutation near QA was isolated (15, 16). We investigate here the electrostatic interactions probed by the stoichiometries of proton uptake in engineered mutants carrying the AlaM247 → Tyr mutation in combination with either a WT or AA QB pocket. The H+/QA and H+/Q0 stoichiometries in the reaction centers of the TyrM247 and the AA + TyrM247 mutants are presented in Fig. 1, a and b. The H+/QA and the H+/Q0 stoichiometries were previously described for the WT (13, 17) and AA mutant reaction centers (13); they are also displayed in Fig. 1, a and b. At neutral pH, the H+/QA and the H+/Q0 curves of the WT and AA mutant reaction centers are superimposable, within the experimental error. However, above pH 7.5–8.0, at variance to the data from the WT reaction centers, in the AA mutant reaction centers, the H+/QA and H+/Q0 proton uptake drops to reach a value close to 0. As displayed in Fig. 1, a and b, the addition of the compensatory mutation AlaM247 → Tyr to the AA QB pocket restores the proton uptake profiles in the QA and Q0 states to those observed for the WT reaction centers. Interestingly, reaction centers carrying the single AlaM247 → Tyr mutation display the same H+/QA curve as the WT reaction centers (Fig. 1a), suggesting that proton uptake at high pH in the AA + TyrM247 mutant is not due to the tyrosine per se. The role played by the association of TyrM247 with its environment in the overall proton uptake response of the protein is highlighted in the notably increased H+/Q0 stoichiometry measured in the TyrM247 single mutant as compared with the WT (Fig. 1b). Indeed, the H+/Q0 values measured in the single mutant varies in the range 0.9–0.8 from pH 6 to 9.5.

Involvement of Hydrogen Bond Networks—The restoration of a WT-like high pH band in both the H+/QA and H+/Q0 stoichiometries by the addition of the AlaM247 → Tyr mutation to RC proteins lacking GluL212 and AspM213 suggests that the proton uptake above pH 8 is not specifically associated with the QB cluster. Instead, this high pH signature reflects the existence of a wide network, extending over the entire cytoplasmic side of the protein, in which strong delocalized interactions take place. To structurally investigate the modifications induced by the mutations, we have crystallized reaction centers of the equivalent mutant of R. sphaeroides (AA + TyrM240).3 The three-dimensional structure of the quinone region of this mutant is presented in Fig. 2. The difference electron density map suggests a position for the tyrosine side chain that is close to the one that was modeled previously (16). In fact, the side chain is located in a cavity that is filled with a cluster of water molecules that is seen in structures of WT reaction centers (18–20). It replaces water molecules W14, W16, and W98 identified in the WT structure of Ermiller et al. (19); Protein Data Bank entry code 1per). Some of these waters are likely to develop hydrogen bonds with the hydroxyl group of TyrM247. Interestingly, this water cluster is connected to water chains closer to QA that join QA and the Q0 cluster to the QA environment. Restoration of wild-type levels of H+/QA and H+/Q0 proton uptake in the AA + TyrM247 mutant reaction centers suggests that TyrM247 induces interactions that

3 P. R. Pocculluli, P. D. Laible, J. L. Ford, D. K. Hanson, and M. Schiffer, unpublished data.
mimic those of the acidic Q_B cluster present in the WT reaction center and that these hydrogen bond networks are involved in this process.

In the WT, the interactions and the associated proton uptake events that differentially stabilize Q_A and Q_B are likely to be ensured by a hydrogen-bonded network that strongly couples the Q_B cluster to the Q_A environment, 18 Å away.

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