INSIGHTS INTO THE ASSEMBLY OF A MULTI-SUBUNIT MEMBRANE PROTEIN COMPLEX USING SITE-SPECIFIC SPIN LABELING ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY ON THE PSAC SUBUNIT IN PHOTOSYSTEM I

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An Abstract of a Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Major Subject: CHEMISTRY

The original of the complete thesis is on file
in the Rensselaer Polytechnic Institute Library

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July 2009
(For Graduation August 2009)

Abstract

Electron paramagnetic resonance (EPR) spectroscopy employs the use of microwave frequencies to excite transitions in unpaired electrons being oriented in an external magnetic field. The sensitivity and specificity of EPR to unpaired electrons makes it an ideal technique for studying protein structure and dynamics on the local amino acid level. For example, site directed spin labeling (SDSL) is used in this study to observe rotational correlation times of a stable nitroxide spin label bonded to a free cysteine residue. In this study, the changes in correlation times provide insight into conformational changes in a protein subunit upon binding to the larger multi-subunit core. Though many multi-subunit proteins exist for study, a photosynthetic protein, Photosystem I, was of interest in this study for implications in better understanding the assembly and conversion of solar energy in a photosynthetic protein complex in cyanobacteria and plants.

The aim of this study was to investigate the assembly of the PsaC subunit in the Photosystem I (PS I) complex by using site-specific spin labeling electron paramagnetic resonance (EPR) spectroscopic techniques. The assembly of the PsaC subunit onto the heterodimeric core was measured from the viewpoint of a stable nitroxide spin label covalently bound to either the native $C34_C$ or the mutated $C75_C$ residue of $PsaC_{WT}$. For the purposes of this study, the subunit assembly of PS I was monitored in three configurations: unbound PsaC, the P_{700}-F_X/PsaC complex, and the P_{700}-F_X/PsaC/PsaD complex. Line broadenings that occurred in EPR spectrum and changes in rotational correlation times of the spin label in the three different configurations were on par with the conformational changes that have been predicted to occur during the assembly

process. The final configuration, the addition of the PsaD subunit to P_{700}-F_X/$PsaC_{WT-C34}$ complexes, generated EPR spectral changes that indicate PsaD's role in affecting the orientation of the PsaC subunit on the PS I heterodimeric core.

To monitor different roles of ionic bridges and hydrogen bonding, PsaC mutant variants were created, where each mutant lacked a key binding contact with the PS I core heterodimer (PsaA/PsaB). Each mutant was observed from a covalently bonded spin label at $C34_C$, the native cysteine. Upon removing the $R65_C$ ionic bonding contact, PsaC bound in an appreciably different configuration on the heterodimeric core than when compared to the $PsaC_{WT}$ bound configuration, which was investigated with the EPR spectrum of the P_{700}-F_X/$R65A_{PsaC-C34}$ complex. Conversely, the $R52_C$ ionic bonding mutant did not show a significant difference in orientation of PsaC on the PS I core when compared to wild-type PsaC. Further mutations explored the significance of hydrogen bond networks in PS I subunit assembly. The $Y80_C$ hydrogen bonding mutant, which was responsible for two symmetry-breaking H-bonds with PsaB subunit of the heterodimer, did not show significant binding changes from wild-type PsaC in either binding extent or from EPR spectra. On the other hand, removing the entire C-terminus, by deleting residues 71-80, produced an EPR spectrum which indicated that PsaC was docking in a noticeably disparate orientation than the wild-type protein. The results of this study are explained in the context of better understanding the conformational dynamics of the PS I stromal assembly process.