Resolving the dynamic structure of chlorosomes in green sulfur bacteria by MAS NMR

Dsouza, L.A.

Chapter 7
General Discussion and Future Prospects

7.1 Structural assessment of the mutant in comparison to WT

This thesis investigates the structural organization of chlorosomes in the *bchQ* mutant strain of *Chlorobaculum tepidum*, with a comparative perspective against both the wild type (WT) and the *bchR* mutant. Recent advances in understanding bacteriochlorophyll (BChl) biosynthesis have identified a number of genetic modifications that typically lead to the formation of single homologs.[1] Notably, Ganapathy *et al.* were the first to elucidate the structure of chlorosomes from the *bchQRU* mutant, which are distinguished by their content of BChl *d* pigments.[2]

The focus of this work lies in exploring the structural properties of *bchQ* chlorosomes, which predominantly contain the single homolog [8Et, 12Et]BChl *c*. This homolog is not only central to the *bchQ* mutant but also the primary pigment found in WT chlorosomes, making it particularly relevant for both experimental and theoretical investigations. A key aim of this thesis is to provide a detailed structural description of this homolog, which has been widely adopted in physical modeling studies. Addressing the assumption that a pure Ethyl-Ethyl homolog and a heterogeneous mixture of homologs would produce equivalent structures, this research seeks to provide the structural foundation necessary to critically evaluate such models. [3,4]

As we progress in interpreting the data from these modeling efforts, the need for a detailed structural foundation that accurately reflects the complexities of the models has become increasingly vital. To achieve this, we have employed three distinct yet complementary methodologies: solid-state nuclear magnetic resonance (NMR), cryo-electron microscopy (cryo-EM), and optical spectroscopy. Each approach contributes unique insights into the chlorosome architecture, paving the way for a more nuanced understanding of the structural properties of the *bchQ* mutant chlorosomes.

We used 2-D homonuclear and heteronuclear correlation experiments to obtain the ^{13}C and ^{1}H assignments and distance constraints. The homogeneous composition of *bchQ* mutant chlorosomes provides the added advantage of well-resolved cross peaks in the 2-D spectra. Two distinct sets of chemical shifts were seen for the carbon and proton resonances for the *bchQ* in a 70:30 ratio, revealing two different components within the system. These two different components can be assigned to hydrogen bonding differences, with 70% hydrogen bond donors and 30% hydrogen bond non-donors.[3,4] Distance constraints that were revealed using CHHC experiments point to the *syn-anti* parallel stacking motif, in line with the results

for *bchQRU* mutant chlorosomes. Cryo-EM results gave us a different stacking distance of 1.49 nm compared to 1.25 nm for WT. The longer stacking distance matches the repeat that was calculated by MD simulations. In addition, the optical data from CD and UV spectroscopy gave insight into the chirality and absorption profile for the *bchQ* chlorosomes. The absorption spectra of the *bchQ* mutant were narrower in comparison to WT, suggesting *bchQ* has less light-harvesting potential than *WT*. This means varying methylation at 8 and 12 positions is important for increasing the absorption cross-section for light harvesting.[1] The broadening of the absorption profile for WT can be attributed to the different microenvironments of the homologs of the BChl *c* that are present. Interestingly, analysis revealed that the chiral angle in the *bchQ* mutant is 90º, compared to 112º in the wild type. This indicates that, in *bchQ*, the molecular stacks are nearly parallel to the tube axis, while in the wild type they are oriented at a distinct angle. These findings, detailed in Chapter 2, add to the growing evidence that the chiral angle is variable and not an essential determinant for chlorosome function.[5]

7.2 Dynamics study of mutant and WT chlorosomes

Another very important objective of this thesis was to study the dynamics of chlorosomes of WT and mutant *bchQ* in detail. This was achieved by using various one-dimensional and two-dimensional NMR measurements. We also employed temperature dependence measurements to study the changes in the site-specific dynamics of BChl *c* molecules. To study the rotational motion of the macrocycle, which is believed to happen in the fast regime, was very challenging to probe on NMR time scales. Due to this rotational mode, chlorosomes are often termed as plastic crystals. The development of the plastic crystal model for the chlorosome has been based on computational studies. In this model, the BChl is portrayed by a minimal model known as a Frenkel Hamiltonian, which encompasses two structural degrees of freedom. These consist of the staggered rotational motion of the BChl macrocycles and the modulation of their separation in the *syn-anti* BChl motifs. Although this model has facilitated the simulation of large numbers of self-assembled BChl in a tubular self-assembly, further experimental validation was necessary to establish that the chlorosome is best described as a plastic crystal in a realistic supramolecular environment. To study this, we made use of REDOR which gave us insights into the rotational motion of the macrocycle. Interestingly for both *bchQ* and WT an angle of 48±4º was estimated to be the librational angle.

7.3 Future Experiments

7.3.1 2-D Measurements on Whole Cells

In the final chapter, I discuss the potential experiments that can be done to get the stacking arrangement of the bacteriochlorophylls inside chlorosomes in whole cells. For instance, chapter 6 gave us preliminary insights about the structure and dynamics of chlorosomes inside a whole cell, but the atomistic structure as a whole could not be resolved since the sample oxidized within a few hours. To obtain 2-D data sets of sufficient resolution, the duration of the experiments tends to be longer than usual 1-D experiments. Therefore, there is a need to make the sample stable for a longer duration and employ techniques such as CHHC to get the distance constraints and perform also cryo-EM to see the behaviour of chlorosomes inside of whole cell.

7.3.2 REDOR studies on *bchR* mutant

Finally, the librational motion of *bchR* mutant, as discussed in chapter 5 can be studied using REDOR, a similar technique employed in chapters 3 and 4, and get an angle for the librational motion of the BChls inside the chlorosomes of this mutant.

References

(1) Chew, A. G. M.; Frigaard, N.-U.; Bryant, D. A. Bacteriochlorophyllide *c* C-8 ^{2} and C-12 ^{1} Methyltransferases Are Essential for Adaptation to Low Light in *Chlorobaculum Tepidum*. *J. Bacteriol.* **2007**, *189* (17), 6176–6184. https://doi.org/10.1128/JB.00519-07.

(2) Ganapathy, S.; Oostergetel, G. T.; Wawrzyniak, P. K.; Reus, M.; Gomez Maqueo Chew, A.; Buda, F.; Boekema, E. J.; Bryant, D. A.; Holzwarth, A. R.; de Groot, H. J. M. Alternating Syn-Anti Bacteriochlorophylls Form Concentric Helical Nanotubes in Chlorosomes. *Proc. Natl. Acad. Sci.* **2009**, *106* (21), 8525–8530. https://doi.org/10.1073/pnas.0903534106.

(3) Erić, V.; Li, X.; Dsouza, L.; Frehan, S. K.; Huijser, A.; Holzwarth, A. R.; Buda, F.; Sevink, G. J. A.; de Groot, H. J. M.; Jansen, T. L. C. Manifestation of Hydrogen Bonding and Exciton Delocalization on the Absorption and Two-Dimensional Electronic

Spectra of Chlorosomes. *J. Phys. Chem. B* **2023**, acs.jpcb.2c07143. https://doi.org/10.1021/acs.jpcb.2c07143.

(4) Li, X.; Buda, F.; de Groot, H. J. M.; Sevink, G. J. A. Contrasting Modes of Self-Assembly and Hydrogen-Bonding Heterogeneity in Chlorosomes of Chlorobaculum Tepidum. *J. Phys. Chem. C* **2018**, *122* (26), 14877–14888. https://doi.org/10.1021/acs.jpcc.8b01790.

(5) Dsouza, L.; Li, X.; Erić, V.; Huijser, A.; Jansen, T. L. C.; Holzwarth, A. R.; Buda, F.; Bryant, D. A.; Bahri, S.; Gupta, K. B. S. S.; Sevink, G. J. A.; De Groot, H. J. M. An Integrated Approach towards Extracting Structural Characteristics of Chlorosomes from a *BchQ* Mutant of *Chlorobaculum Tepidum*. *Phys. Chem. Chem. Phys.* **2024**, 10.1039.D4CP00221K. https://doi.org/10.1039/D4CP00221K.