Charge Separation and L209 Mutation Give Rise to Similar Conformational Changes in the Reaction Center from *Rhodobacter sphaeroides*

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**Introduction**

Site-directed mutagenesis at position L209 is a suitable tool to study the electron and proton transfer reactions in the neighborhood of the secondary quinone Q₉ from *Rhodobacter sphaeroides* RCs. The native proline residue is located 9 Å away from the Q₉ molecule and can be assumed not to interact directly with Q₉ even when the proline is replaced by aromatic amino acid residues. Large structural changes in the backbone around the position L209 are not expected, since Pro L209 is not conserved in *Rhodopseudomonas viridis* RCs where it is replaced with an alanine residue.

**Materials and Methods**

The mutant RCs were purified and crystallized as reported earlier (Baciou and Michel 1995; Ermelr et al. 1994; Fritzsch 1998). RC crystals with a maximum size of 2.0 x 1.0 x 1.0 mm³ grew within about two weeks. They belong to space group P3₁2₁ with unit cell dimensions a = b = 141.7 Å, c = 187.2 Å. Data collection and model refinement have been described in Kuglstatter et al. (2001).

**Results**

In the RC structures from the L209PY (PDB entry code 1F6N), the L209PF (1FNP), and the L209PE (1FNQ) variants, the arrangements of the protein backbone are conserved compared to the wild-type RC (1PCR). Structural shifts in the RC variants are observed in the vicinity of the exchanged amino acid residues and around the binding pocket of Q₉ (Figure 1). The side chains of the introduced Tyr L209 and Phe L209 point towards Asp L213 and induce a shift of the Asp L213 carboxy group by ~1.7 Å compared to its position in the wild type RC (Figure 1a and 1b). In addition, Glu H173 and Thr L226 are displaced by ~2.6 Å and ~1.2 Å, respectively. A hydrogen bond not observed in the wild-type RC is formed between the Glu H173 and Arg H177 side chains with a bond length of ~2.6 Å. In the L209PY mutant RC, a remarkable shift of 4 Å is observed for the secondary quinone molecule that assumes a position *proximal* to Fe²⁺ in the neutral state while it is located at the *distal* binding site in the neutral native RC (Figure 1a).
Fig. 1. Electron density map (1.0 σ) near the Q₈ binding site. The wild type structure is shown in red (PDB entry code 1PCR). (a) yellow: L209PY RC, (b) green: L209PF RC, (c) blue: L209PE RC.
The structural data do not allow us to determine the orientation of the UQ\textsubscript{10} ring in the Q\textsubscript{B} binding site. In our model of the L209PY variant, the keto oxygen of Q\textsubscript{B}, which is bound distally to Fe\textsuperscript{2+}, is in hydrogen bonding distance to the Ser L223 hydroxy oxygen and to the Ile L224 peptide nitrogen, the distal methoxy oxygen forms a hydrogen bond to the Gly L225 peptide nitrogen, and the proximal keto oxygen forms a hydrogen bond to His L190. In the L209PF variant, the best fit of the Q\textsubscript{B} molecule is obtained for a position of the quinone head-group between the proximal and the distal binding sites (Figure 1b).

In contrast to the L209PY and L209PF mutant RCs, no significant structural changes are observed in the L209PE structural model (Figure 1c). Glu L212 and the introduced Glu L209 are 2.8 Å apart and the relative orientations to each other indicate the formation of a hydrogen bond between these residues, suggesting that at least one of them is protonated.

**Fig. 2.** Hydrogen bonding pattern at the Q\textsubscript{B} binding sites of (a) L209PY RC, (b) L209PF RC, (c) L209PE RC, and (d) charge-separated wild-type RC (PDB entry code 1AIG; Stowell et al. 1997). The Ile L224 side chains are omitted for clarity. Distances are given in Å.
Discussion

In the neutral state of the wild-type RC, QB is predominantly bound at a position distal to Fe^{2+} (Ermler et al. 1994, Stowell et al. 1997) whereas it is located at the proximal position in the charge-separated, native RC (Stowell et al. 1997) as well as in the neutral L209PY RC (Kuglstatter et al. 2001). It has been proposed that the first electron transfer to QB measured in native RCs might be rate limited by a gating step attributed to the translation movement of QB from the distal to the proximal binding position (Stowell et al. 1997, Graige et al. 1998). However, in the L209PY mutant RC, the rate of the first electron transfer to QB does not differ significantly from the rate of the wild-type RC (Baciou and Michel 1995) suggesting that the rate limiting step of the first electron transfer is not correlated to the postulated movement of the QB molecule from the distal to the proximal position after charge separation.

In the L209PY and L209PF mutant RCs, the presence of the bulky aromatic groups between the side chains of Asp L213, Glu H173, and Thr L226 causes very similar conformational changes of these residues. In both RC variants, the lengthening of the hydrogen bonds between Glu H173 and Thr L226 is obviously offset by the formation of a hydrogen bond between Glu H173 and Arg H177 that is not present in the wild-type RC. This similarly organized hydrogen bonded pattern in both aromatic variants is consistent with the similar proton uptake kinetics of both mutant RCs (Baciou and Michel, 1995; Tandori et al., submitted). The first and second electron transfer rates, however, are different for these two mutants (Baciou and Michel, 1995). We assume that the different electron transfer rates result from different effects of the introduced aromatic side chains (Tyr L209 or Phe L209) on the protonation states of the surrounding ionizable residues, in particular of Asp L213.

Taken as a whole, our findings indicate that the structural organization of the hydrogen bonded network determines the proton transfer rates while the electrostatic properties related to the protonation state of the ionizable residues present in the QB pocket influence the electron transfer reactions.

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