Review

Breaking biological symmetry in membrane proteins: The asymmetrical orientation of PsaC on the pseudo-C\textsubscript{2} symmetric Photosystem I core

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Abstract. The elucidation of assembly pathways of multi-subunit membrane proteins is of growing interest in structural biology. In this study, we provide an analysis of the assembly of the asymmetrically oriented PsaC subunit on the pseudo C\textsubscript{2}-symmetric Photosystem I core. Based on a comparison of the differences in the NMR solution structure of unbound PsaC with that of the X-ray crystal structure of bound PsaC, and on a detailed analysis of the PsaC binding site surrounding the F\textsubscript{X} iron-sulfur cluster, two models can be envisioned for what are likely the last steps in the assembly of Photosystem I. Here, we dissect both models and attempt to address heretofore unrecognized issues by proposing a mechanism that includes a thermodynamic perspective. Experimental strategies to verify the models are proposed. In closing, the evolutionary aspects of the assembly process will be considered, with special reference to the structural arrangement of the PsaC binding surface.

Keywords. PsaC, Photosystem I, C\textsubscript{2}-symmetry, PsaD, stromal proteins, reaction center, iron-sulfur cluster.

Introduction

Photosynthesis is the process in which sunlight is captured and converted into chemical bond energy by plants, algae and photosynthetic bacteria. The basic reaction involves the light-driven transfer of electrons across the photosynthetic membrane, generating an electrochemical gradient that is subsequently used for carbon fixation. Oxygenic photosynthetic organisms, including cyanobacteria, algae and plants, are capable of splitting water to extract electrons, liberating oxygen as a by-product. Anoxygenic phototrophs such as purple bacteria and green sulfur bacteria derive their electrons from organic and inorganic molecules and hence do not evolve oxygen. Oxygenic photosynthesis is driven by two protein-pigment complexes termed reaction centers (RCs). Photosystem II operates at the oxidizing end of the redox scale and incorporates the water-splitting manganese enzyme that extracts electrons from water. The electrons are subsequently transferred to Photosystem I (PS I) via a membrane-bound cytochrome b\textsubscript{6}f complex. PS I operates at the reducing end of the redox scale and catalyzes the reduction of NADP\textsuperscript{+} to NADPH, thereby generating the reducing equivalents that are required for carbon fixation.
Photosystem I

Photosystem I (PS I) is a membrane-bound protein-pigment complex that contains pigment molecules to absorb light energy and redox cofactors to carry out trans-membrane electron transfer. A model based on the 2.5 Å resolution X-ray crystal structure of the trimeric PS I reaction center from the cyanobacterium, Thermosynechococcus elongatus (PDB ID: 1JB0) has provided invaluable structural information on the three-dimensional arrangement of the pigments, proteins and cofactors (Fig. 1) [1]. Each monomer of the cyanobacterial PS I complex contains 12 protein subunits, 96 chlorophyll a (Chl a) molecules, 22 carotenoids, four lipids, two phyloquinones and three [4Fe-4S] clusters. PsaA (83 kDa) and PsaB (83 kDa) form the heterodimeric core and contain the majority of the antenna molecules and redox cofactors. PsaC (9 kDa) is a membrane-extrinsic (stromal) subunit that contains the two terminal electron acceptors in the electron transfer chain. PsaD (15 kDa) and PsaE (8 kDa) are stromal subunits and, along with PsaC, provide a docking site for the soluble electron acceptors ferredoxin and flavodoxin [2, 3]. Cyanobacterial PS I contains seven additional subunits (PsaF, PsaI, PsaJ, PsaK, PsaL, PsaM and PsaX) whose roles are not well established. Some, including PsaI, PsaK, PsaL, PsaM and PsaX, function as additional antenna proteins by binding Chl a molecules [4]. Others, such as PsaL [5, 6], along with PsaI [7, 8], contribute to the trimerization of cyanobacterial PS I.

The electron transfer chain starts at a special pair of Chl a molecules termed P700, named for its peak absorbance in the visible region. When P700 becomes excited to the singlet state, its electron is transferred to A0, a Chl a monomer. An additional molecule of Chl a (A) is located between P700 and A0, and may act as an electron transfer intermediate [4]. The initial P700+A0 charge-separated state is stabilized by rapid transfer of the electron to a bound phyloquinone, A1, and then to a series of [4Fe-4S] clusters, termed FX, FA and FB [9]. The latter function as an electron transfer wire, shuttling the electron to the stromal surface and making it available to ferredoxin or flavodoxin. The terminal acceptors, FA and FB are located in the PsaC subunit. Because the terminal electron acceptors are iron-sulfur clusters, and because the core is composed of the PsaA/PsaB subunits, PS I is termed a heterodimeric Type I RC.

This article will focus on the assembly of the stromal subunits and, in particular, on the final steps in the association of the PsaC subunit with the membrane-bound PS I core. Based on a comparison of the NMR solution structures of unbound PsaC and the X-ray crystal structure of bound PsaC, and on a detailed analysis of the PsaC binding region on the PsaA/PsaB surface, two models can be envisioned for the assembly of PsaC onto the PS I core. Both models will be described in detail, and their strengths and weaknesses will be addressed in the context of a thermodynamic perspective. Experimental strategies to verify these models will also be discussed. Finally, the evolutionary aspects of the assembly process will be considered, with special reference to the structural changes in the PsaC binding surface.

Symmetry Elements in the PsaA/PsaB Heterodimer

The overall symmetry within the PsaA/PsaB heterodimer is evident even in a cursory analysis of the amino acid sequences of the protein subunits. PsaA and PsaB in Thermosynechococcus elongatus contain an almost identical number of amino acids and have comparable molecular masses. Although there are certain differences in the amino acid sequences, particularly in N-terminal half of the protein associated with the light-harvesting chlorophylls, there is a high degree of similarity in the C-terminal half of the protein in the vicinity of the redox cofactors. For instance, the FX iron-sulfur cluster is ligated by two cysteines each from PsaA and PsaB [10, 11, 12], which are located in homologous regions on each subunit. The 2.5 Å resolution crystal structure of PS I further reinforces the high degree of symmetry within the PsaA/PsaB heterodimer [1]. Each subunit is comprised of 11 transmembrane α-helices that are connected via loop regions on the stromal and luminal surfaces. The symmetry between PsaA and PsaB

Figure 1. Side view of the arrangement of all proteins in one monomer of PS I (PDB ID: 1JB0), with the main subunits indicated.