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Electron Transfer between Membrane Complexes and Soluble Proteins in Photosynthesis†

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ABSTRACT

Photosynthesis consists of a series of endergonic redox reactions, with light as the source of energy, chlorophyll as the energy converter, and electrons flowing through membrane and soluble proteins. Here, we give an account of the most recent results on the structure−function relationships of the membrane-embedded complexes cytochrome b_6−f and photosystem I and of the two soluble proteins (cytochrome c_6 and plastocyanin) that serve as alternative electron carriers between them. Particular attention is paid to the evolutionary aspects of the reaction mechanism and transient protein−protein interactions between the membrane complexes and their partners in cyanobacteria, eukaryotic algae, and plants.

Introduction

Photosynthesis is usually identified with the typical process of carbon dioxide fixation performed by plants. Such a reaction involves reduction of CO_2 by water, with the concomitant formation of glucose, which is incorporated into cell metabolism, and molecular oxygen, which is released to the atmosphere as a waste product. However, in a wider (and more precise) context, photosynthesis means reduction and further assimilation not only of carbon, but also of inorganic species of two other primordial bioelements, nitrogen and sulfur, which are all required for the synthesis of macromolecules (Figure 1).[1,2]

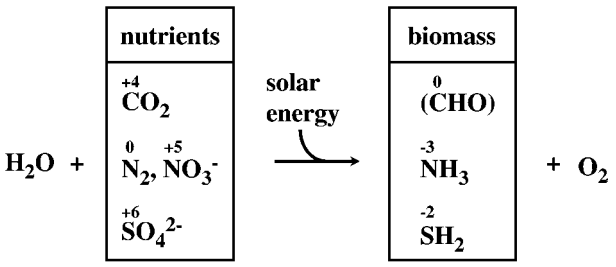

FIGURE 1. Overall reaction of photosynthesis. The inorganic species that serve as nutrients to plants are reduced by water and incorporated into cell biomass, with the concomitant release of dioxygen.

The role of water is just to provide electrons for such redox reactions. However, as the water molecule is a weak reducing agent, the electrons have to be further energized by photons in a rather complex process driven by chlorophyll, the typical light-absorbing pigment of plant kingdom.

In addition to redox energy, most of the reactions for C, N, and S assimilation require a supply of energy from ATP (adenosine triphosphate), the well-known "energy currency" of living cells. Such a transduction of solar energy into phosphate-bond energy (∼P) is known as photophosphorylation. Photosynthesis thus consists of two sequential phases: (i) chlorophyll-dependent water photooxidation, with the yield of assimilatory power (high-energy electrons and ATP); and (ii) assimilatory reduction of primordial bioelements, with solar energy being stored as useful chemical energy into biomass. Light reactions are performed by a number of membrane protein complexes that work in series and constitute the photosynthetic electron transport chain, but dark reactions are mainly part of cell metabolism. An outstanding introduction to photosynthesis has recently been published.[3]

Oxygen-evolving photosynthetic organisms comprise not only plants, but also algae and cyanobacteria. Most other primitive photosynthetic organisms (green sulfur and purple bacteria) use some reduced compound (H_2S, for instance) rather than water as the source of electrons and, in consequence, they do not release oxygen. In both cases, the light-energy transducer is chlorophyll, which is in membrane-embedded multimeric protein complexes called photosystems. Anoxygenic photosynthesis requires just one photosystem, but oxygenic photosynthesis needs the sequential participation of two photosystems, the so-called photosystems I and II (PSI and PSII, respectively). The 3D structure of a bacterial photosystem was first solved by Deisenhofer, Huber, and Mitchel between 1982 and 1984.[4] Structural information on PSI and PSII has been available for over a decade, with their crystal structures being recently solved at 2.5 and 3.8 Å, respectively.[5,6]

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† Dedicated to Dr. Manuel Losada on the occasion of his appoinment as Emeritus Professor.

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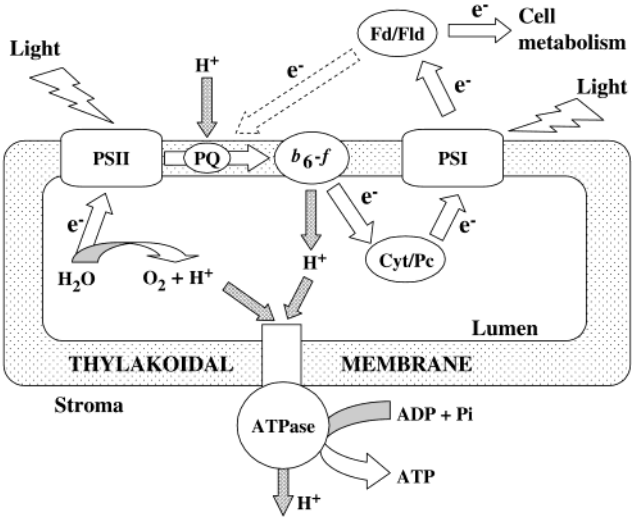

FIGURE 2. Photosynthetic electron transport chain. In the noncyclic flow, electrons are transferred from water to ferredoxin (Fd) or flavodoxin (Fld) by the three membrane complexes photosystem II (PSII), cytochrome b_6-f, and photosystem I (PSI), which are connected by the mobile carriers plastoquinone (PQ) and cytochrome c_6 (Cyt) or plastocyanin (Pc). In the cyclic flow, Fd and Fld donate electrons back to cytochrome b_6-f via the PQ pool. The resulting proton gradient is used by the ATP synthase (ATPase) to form ATP.

Oxygenic Photosynthesis

In oxygen-evolving photosynthetic organisms, electrons flow from water ($E_{m,7}$, 0.82 V), inside the thylakoidal lumen, to ferredoxin ($E_{m,7}$, −0.42 V), which is a [2Fe−2S] iron-sulfur protein that acts as the starting point for the distribution of energized electrons into metabolic reactions outside the thylakoid. The electrons are thus driven in an uphill reaction from the redox level of the water/dioxygen couple to the redox level of the hydrogen electrode. Under iron starvation, ferredoxin can be replaced in some organisms by flavodoxin, a flavin-containing protein.

The well-known Z-scheme of photosynthesis involves two light reactions, one for oxidizing water and the other for reducing ferredoxin and/or flavodoxin.[7] Water lysis is driven by PSII, whereas ferredoxin and flavodoxin reduction is performed by PSI. Each electron from water thus needs two photons to reach the hydrogen electrode level, with each light reaction taking place in a chlorophyll-containing membrane complex. A third membrane-embedded electron-transfer complex is the cytochrome b_6-f complex, which functions between PSII and PSI (Figure 2).

The three complexes are connected by mobile redox carriers. The transfer of electrons from PSII to cytochrome b_6-f is mediated by lipophilic quinones, the so-called plastoquinones (PQ), which move freely in the membrane, while the transport of electrons from cytochrome b_6-f to PSI is carried out by a soluble metalloprotein (cytochrome c_6 or plastocyanin), inside the thylakoidal vesicle.

Reduction and oxidation of the cytochrome b_6-f complex takes place on the external and internal membrane sides, respectively, and involves the translocation of protons from outside to inside, with the concomitant formation of an electrochemical proton gradient (ΔpH) across the membrane. Water photolysis also releases protons inside the lumen, thereby contributing to the proton gradient. The ΔpH is used by the ATP synthase for the synthesis of ATP, according to the chemiosmotic hypothesis put forward by Mitchell.[8]

With the three membrane complexes operating in series, electrons flow from water to ferredoxin and/or flavodoxin. This is called "linear" (or "noncyclic") flow. Alternatively, ferredoxin and flavodoxin can donate electrons back to cytochrome b_6-f via the quinone pool. As the electrons circulate around PSI, this is called "cyclic" electron flow (Figure 2). Whereas the linear flow is used to generate both components of the assimilatory power (redox energy and ATP), the aim of the cyclic flow is the transduction of light energy into ATP.

Hereinafter, we focus on a part of the electron transport chain to review our current understanding of the redox interactions between membrane complexes and soluble proteins, one of the most active fields in modern biochemistry. This Account thus provides an overview of the most relevant and recent findings in the structure−function relationships of the two membrane protein complexes cytochrome b_6-f and PSI, as well as of the soluble metalloproteins cytochrome c_6 and plastocyanin that serve as alternative carriers of electrons from the former to the latter.

Cytochrome b_6-f

The cytochrome b_6-f complex is an electron transfer and proton translocating membrane enzyme acting as a redox link between photosystems I and II.[9,10] It forms dimeric complexes, each monomer (ca. 105 kDa) containing 7 protein subunits and 11−12 transmembrane α-helices. In each monomer, the redox cofactors are distributed in three subunits and arranged in two chains. The high redox potential chain is formed by (i) the Rieske protein, which contains an unusual high potential [2Fe−2S] iron−sulfur cluster ($E_{m,7}$, 300 mV), and (ii) cytochrome *f* ($E_{m,7}$, 350 mV), which is an unusual *c*-type cytochrome (see below). The low potential chain is formed by cytochrome b_6, which possesses two heme groups with bis-histidinyl axial coordination: hemes b_L ($E_{m,7}$, −150 mV) and b_H ($E_{m,7}$, −30 mV). The Rieske protein and cytochrome *f* have some solvent-exposed globular domains.[9−11]

The 3D structure of cytochrome b_6-f is not yet available, although large crystals of a defined protein−detergent−lipid complex diffracting at 3.4 Å have recently been obtained.[12] Most of the structure−function relationships of cytochrome b_6-f have thus been inferred from the analogous mitochondrial cytochrome bc_1 complex. Both complexes exhibit a high degree of similarity, with cytochromes *f* and c_1 presenting the greatest differences.[11]

The electron transfer/proton translocation mechanism of cytochrome b_6-f is described by the classical Q-cycle mechanism.[13] This model assumes that electrons pass from plastoquinol (reduced plastoquinone, PQH_2) to

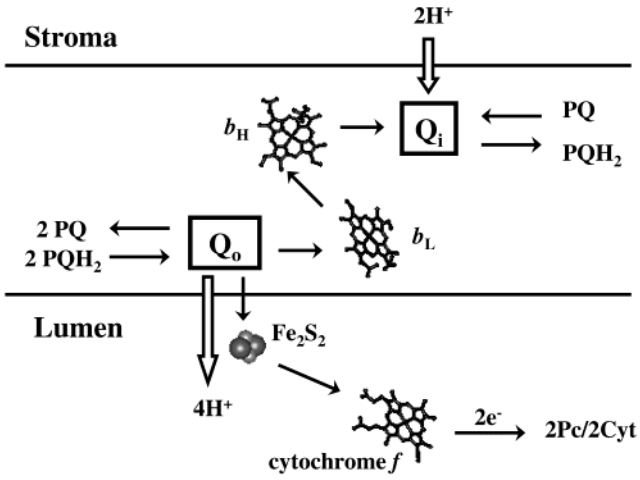

FIGURE 3. The Q-cycle model. The electron flow in the cytochrome b_6-f complex from PQH_2 to plastocyanin (Pc) or cytochrome c_6 (Cyt) is coupled to proton translocation.

plastocyanin (or cytochrome c_6) through sequential one-electron reactions. In fact, the electrons from PQH_2 are alternatively transferred along the two chains of cytochrome b_6-f.[9,11] There are three control sites: (i) the plastoquinol oxidase, or site Q_o, close to the inner aqueous phase, (ii) the plastoquinone reductase, or site Q_i, on the other side of the membrane, and (iii) the cytochrome f/plastocyanin oxidoreductase site.

The Q-cycle for cytochrome b_6-f would thus operate as follows: At site Q_o, two PQH_2 molecules are fully oxidized and deprotonated to yield PQ, with four electrons being alternatively introduced into the two redox chains and four protons being released to the lumen. At site Q_i, one PQ molecule is fully reduced to regenerate one PQH_2 molecule by the low potential chain, with the concomitant uptake of two protons from the stroma. Finally, two plastocyanin (or cytochrome c_6) molecules are reduced by sequential transfer of two electrons via the high potential chain.[9,11] The net balance is that two electrons are donated to two metalloprotein molecules and four protons are translocated across the membrane (Figure 3). The PQH_2 oxidation rate constant is ca. 200 s^{-1}, the [2Fe-2S]/cytochrome f/plastocyanin reactions occur at rates about 10 times faster (>3000 s^{-1}), and electron transfer between b_L/b_H hemes can be even faster (ca. 10^5 s^{-1}).[11,14] PQH_2 oxidation seems thus to be the rate-limiting step not only for cytochrome b_6-f but also for electron transfer through the noncyclic chain.[15]

Other models have been formulated but to some degree converge with the Q-cycle.[11,16] A intriguing part of the functional cycle of cytochrome b_6-f is the rotation of the soluble domain of the Rieske protein around a flexible putative polyglycine linker upon reduction, thus translating the [2Fe−2S] cluster in a 15 Å movement from the quinol binding site into proximity with cytochrome f.[17] As the electron transfer rates strongly depend on distance, such a movement can be a crucial factor to achieve the bifurcation of electrons in two chains, first by reducing the [2Fe−2S] cluster by plastoquinol, then moving the iron-sulfur cofactor away and facilitating the reduction of cytochrome b_L by plastosemiquinone. An internal chain of five water molecules at cytochrome f has indeed been proposed to form a "proton wire" and act as the exit-port for proton translocation.[18]

Cytochrome f is an unusual c-type cytochrome; not only is its β-sheet secondary structure unrelated to other c-type cytochromes and its internal water chain unique, but it has an unusual histidine-tyrosine heme axial coordination.[18] It is also noteworthy that its content in electrostatically charged amino acids varies depending on the organism from which it is isolated, and so its isoelectric point can be either acidic (in some cyanobacteria) or basic (in plants and green algae). The relevance of such electrostatic differences will be discussed later when we consider the interactions of cytochrome f with plastocyanin and cytochrome c_6.

Photosystem I

The heteromultimeric complex PSI is a ligth-driven oxidoreductase that transfers electrons from the lumenal donors plastocyanin or cytochrome c_6 to the stromal acceptors ferredoxin or flavodoxin.[19] The number of cofactors in PSI is one of the largest found up to now in any protein−cofactor complex.[20]

PSI can be isolated either as monomers or homotrimers, although the naturally occurring form seems to be the trimer.[21,22] The 3D structure of trimeric PSI has been recently solved at 2.5 Å in the cyanobacterium *Synechococcus elongatus*.[6] Each monomer (ca. 356 kDa) contains 127 cofactors (96 chlorophylls, 2 phylloquinones, 3 [4Fe−4S] clusters, 22 carotenoids, 4 lipids) and consists of 12 proteins, of which 9 subunits have transmembrane α-helices (Figure 4).[23] The 3D structure of eukaryotic PSI is not yet available, but it seems to be similar to cyanobacterial PSI.[24]

The core is formed by subunits PsaA and PsaB, with a molecular mass of 83 kDa and 11 α-helices each. The PsaA/PsaB heterodimer harbors most of the redox cofactors and a large number of antenna chlorophylls and carotenoids. A hollow at the lumenal side of PsaA/PsaB, close to the symmetry axis, seems to be the binding site for plastocyanin and cytochrome c_6.[25−27] At the cytoplasmic side, subunit PsaC (9 kDa) harbors two of the three [4Fe-4S] clusters (F_A and F_B), and subunits PsaD and PsaE, which are both surrounding subunit PsaC, could contribute to the binding of ferredoxin or flavodoxin.[25]

Subunit PsaF (15 kDa), with one transmembrane α-helix and a globular domain protruding toward the lumenal side, seems to be involved in the correct docking of plastocyanin in eukaryotic PSI, but not in some cyanobacteria.[28] Subunit PsaF could thus play a significant role in the interaction of PSI with any (or both) of its two electron donor proteins. This hypothesis is supported by the fact that the isoelectric point of PsaF is quite variable, depending on the organism (it is ca. 9.0 in plants and green algae and ca. 5.5 in some cyanobateria), but is just the opposite to that of plastocyanin and cytochrome c_6 in any given organism (see below).[29]

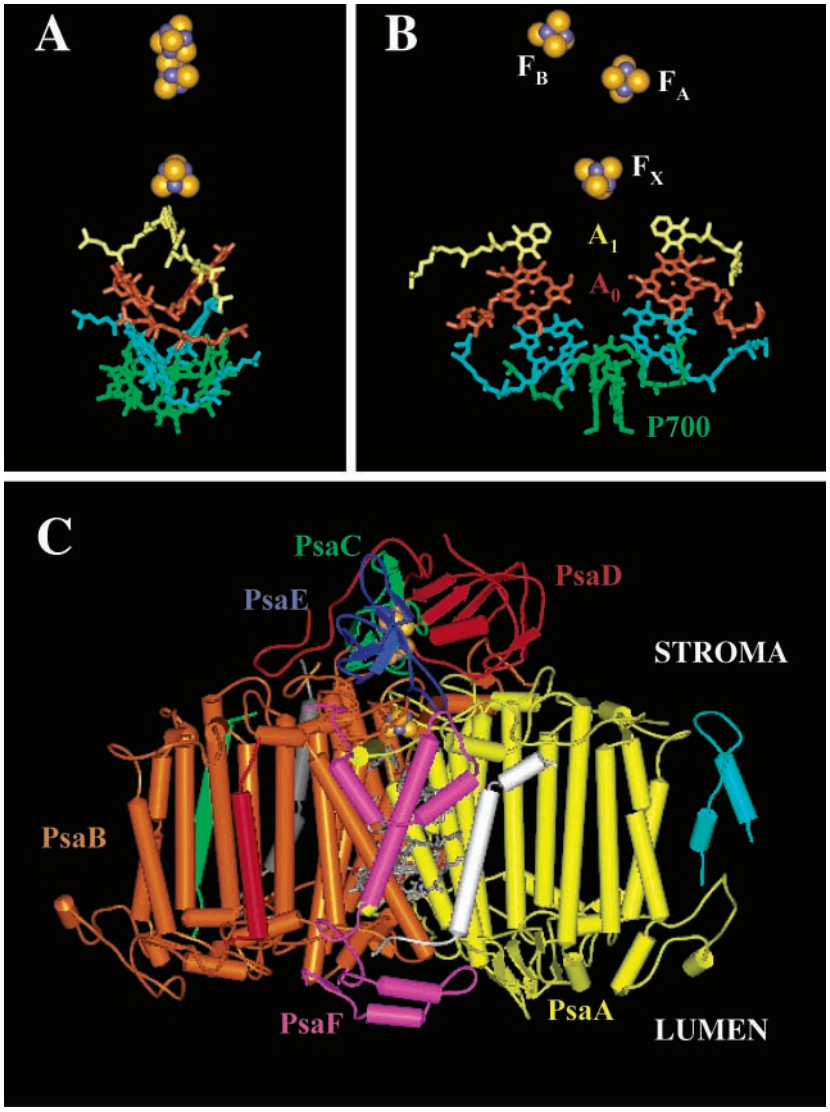

FIGURE 4. Photosystem I (1JB0). The redox components of the reaction center are in panel A, as well as in panel B upon ca. 90° rotation. Details on the description of the reaction center components are in the text. The 3D structure of the monomeric PSI is in panel C, where each subunit is in different color and α-helices are represented by cylinders.

Most of the chlorophylls and carotenoids form an antenna system. The electron-transfer chain in the PSI reaction center is formed by six chlorophyll molecules (including a special pair), two phylloquinones, and three [4Fe−4S] clusters, denoted F_X, F_A, and F_B (Figure 4). The special chlorophyll pair of PSI (P700) is a heterodimer of chlorophylls *a* and *a′*. Like in other photosystems, the redox cofactors of PSI are distributed in two symmetric branches, A and B, which run from P700, close to the lumenal membrane side, to the first [4Fe−4S] cluster (F_X), on the stromal side.

The redox reactions start upon excitation of P700 to P700*. Spectroscopic analyses have identified a primary electron acceptor (A_0), followed by a series of secondary electron acceptors (A_1, F_X, F_A, and F_B) (Figure 4). According to a simplified scheme, P700* transfers the energized electron to the chlorophyll acceptor A_0 in 1−2 ps, and the latter reduces the phylloquinone component A_1 in ca. 30 ps, and this in turn reduces the iron-sulfur center F_X in 15−200 ns.[30] Electron transfer from F_X to F_B via F_A seems to occur in less than 500 ns. Finally, the electron is transferred on the other side of the membrane to ferredoxin, in less than 1 μs, or to flavodoxin, for which reduction of flavin semiquinone to hydroquinone takes ca. 10 μs.[31] The cycle is closed by the transfer of one electron from cytochrome c_6 or plastocyanin to oxidized P700 ($P700^+$). Recent data suggest that the slower and faster phases of A_1^- reoxidation could be assigned to the A and B electron-transfer branches, respectively, but whether one or both branches are active in PSI is under debate.[30]

Reduced ferredoxin and flavodoxin introduce low redox potential electrons into cell metabolism for the further assimilation of the primordial bioelements carbon, nitrogen, and sulfur. Some of the enzymes involved in such redox reactions accept electrons directly from ferredoxin and flavodoxin, but some others accept electrons from reduced nicotinamide−adenine−dinucleotide−phosphate (NADPH), which is in its turn reduced by the enzyme ferredoxin/flavodoxin−$NADP^+$ oxidoreductase (FNR). When

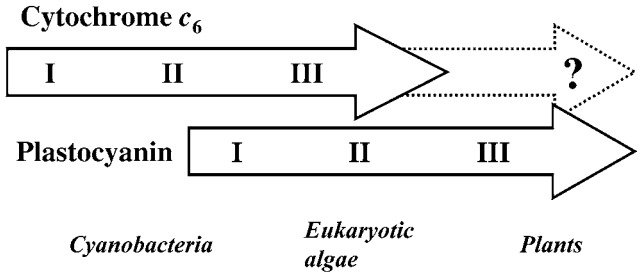

FIGURE 5. Evolution of cytochrome c_6 and plastocyanin. The heme protein appeared first, before the copper protein in cyanobacteria, but lost its original function in plants. The kinetic mechanism of photosystem I reduction by either cytochrome c_6 or plastocyanin would have evolved from type I to type II and III. See text for further explanation.

cyclic flow is operating, ferredoxin, and flavodoxin donate electrons to the cytochrome b_6-f complex.[32] The molecular mechanism of such a cyclic pathway still remains under discussion, as several proteins (including FNR) have been proposed as plausible candidates to drive the electron transfer from ferredoxin and flavodoxin to the plastoquinone pool.[33]

Cytochrome c_6 and Plastocyanin

The electron transfer from cytochrome b_6-f to PSI is mediated by either cytochrome c_6 or plastocyanin. Until very recently, it was widely believed that plants produce only plastocyanin, whereas eukaryotic algae and cyanobacteria synthesize either cytochrome c_6 or plastocyanin, depending on copper bioavailability.

A few years ago, we proposed that cytochrome c_6 was first "discovered" by Nature, when iron was much more available than copper because of the reducing character of the Earth's atmosphere.[29,34] As the atmospheric molecular oxygen concentration was rising because of photosynthetic activity, the relative bioavailabilities of iron and copper were going down and up, respectively, and cytochrome c_6 was replaced with plastocyanin. In plants, copper is not a limiting element, and so cytochrome c_6 would have disappeared, whereas plastocyanin would have become a constitutively synthesized protein (Figure 5). Many cyanobacteria and eukaryotic algae still retain their capacity to synthesize both plastocyanin and cytochrome c_6 to adapt their metabolism to changing aqueous environments.

The recent discovery of a modified cytochrome c_6 in several plants and the proposal that it could be able to play the same role as plastocyanin in the photosynthetic electron transport chain have opened a lively debate.[35,36] With this backdrop, we performed a structural and functional analysis of plant cytochrome c_6 compared with plant plastocyanin and algal cytochrome c_6 and concluded that the plant heme protein is not an effective donor to its own PSI.[37] In fact, the physicochemical parameters and surface electrostatic potential distribution of cytochrome c_6 and plastocyanin from plants are so different that the latter cannot be replaced by the former. We thus proposed that the true function of plant cytochrome c_6 may be

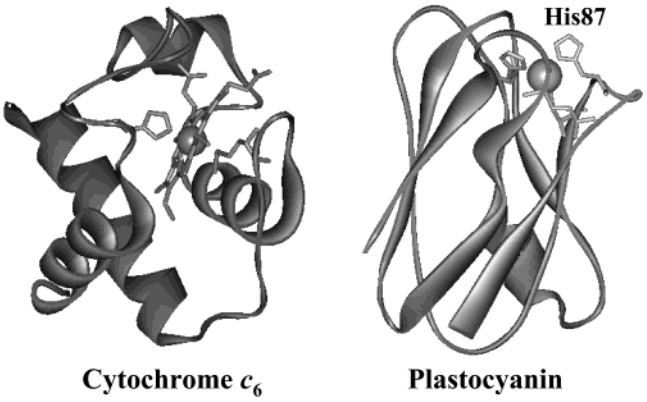

FIGURE 6. Tertiary structures of cytochrome c_6 and plastocyanin, with metal atoms in space-filling representations, porphyrin ring and metal ligands in sticks, and polypeptide chains in flat ribbons.

related to an extra loop of 12 residues that is a unique feature of the plant heme protein.[37] Recent in vivo experiments with plants mutated in both of the two plastocyanin-coding genes also demonstrate that only plastocyanin can donate electrons to PSI in plants.[38]

Most of our investigations have been aimed toward establishing the structure−function relationships that allow cytochrome c_6 and plastocyanin to play the same role despite their different structures.[29] Nowadays, cytochrome c_6 and plastocyanin are very well characterized, both at the structural and functional levels. At the Protein Data Bank, there are a great number of 3D structures of cytochrome c_6 and plastocyanin from different photosynthetic organisms. The former is a typical class I *c*-type cytochrome, formed by four α-helices and a heme group, in which the iron atom exhibits a histidine−methionine axial coordination and the porphyrin ring is covalently bound to the polypeptide chain by two cysteines. The latter consists of a β-barrel formed by eight β strands, along with a small α-helix, and a redox center typical of Type I blue copper proteins, in which the metal atom is coordinated by two histidines, one methionine, and one cysteine in a tetrahedral geometry (Figure 6).

However, the two proteins share a number of physicochemical parameters: the molecular mass is ca. 10 kDa, the midpoint redox potential value is ca. 350 mV at pH 7, and the isoelectric point is practically identical within the same organism, even though it can vary from one organism to another. Such a difference in the isoelectric point is one of the most interesting aspects of the comparative analysis of these two proteins, as it is a direct consequence of the relative abundance of acidic and basic residues in each. In fact, the two proteins are negatively charged in eukaryotes (plants and green algae), but are neutral or positively charged in prokaryotes (cyanobacteria). This makes the proteins exhibit a peculiar distribution of their surface electrostatic potential, which has a fundamental role in driving their attractive movement toward and forming the transient complex with cytochrome b_6-f and PSI.[29]

On the surfaces of cytochrome c_6 and plastocyanin, a striking homology can be found between the arrangements not only of charged but hydrophobic residues. In fact, each protein possesses two equivalent interaction areas, one electrostatic for complex formation (site 2) and the other hydrophobic for electron transfer (site 1), which are used by both cytochrome c_6 and plastocyanin for the interaction with both cytochrome b_6–f and PSI.[29,39–41] At site 1, the redox pathway for electrons going into and out the respective metal atom is formed by the surface-accessible ring C of the heme group in cytochrome c_6 and copper-ligand His87 in plastocyanin (Figure 6). Therefore, the similar physicochemical features of cytochrome c_6 and plastocyanin allow them to interact with the same redox partners with identical kinetic efficiency and replace each other inside the cells, despite their intrinsic different structures.

Cytochrome b_6–f Oxidation

Cytochrome c_6 and plastocyanin are reduced upon binding to the cytochrome b_6–f complex at its third control site (see above). Kinetic and site-directed mutagenesis studies have shown that plastocyanin interacts with cytochrome *f* by means of both hydrophobic and electrostatic forces in plants, whereas nonpolar interactions dominate in some cyanobacteria.[14,42] In plants, native plastocyanin and cytochrome *f* in solution form a complex capable of high electron-transfer rates, with a half-time of ca. 200 μs.[14] At present, there are no kinetic data available on the redox reaction between cytochrome c_6 and cytochrome *f*, a fact that is due to the overlapping of the spectral bands of both *c*-type cytochromes that impedes the experimental recording of any redox change.

NMR chemical-shift perturbation analysis has been used to investigate the structure in solution of the complex formed between cytochrome *f* and plastocyanin from plants and cyanobacteria,[41,43] with the results obtained being in agreement with the kinetic data, as well as of the complex formed between cyanobacterial cytochrome *f* and cytochrome c_6.[40] As a general rule, the protein–protein interface of the two complexes of cytochrome *f* with cytochrome c_6 and plastocyanin is similarly formed by complementary hydrophobic regions around the respective redox centers; in eukaryotic organisms, opposite charged patches (one positive, the other negative) are likewise involved. In the case of cytochrome *f* and plastocyanin in particular, tyrosine 1 of the heme protein (which is a heme iron ligand) and histidine 87 of the copper protein (which is a copper ligand) are in proximity to one another at the protein–protein interface, thus forming a short "electric wire" for electrons flowing from iron to copper atoms (Figure 7).[41]

Photosystem I Reduction

The reaction mechanism of PSI reduction has been extensively analyzed from an evolutionary point of view in a wide variety of organisms, thereby resulting a hierarchy of kinetic models with a significant increase in efficiency.[15,29] PSI reduction by the donor proteins cytochrome c_6 or plastocyanin, isolated from different sources, can thus follow either an oriented collisional mechanism (type I), a mechanism requiring complex formation (type II), or complex formation with rearrangement of the interface (type III):

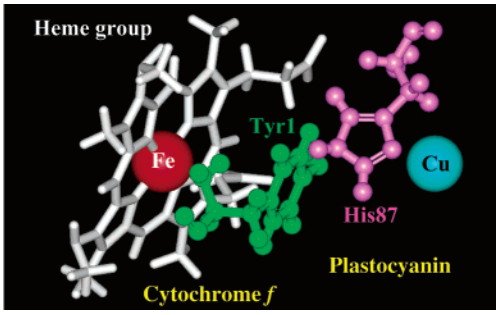

FIGURE 7. Electron-transfer pathway in the transient complex formed between cytochrome *f* and plastocyanin (2PCF). Tyrosine 1 and histidine 87, which are both metal ligands of the heme and copper proteins, respectively, are accommodated in front of each other at the protein–protein interface to allow the electrons go from the iron atom to the copper atom.

$$PSI_{ox} + Donor_{red} \rightarrow PSI_{red} + Donor_{ox} \quad (1)$$

$$PSI_{ox} + Donor_{red} \leftrightarrows [PSI_{ox}/Donor_{red}] \rightarrow PSI_{red} + Donor_{ox} \quad (2)$$

$$PSI_{ox} + Donor_{red} \leftrightarrows [PSI_{ox}/Donor_{red}] \leftrightarrows [PSI_{ox}/Donor_{red}]^* \rightarrow PSI_{red} + Donor_{ox} \quad (3)$$

in which the asterisk denotes a transient complex whose redox centers are properly oriented to allow an efficient, fast electron transfer. The type I model is found in some cyanobacteria, in which the donor proteins do not form a kinetically detectable transient complex with PSI because of electrostatic repulsion between partners. The type II model is found in some other cyanobacteria, in which the transient complex can be either electrostatic or hydrophobic in nature. The type III model is observed mostly in eukaryotic organisms, in which the intermediate complex is first formed by electrostatic attractions and the further reorientation mainly involves hydrophobic interactions. The kinetics of PSI reduction are typically monophasic for the type I and II reaction mechanisms, but biphasic for the type III model. In the latter, the lifetime of the first, fast phase is within the range of several microseconds, and that of the second, slower phase is around 2 orders of magnitude higher. This can be interpreted by assuming that the kinetic mechanism of each protein in the whole range of photosynthetic organisms has evolved from type I to type II and III to reach the maximum efficiency (see Figure 5).

A wealth of functional information derived from site-directed mutants of cytochrome c_6 and plastocyanin—in which any given amino acid is replaced by some other at both the electrostatically charged and hydrophobic patches—from eukaryotic and prokaryotic systems has clearly established the involvement of these two surface

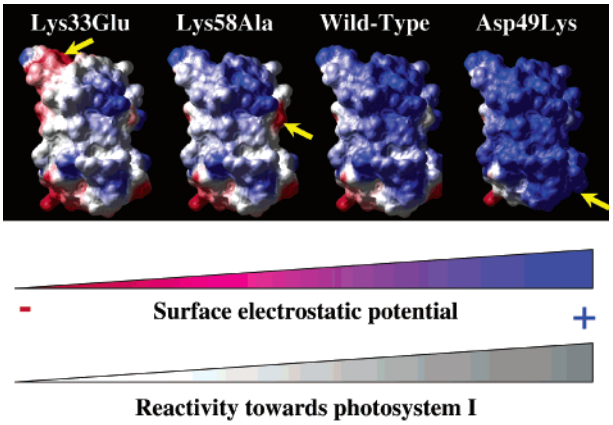

FIGURE 8. Surface electrostatic potential distribution of wild-type and mutant plastocyanin of *Anabaena* compared with their respective reactivity toward photosystem I. The yellow arrow points to the specific amino acid that is replaced in each mutant.

areas in complex formation and electron transfer, respectively.[14,29] For illustrative purposes, Figure 8 shows how PSI reduction by the positively charged plastocyanin from the cyanobacterium *Anabaena* is modified by replacing some specific residues at the protein surface: the more positive the surface electrostatic potential, the higher the protein reactivity. A comparative site-directed mutagenesis analysis of cyanobacterial cytochrome c_6 and plastocyanin has revealed the existence of a single arginyl residue in their respective amino acid sequences that is similarly located between sites 1 and 2 of each protein and appears to play the same crucial role in the electron transfer to PSI.[39]

The transient complex between cyanobacterial cytochrome c_6 and PSI has been recently investigated by NMR in solution.[44] The titration of cytochrome c_6 samples with increasing amounts of PSI induces two different effects on the NMR signals of cytochrome c_6: a line broadening for residues at site 1 and a slow exchange for residues at site 2. These findings, which have been interpreted by assuming that the interaction of the heme protein with PSI is tight at site 2 but weaker at site 1, speak in favor of a type III model, with cytochrome c_6 forming an initial electrostatic complex with PSI via site 2 to further pivot around the complementary electrostatic patch in PSI and generate an ensemble of changing conformations as to finally yield a productive electron-transfer complex via site 1.[44]

In summary, the reaction mechanism of PSI reduction can vary from one organism to another but is the same for the two proteins isolated from the same source.[29,39] The two metalloproteins can thus be presented as an excellent case study of biological evolution, which is not only convergent (two structures playing the same role) but also parallel (two proteins varying in a concerted way from one organism to another).

Concluding Remarks

In the past few years, a number of results have accumulated in photosynthesis research that allow us to obtain further insight into our understanding of the operating mechanism of biological solar energy converters. In particular, electron transfer between membrane complexes and soluble proteins has been extensively analyzed, both at the structural and functional level. However, many important questions remain unanswered. The mechanism of electron/proton coupling in cytochrome b_6–f, along with cyclic electron flow around PSI and the relative activity of the A and B branches in PSI, are relevant aspects that deserve further efforts to become fully elucidated. The physiological role of the recently discovered plant cytochrome c_6, which appears to have evolved to lose its original function and adapt to a new use inside plant cell metabolism, likewise remains an interesting point in photosynthesis.

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