FINAL FOR SUBMISSION

COMPENSATION FOR L212GLU IN BACTERIAL REACTION CENTERS

D. K. Hanson[1], Y.-L. Deng[1], P. Sebban[2], and M. Schiffer[1]. [1]Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, Argonne, IL USA; [2]Centre de Génétique Moléculaire, CNRS, Gif/Yvette, France

1. Introduction

In wild-type bacterial reaction centers (RC), residue L212Glu, which is located about 5 Å away from Q_B, is involved in the delivery of the second proton to Q_B^{2-} [1-4]. We previously constructed the L212Glu-L213Asp → Ala-Ala double mutant of *Rhodobacter capsulatus*, and it is incapable of photosynthetic growth (PS^-) due to interruption of the proton transfer pathway to Q_B[3,4]. We have isolated several photocompetent (PS^+) phenotypic revertants of this L212-L213AA double mutant [3-7]. The compensatory mutations that restore function in these strains are diverse and show that neither L212Glu nor L213Asp is absolutely required for efficient light-induced electron or proton transfer. Genotypic revertant and second-site mutations, located within the Q_B binding pocket or at more distant sites, can compensate for mutations at L212 and L213 to restore photocompetence. One of the phenotypic revertants of the L212Ala-L213Ala double mutant carries a genotypic reversion of L213Ala to Asp; the Ala substitution at L212 remains. We were intrigued that this L212Glu → Ala mutant of *R. capsulatus* is photocompetent, while the L212Glu → Gln mutant of *R. sphaeroides* is not, particularly since the sequence identity in the Q_B site of these two strains is 90% [8]. To this end, we constructed the L212Glu → Gln mutant in *R. capsulatus*, and it is also PS^-. To determine the function that is lost in the L212Gln mutant but restored by Ala at that site, we selected four PS^+ revertants from the L212Gln strain.

2. Results and Discussion

None of the revertants carried the single base transversion that would restore the wild-type Glu codon; each carried a different second-site substitution in addition to the original L212Gln mutation. Complementation mapping [5] was used to determine that this single second-site mutation was responsible for restoration of the PS^+ phenotype. The compensatory mutations carried by the phenotypic revertants are: Rev2: L228Gly → Asp; Rev4: M231Arg → Cys; Rev5: L227Leu → Phe; Rev6: L231Arg → Cys. Among the suppressor mutations of the L212Ala-L213Ala double mutant were some mutations that are similar to Rev4, that have been shown to restore the PS^+ phenotype by increasing the negative environment surrounding Q_B, sometimes from a large distance [3,4,7]. Therefore, we have also constructed plasmids that coupled some of these other compensatory mutations with the L212Glu → Gln mutation: L212Gln + M5Asn → Asp; L212Gln + M43Asn → Asp; L212Gln + M231Arg → Leu. These substitutions also restored the PS^+ phenotype to varying extents. Their relative growth rates under photosynthetic conditions are summarized in Table 1.

Table 1. Relative photosynthetic growth rates of strains carrying mutations which compensate for loss of L212Glu.

Strain	Growth
Wild-type	++++++
L212Glu → Gln[e]	–
Rev2 (L212Gln + L228Gly → Asp)[s]	++
Rev4 (L212Gln + M231Arg → Cys)[s]	++++
Rev5 (L212Gln + L227Leu → Phe)[s]	+++++
Rev6 (L212Gln + L231Arg → Cys)[s]	+++
L212Gln + M5Asn → Asp[c]	+++
L212Gln + M43Asn → Asp[c]	+
L212Gln + M231Arg → Leu[c]	++++

[e]site-specific mutation
[s]spontaneous phenotypic revertant
[c]constructed

It is likely that the photocompetence of the L212Glu → Ala strain, as opposed to the PS$^-$ phenotype of the L212Glu → Gln mutant, is due to the size difference between the side chains. The Ala substitution could accomodate a water molecule which could assume the role played by L212Glu in proton delivery in the wild type, similar to observations with the L223Ser → Ala (PS$^-$) and L223Ser → Gly (PS$^+$) mutants of *R. sphaeroides* [9,10].

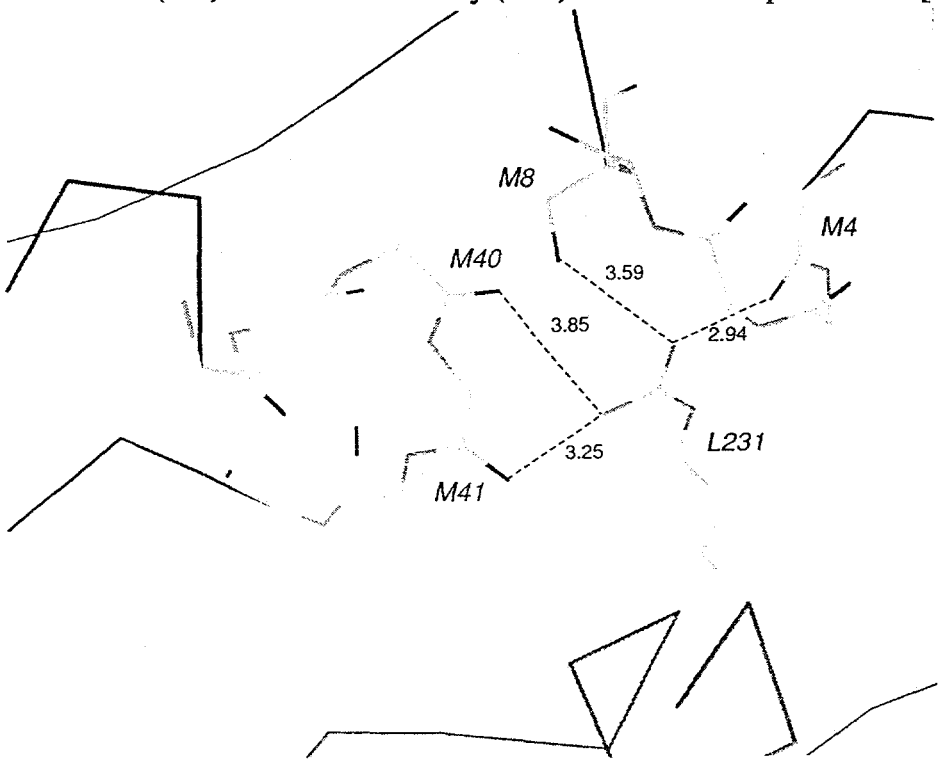

Figure 1. Molecular model, using the *R. sphaeroides* RC structure [11] of hydrogen bonds between L231Arg (→ Cys in Rev6) and residues in two loops of the M chain.

The mechanism(s) of suppression of these second-site mutations is not yet clear, but it may involve some displacement of the protein backbone to allow a water molecule to be accomodated near Q_B. Residues L227Leu and L228Gly are conserved in five of the six strains of photosynthetic bacteria whose RC genes sequences are known; L231Arg and M231Arg are strictly conserved. Modeling of these substitutions using the *R. sphaeroides* RC structure [11] shows that some of these residues lie at the interface of the L and M chains, thus the mutations might cause chain rearrangement as was observed for the L222Tyr → Phe mutant of *R. viridis* [12]. In particular, residue L231Arg [Fig. 1] forms hydrogen bonds to the backbone carbonyl oxygen atoms of residues M4, M8, M40, and M41, thus acting to tether this segment of the L chain near Q_B to two different loops of the M subunit that are part of the second sphere of residues that sequesters Q_B from the external aqueous environment. Loss of these hydrogen bonds could lead to a major rearrangement of this region of the RC, which could change the orientation of protonatable residues and the structure of internal water molecules near Q_B to generate a substitute proton delivery pathway.

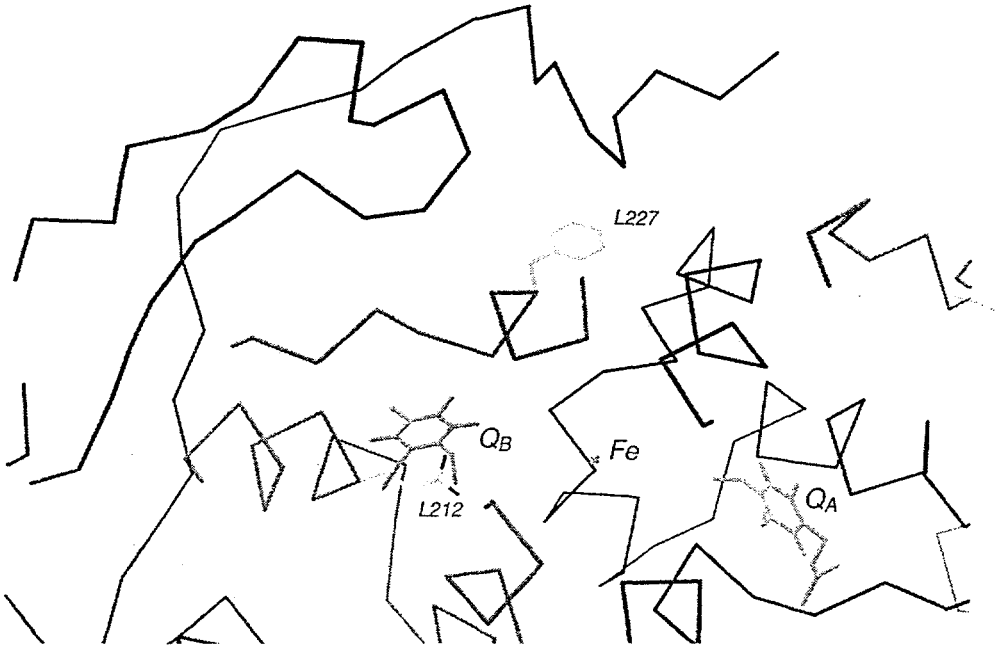

Figure 2. Molecular model, using the *R. sphaeroides* RC structure [11] showing replacement of L227Leu by Phe.

Modeling of the replacement of residue L227Leu with Phe using the program "O" [13] shows that this large aromatic residue can be tolerated in this region of the structure [Fig. 2]. This residue could lie within 3.5 Å of L231Arg. π-orbital interactions between L227Phe and L231Arg could cause L227Phe to be pulled slightly towards the Arg, thus causing a displacement of that chain segment away from the quinone. The L228Gly → Asp replacement is difficult to accomodate without rearrangement of the L chain backbone.

The replacement of M231Arg by a non-protonatable residue has been observed before as a mutation that compensates for the loss of L213Asp [3,6]. Loss of M231Arg breaks a salt bridge between that residue and two glutamic acid residues of the H chain. The pH dependence of K_2, the Q_A-Q_B ↔ Q_AQ_B- equilibrium constant, is altered by this mutation, revealing differences in the pKs of titratable groups in this strain compared to the wild type

[3,7]. This residue is 15 Å away from Q_B, and we have proposed that the breakage of the salt bridge as a result of the mutation causes realignments of salt bridges within the network of charge-charge interactions in this region of the RC, thereby partially relocating the negative charge much closer to the quinone [7]. An analogous mechanism may be proposed to explain the electrostatic effects of the M5Asn → Asp (13 Å away from Q_B) and M43Asn → Asp (9 Å away from Q_B) substitutions in restoring proton transfer to Q_B in strains which contain Ala residues at L212 and L213 [7, and Hanson et al., unpublished]. When they are coupled with the L212Glu → Gln mutation, these substitutions must be acting in a comparable manner to restore proton transfer, thereby suppressing the PS^- phenotype. In the case of RCs carrying these electrostatic substitutions, the proton transfer pathways must be different than that of the wild type, or that of the L212Ala mutant where function of L212Glu might be restored by a water molecule.

We thank George Johnson for the figures. M. S., Y.-L. D, and D. K. H. were supported by U. S. Dept. of Energy, OHER Contract No. W-31-109-ENG-38 and by U. S. PHS Grant GM36598. P. S. was supported by NATO grant CRG920725 and NSF-CNRS grant CDP900350.

References

1 Paddock ML, Rongey SH, Feher G, Okamura MY (1989) *Proc. Natl. Acad. Sci. USA* 86, 6602-6606.
2 Takahashi E, Wraight CA (1992) *Biochemistry* 31, 3, 55-866.
3 Hanson DK, Baciou L, Tiede DM, Nance SL, Schiffer M, Sebban P (1992) *Biochim. Biophys. Acta* 1102, 260-265.
4 Maróti P, Hanson DK, Baciou L, Schiffer M, Sebban P (1994) *Proc. Natl. Acad. Sci. USA* 91, 5617-5621.
5 Hanson DK, Nance SL, Schiffer M (1992) *Photosyn. Res.* 32, 147-153.
6 Hanson DK, Tiede DM, Nance SL, Chang C-H, Schiffer M (1993) *Proc. Natl. Acad. Sci. USA* 90, 8929-8933.
7 Sebban P, Maróti P, Schiffer M, Hanson DK (1995) *Biochemistry* 34, 8390-8397.
8 Schiffer M, Chan C-K, Chang C-H, DiMagno TJ, Fleming GR, Nance S, Norris J, Snyder S, Thurnauer M, Tiede DM, Hanson DK. (1992) In: *The Photosynthetic Bacterial Reaction Center: Structure, Spectroscopy, and Dynamics* (J. Breton and A. Vermeglio, eds.), NATO ASI Series, Plenum Press, London, pp. 351-361.
9 Paddock ML, McPherson PH, Feher G, Okamura MY (1990) *Proc. Nat. Acad. Sci. USA* 87, 6803-6807.
10 Paddock ML, Feher G, Okamura MY (1995) *Biophys. J.* 68, A246.
11 Chang, C-H, El-Kabbani O, Tiede D, Norris J, Schiffer M (1991) *Biochemistry* 30, 5352-5360.
12 Sinning I (1992) *TIBS* 17, 150-154.
13 Kleywegt GJ, Jones TA (1994) *Acta Crystallogr.* 50, 178-185.