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Review

Protein–cofactor interactions in bioenergetic complexes: The role of the A_{1A} and A_{1B} phylloquinones in Photosystem I

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ABSTRACT

This review focuses on phylloquinone as an indispensable link between light-induced charge separation and subsequent charge stabilization in Photosystem I (PS I). Here, the role of the polypeptide in conferring the necessary kinetic and thermodynamic properties to phylloquinone so as to specify its functional role in PS I electron transfer is discussed. Photosynthetic electron transfer and the role of quinones in Type I and Type II reaction centers are introduced at the outset with particular emphasis on the determination of redox potentials of the cofactors. Currently used methodologies, particularly time-resolved optical spectroscopy and varieties of magnetic resonance spectroscopy that have become invaluable in uncovering the details of phylloquinone function are described in depth. Recent studies on the selective alteration of the protein environment and on the incorporation of foreign quinones either by chemical or genetic means are explored to assess how these studies have improved our understanding of protein–quinone interactions. Particular attention is paid to the function of the H-bond, methyl group and phytyl tail of the phylloquinone in interacting with the protein environment.

1. Electron transfer in Photosystem I

Life on planet Earth is sustained to a large extent by oxygenic photosynthesis. In this highly evolved process, solar energy is used to convert CO_2 and H_2O into carbohydrates, releasing O_2 to the atmosphere as a by-product. Photosynthetic organisms are able to perform this task so efficiently that the composition of the atmosphere underwent a complete transition ~2.3 billion years ago as a result of the arrival of cyanobacteria some ~400 million years earlier [1,2]. Indeed, it is the production of O_2 by photosynthetic prokaryotes that has made the emergence of highly advanced forms of eukaryotic life possible some 1.4 billion years ago [3–5]. To this day, oxygenic photosynthesis remains the sole source of O_2 in the atmosphere. All of this photosynthetic activity has generated enough biomass to provide all of the stored carbon-based fuel currently used by humankind. Many of those working in the field of bioenergetics believe that the economic deployment of solar biofuels will depend on the construction of artificial systems that closely mimic the process of natural photosynthesis. For this reason, how photosynthetic organisms convert light into chemical bond energy has become a compelling topic of study.

The purpose of a photosynthetic reaction center is to generate and stabilize a charge-separated state over hundreds of milliseconds, which constitutes a biochemically relevant period of time [6]. It carries out charge separation using the energy of a photon to generate a hole-electron pair against a highly unfavorable thermodynamic gradient, and stabilizes this state by transferring the electron and/or the hole through a series of cofactors to lengthen the lifetime of the donor-acceptor pair. In general, two types of photosynthetic reaction centers exist and are classified depending on the nature of the terminal electron acceptor [7]. Type I reaction centers use a bound iron–sulfur cluster and operate at the reducing end of the redox scale [8,9]. Type II reaction centers use a lipophilic quinone molecule and operate at the oxidizing end of the redox scale [10]. During oxygenic photosynthesis, the Type II reaction center, Photosystem II (PS II), and the Type I reaction center, Photosystem I (PS I), function in series to oxidize water and reduce $NADP^+$, respectively [11]. Conceptually, the process is initiated at PS II, where light-induced charge separation generates a strong oxidant that prompts a catalyst consisting of four Mn and one Ca to decompose H_2O into O_2, protons and electrons [12–20]. Concomitantly, light-induced charge separation in PS I generates a strong reductant that ultimately reduces $NADP^+$ to NADPH [21]. The energy of the proton gradient, generated as a result of H_2O oxidation and by passage of the electron through the cytochrome b_6f complex, is conserved in the generation of ATP from ADP and inorganic phosphate. The process culminates when CO_2 is converted into

Abbreviations: PS I, Photosystem I; PS II, Photosystem II; ATP, Adenosine triphosphate; NADPH, Nicotinamide adenine dinucleotide phosphate; RC, Reaction center; EPR, Electron paramagnetic resonance; ENDOR, Electron nuclear double resonance; FTIR, Fourier transform infrared; hfc, Hyperfine coupling; H-bond, Hydrogen bond; ESEEM, Electron spin echo envelope modulation

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carbohydrate using the bond energy of ATP and the reducing power of NADPH [22].

In this article, we focus on phylloquinone as an indispensable link between light-induced charge separation and subsequent charge stabilization in PS I. Our goal is to describe the role of the polypeptide in conferring the necessary kinetic and thermodynamic properties to phylloquinone so as to specify its functional role in PS I electron transfer. We begin by describing photosynthetic electron transfer in general and the role of quinones in Type I and Type II reaction centers in particular. We outline the currently used methodologies, particularly time-resolved optical spectroscopy and varieties of magnetic resonance spectroscopy that have become invaluable in uncovering the details of phylloquinone function. We describe recent studies on the selective alteration of the protein environment and on the incorporation of foreign quinones either by chemical or genetic means, and how these studies have improved our understanding of protein–quinone interactions. Particular attention is paid to the function of the H-bond, methyl group and phytyl tail of the phylloquinone in interacting with the protein mileu. The aim of this article is to place the most current work in the field within the context of protein–cofactor interactions; interested readers are asked to consult several recent articles for a more complete treatment of the role of the quinones in biology [23–32].

1.1. Pseudo-C_2 symmetry and the bifurcating pathway of electron transfer cofactors

The electron density map of trimeric PS I from the cyanobacterium *Thermosynechococcus elongatus* has been solved to a resolution of 2.5 Å [33,34], thereby allowing the architecture of pigments, cofactors and proteins to be accurately modeled (Fig. 1). Each PS I monomer (hereafter known as the PS I complex) is comprised of twelve subunits (PsaA to PsaF, PsaI to PsaM and PsaX), 96 chlorophyll *a* (Chl *a*) molecules, 22 carotenoids, two phylloquinones (PhQ)[1], three [4Fe–4S] clusters, four lipids and a number of bound water molecules. The PsaA and PsaB polypeptides assemble as a heterodimer and provide ligands for the majority of the electron transfer cofactors, which are located on both sides of a pseudo-C_2 axis of symmetry. Thus, a common overall binding frame exists for the two branches of electron transfer cofactors. The arrangement of the electron transfer cofactors in the PS I complex is depicted in Fig. 2. The individual cofactors are labeled with their respective spectroscopic and structural names. The use of 'A' or 'B' in the structural name specifies the protein (PsaA or PsaB) that ligates the cofactor. The spectroscopic names refer to the electron transfer pathway in which each cofactor participates. For instance, the cofactor Q_KA (A_{1A}) is bound by the PsaA subunit and is therefore on the A-branch.

Light-induced charge separation is initiated within the six excitonically coupled Chl molecules, which form the photoactive core of the PS I complex; Chl *a*, ligated by PsaB and Chl *a*′, the 13^2 epimer of Chl *a* ligated by PsaA that comprise P_{700} [36], and four Chl *a* molecules that comprise A_A, A_B, A_{0A}, and A_{0B}. When light is absorbed by any of the 90 antenna Chls [37], the excited state migrates to these six Chls, initiating charge separation and resulting in the formation of what has historically been considered as the primary radical pair $P_{700}^+A_{0A}^-$ (or $P_{700}^+A_{0B}^-$). The charge separation event occurs within ~3.7 ps [38,39], but the details of the process remain obscure. Owing to the relatively large distance between P_{700} and A_{0A} (or P_{700} and A_{0B}), it is generally assumed that the accessory Chl A_A (or A_B) plays a role as

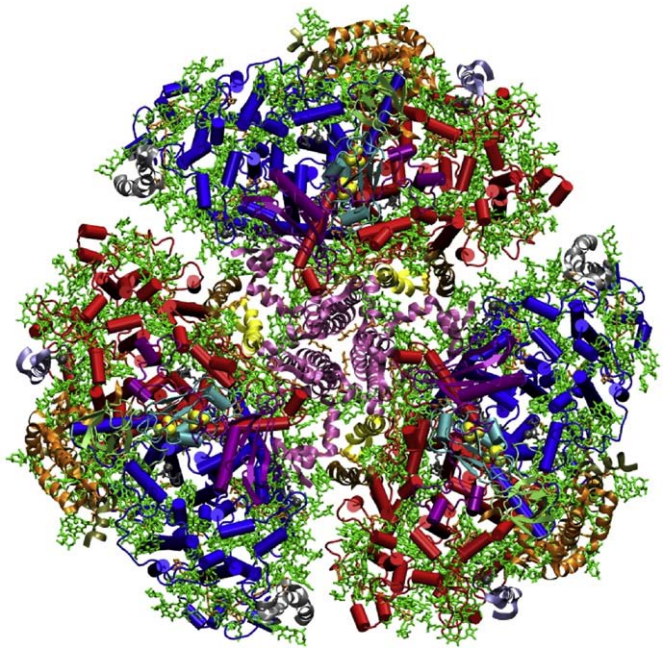

Fig. 1. Structural model of trimeric PS I at 2.5 Å resolution viewed from the stromal side onto the membrane plane (PDB ID 1JB0). The twelve proteins (PsaA–PsaF, PsaI–PsaM and PsaX) that comprise the PS I complex and the antenna system are shown.

a transient electron transfer intermediate. Recently, a new model [40] based on the analysis of ultrafast transient absorbance data has been proposed in which the initial charge separation occurs between A_A and A_{0A} (or A_B and A_{0B}), resulting in the primary radical pair $A_A^+A_{0A}^-$ (or $A_B^+A_{0B}^-$). P_{700} quickly donates an electron to A_A^+ (or A_B^+), thereby initiating the first step in the stabilization of the charge-separated states and resulting in the first readily observable state, $P_{700}^+A_{0A}^-$ (or $P_{700}^+A_{0B}^-$). In this model [40], the initial charge separation would have to be a shallow trap for the exciton (i.e. it would need to be in equilibrium with the Chl* excited state) to explain the presence of fluorescence components that are longer lived than the modeled trapping lifetime. The identification of the initial charge-separated state is critical for the development of strategies to selectively control the pathway of electron transfer through the A- and B-branch cofactors. The distinction between the two pathways stems partly from the inequivalence of the Chl *a*/Chl *a*′ molecules and partly from the inequivalency of the protein environment; both contribute to the asymmetrical distribution of the positive charge on P_{700}^+. According to EPR studies, the positive charge is localized largely on the Chl *a* molecule that is ligated by PsaB [41–43]. (However, see refs. [44,45] for an alternative view from FTIR studies that the charge may be more evenly distributed among the Chl *a*/Chl *a*′ special pair). Small differences in the distance and environment between the A- and B-side cofactors could affect the electronic coupling, and the resulting difference in energies could contribute to the inherent asymmetry in the two pathways. Following initial charge separation, the electron is transferred within ~30 ps to A_{1A} (or A_{1B}), resulting in the formation of the $P_{700}^+A_{1A}^-$ (or $P_{700}^+A_{1B}^-$) radical pair that initiates the process of stabilizing the charge-separated state over longer periods of time [38,46–50].

Due to the pseudo-C_2 symmetric arrangement of electron transfer cofactors, there has been considerable debate about whether the A- and/or B-side branches are active in electron transfer. However, there is an emerging consensus that electron transfer in PS I follows a bidirectional scheme [51–63]. The residual issues involve uncovering the protein factors that lead to the initial asymmetry that results in different amounts of A-branch and B-branch electron transfer in PS I from different organisms. Regardless of the actual pathway, it must be

[1] Although *Synechococcus* sp. PCC 7002, *Gleobacter violaceus* and *Cyanidium caldarium* contain menaquinone-4 (2-methyl-3-all-*trans*-tetraisoprenyl-1,4-naphthalenedione) and *Anacystis nidulans* and *Euglena gracilis* contains 5-OH-phylloquinone [35], we will use the term 'phylloquinone' (2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-1,4-naphthalenedione) to denote a generic naphthoquinone in the A_{1A} and A_{1B} sites of PS I.