EFFECTS OF CAVITIES IN THE BACTERIAL REACTION CENTER

M. Schiffer, Y.-L. Deng, A. Marrufo, & D.K. Hanson.
Center for Mechanistic Biology & Biotechnology,
Argonne National Laboratory, Argonne, IL 60439, USA

1. Introduction

A site-specific double mutant of *Rhodobacter capsulatus*, in which the large aromatic residues M208Tyr and L181Phe in the interior of the photosynthetic reaction center (RC) complex were replaced by smaller theonine residues, showed a dramatic reduction in the number of assembled complexes and was incapable of photosynthetic growth. The cavity created by the smaller side chains interferes mostly with the assembly of the complex. Phenotypic revertants were recovered in which a spontaneous second-site mutation restored photocompetence in the presence of the original site-specific mutations. In these strains, an Ala to Pro substitution in a neighboring transmembrane helix (at M271) resulted in an increased yield of RC complexes. To test the hypothesis that the original phenotype was due to a cavity, other mutants were constructed where L180Phe and M207Leu were replaced with alanines that created similar-sized voids at other positions in the membrane-spanning interior. The L180Ala-M207A mutant had the same phenotype. Coupling of the above proline substitution to these new cavity mutants also resulted in photocompetant strains that carry increased levels of RC complexes. Therefore, the proline substitution at M271 serves as a global suppressor of the phenotype caused by these internal cavities. The proline substitution slightly increases the thermal stability of the complex at higher temperatures, but both mutant and suppressor strains have about the same stability at the optimal culture temperature, where both are less stable than the wild-type strain. Therefore, the proline substitution may suppress the nonphotosynthetic phenotype of cavity mutants by facilitating folding of the nascent polypeptides as they assemble with cofactors to form the transmembranar RC complex. The proline replacement occurs at a pre-existing kink in a transmembrane helix where it can be accommodated without introducing a strain in the structure. The function of prolines in transmembrane helices might be to promote folding and/or assembly in general (1).

2. Result and Discussion

To further our understanding of the effect of cavities, we studied phenotypic revertants of cavity generating mutants, and have created voids at additional sites in the RC with site-specific mutagenesis.

i. We have selected and genotypically characterized phenotypic revertants of the PS^- L180Ala - M207Ala double mutant. Surprisingly, none carried the M271Ala $\rightarrow$ Pro mutation, which we found previously to suppress its PS^- phenotype. Two independently isolated revertants carried the M207Ala $\rightarrow$ Glu mutation (L180Ala was still present), while two others carried a suppressor mutation, M211Ala $\rightarrow$ Val, and retained the L180Ala-M207Ala site-specific mutations. Both M207Glu and M211Val could be acting to suppress the PS^- phenotype by partially filling the cavity created by the original L180Ala-M207Ala mutations, suggesting that indeed the cavity was causing the problem with this mutant.

ii. The L187Phe $\rightarrow$ Ala and M214Leu $\rightarrow$ Ala mutations, when combined, created a cavity at the L-M subunit interface at the level of the bacteriopheophytins. This cavity does not significantly alter the function or assembly of the RC complex. Though this mutant is analogous to the previously described AA mutant (L180Phe $\rightarrow$ Ala + M207Leu $\rightarrow$ Ala), the cavity created (Figure 1) is not as deeply buried in the complex, and it is further away from the special pair.

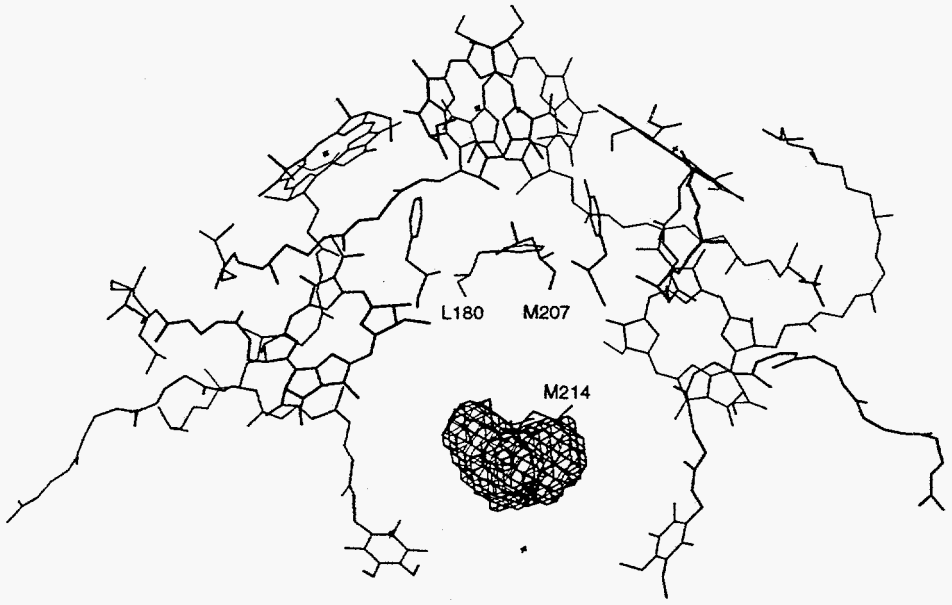

Figure 1. Cavity generated by the replacement of L187Phe and M214Leu by alanines, calculated and displayed by program VOIDOO (2) with the wild-type *Rhodobacter sphaeroides* reaction center structure (3). Side chains of wild-type residues are shown.

iii. We also replaced conserved tryptophan residues at the symmetry-related positions L156 and M183 (Figure 2), which are located near the special pair close to the periplasmic surface of the RC, with phenylalanines or alanines. The phenylalanine substitution slightly affected the growth phenotype, but more severe consequences resulted from substitution with the smaller alanines, especially the mutation at L156 on the active side.

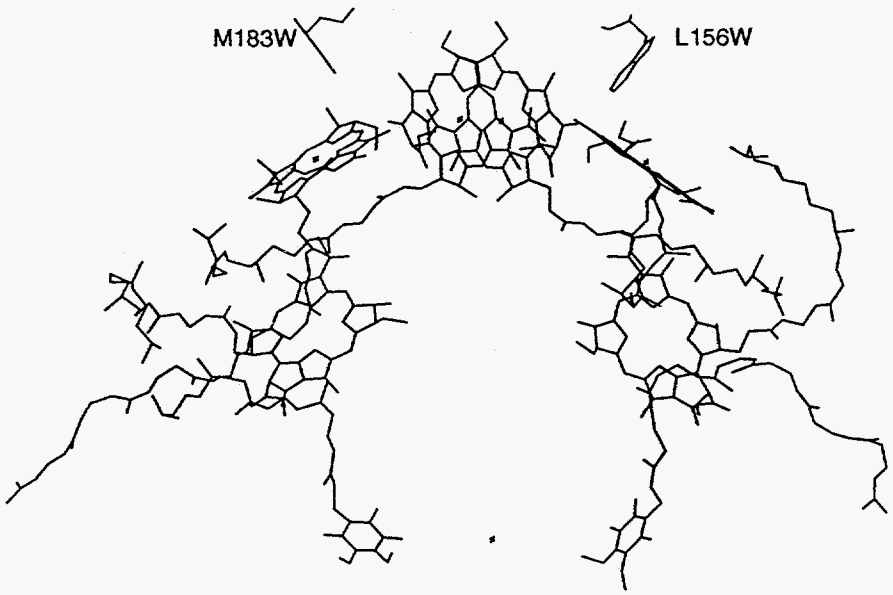

Figure 2. Location of the large M183 and L156 tryptophan residues near the periplasmic surface of the reaction center. Cavities which border the "special pair" bacteriochlorophyll dimer (primary electron donor) are generated by replacement of these residues with alanines.

The photocompetence of the family of alanine mutants is: L156Ala-M183Ala < L156Ala < M183Ala < wild type. The L156Ala-M183Ala double mutant is incapable of photosynthetic growth and contains approximately 36% the amount of RC complexes as the wild type. We coupled the M271Ala → Pro global suppressor mutation with the L156Ala-M183Ala double mutant to see if it enhanced the yield of RCs in this strain, but no increase in the number of RC complexes was observed. This shows that the effect of the cavity created by the Trp to Ala substitution is different in these mutants than in the cavity generating mutants we described previously. Selection of phenotypic revertants is in progress.

3. Conclusions

Our results indicate that the effect of a cavity in the reaction center depends on its location. Cavities near the special pair interfere with photosynthetic growth, while similar cavities distant from the special pair do not. We have observed previously that lack of photosynthetic growth can depend on interference with RC function (4), or interference with assembly (1) which results in insufficient numbers of RCs in the photosynthetic membrane. The substitution of proline at M271, at a bend of the helix, appears to counteract a defect in folding and/or assembly of the complex (1). Since it does not restore photosynthetic growth lost by the Trp to Ala mutation at L156 and M183, therefore the mutations at M183 and especially at L156 are likely to influence the RC function. Spectroscopic studies of these mutants will be necessary to show the functional effects of these mutations. Since these mutations are located between the special pair of bacteriochlorophylls and the monomeric bacteriochlorophylls they might influence the electron transfer between them, and shed new light on the very fast 3ps electron transfer step.

4. Acknowledgement

We thank George Johnson for making the figures. This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38 and by Public Health Service Grant GM36598.

5. References

1 Schiffer, M., Ainsworth, C.F., Deng, Y.-L., Johnson, G., Pascoe, F.H. and Hanson, D.K. (1995) J. Mol. Biol. in press

2 Kleywegt, G.J. and Jones, T.A. (1994) Acta Crystallogr. 50, 178-185

3 Chang, C.-H., El-Kabbani, O., Tiede, D.M., Norris, J. and Schiffer, M. (1991) Biochemistry 30, 5352-5360

4 Hanson, D.K., Tiede, D.M., Nance, S.L., Chang, C.-H. and Schiffer, M. (1993) Proc. Nat. Acad. Sci.USA 90, 8929-8933