Regulation of photosynthesis by ion channels in cyanobacteria and higher plants

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HIGHLIGHTS

• Ion channels regulate photosynthesis in cyanobacteria and higher plants.
• Some organelar ion channels are identified from molecular point of view.
• Genetic approach identifies channels important for light utilization.

GRAPHICAL ABSTRACT

Ion channels

Photosynthesis

Photosynthesis converts light energy into chemical energy, and supplies ATP and NADPH for CO₂ fixation into carbohydrates and for the synthesis of several compounds which are essential for autotrophic growth. Oxygenic photosynthesis takes place in thylakoid membranes of chloroplasts and photosynthetic prokaryote cyanobacteria. An ancestral photoautotrophic prokaryote related to cyanobacteria has been proposed to give rise to chloroplasts of plants and algae through an endosymbiotic event. Indeed, photosynthetic complexes involved in the electron transport coupled to H⁺ translocation and ATP synthesis are similar in higher plants and cyanobacteria. Furthermore, some of the protein and solute/ion conducting machineries also share common structure and function. Electrophysiological and biochemical evidence support the existence of ion channels in the thylakoid membrane in both types of organisms. By allowing specific ion fluxes across thylakoid membranes, ion channels have been hypothesized to either directly or indirectly regulate photosynthesis, by modulating the proton motive force. Recent molecular identification of some of the thylakoid-located channels allowed to obtain genetic proof in favor of such hypothesis. Furthermore, some ion channels of the envelope membrane in chloroplasts have also been shown to impact on this light-driven process. Here we give an overview of thylakoid/chloroplast located ion channels of higher plants and of cyanobacterium Synechocystis sp. PCC 6803. We focus on channels shown to be implicated in the regulation of photosynthesis and discuss the possible mechanisms of action.

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Introduction

During photosynthesis, taking place in the thylakoid membrane, photons are absorbed by the antenna pigments and the excitation energy is transferred from the site of absorption to the reaction centers. In prokaryotic photosynthetic organisms such as cyanobacteria, thylakoid membranes enclosing the lumen compartment, are not isolated from the cytosol. Instead, in eukaryotic photosynthetic organisms, including algae and higher plants, the thylakoids are surrounded by the stroma and two envelope membranes, forming together the chloroplast as bioenergetic organelle. In higher organisms but not in cyanobacteria [1], thylakoids are organized into stacked membranes called grana. The sites of photon absorption are the light-harvesting complexes of the thylakoid membrane which contain protein-bound chlorophyll and carotenoids, and the phycobilisomes containing phycobilins as pigments in higher plants and cyanobacteria, respectively.

Photosynthetic electron transport and proton motive force

In the reaction center of photosystems, excitation is converted into charge separation, which drives electron flow from photosystem II (PSII) to photosystem I (PSI) via the cytochrome b6f complex [e.g. 2]. Most components of these macromolecular complexes are highly conserved between cyanobacteria and eukaryotic photosynthetic organisms. In addition, in cyanobacteria the thylakoid membrane is also the site of respiration [3]. In general, the net result of the light-driven electron transport is the oxidation of water molecules, molecular oxygen evolution, the reduction of NADP\(^+\), and generation of a proton gradient (\(\Delta \Psi\)) across the membrane with an acidic pH generated on the luminal side. The electrical compensations of light-driven H\(^+\) uptake into thylakoids is crucial for initiating photoprotection of the photosynthetic apparatus through energy dependent non-photochemical quenching (qE), a process that thermally dissipates the excess absorbed light energy, thereby limiting the production of reactive oxygen species (ROS) [e.g. 3–5, 8]. qE, a component of non-photochemical quenching (NPQ), is also called energy-dependent excitation quenching, because thermal dissipation is stimulated by \(\Delta \Psi\), which builds up across the thylakoid membrane during photosynthetic electron transport. Since qE involves the de-excitation of singlet excited chlorophyll, therefore it is also called feedback de-excitation. ATP synthase mediates exit of protons from the thylakoid lumen. Its malfunction was recently shown to increase the steady-state proton motive force, resulting in strong lumen over-acidification. This was found to substantially inhibit linear electron flux, causing NPQ activation even upon exposure to low intensity light [9].

ION fluxes across thylakoid membranes might contribute to regulation of photosynthesis (and respiration in cyanobacteria) by modulating the electric component of the transthylakoid proton motive force. Approximately 50% of the steady-state trans-thylakoid pmf is ascribed to the electric field both in higher plants and eukaryotic algae (e.g. [4, 10]). The exact mechanism of how pmf is partitioned into \(\Delta \Psi\) and \(\Delta \mathrm{pH}\) is still unclear, but at least three factors seem to be important, including the capacitance of the thylakoid membrane (which determines the \(\Delta \Psi\) generated for the transfer of a charge across the membrane) and the proton-buffering capacity of the lumen (which sets the actual value of luminal pH following proton translocation). A recent study reported evidence that permeant buffers, such as putrescine, are able to dissipate the \(\Delta \mathrm{pH}\) component, to favor \(\Delta \Psi\), and thus, to adjust the \(\Delta \Psi/\Delta \mathrm{pH}\) ratio. Elevated putrescine level in infiltrated leaves caused a decreased qE [11]. The third factor concerns the ionic composition on the two sides of the thylakoid membrane. Influx of protons into the lumen causes accumulation of positive charges, thus a development of inside-positive membrane potential. Ion movement determines the degree to which the \(\Delta \Psi\) component can be dissipated. In higher plants, it has been postulated that the influx of cations from the lumen toward the stroma or influx of anions in the opposite direction would permit dissipation of the transmembrane electrical potential while conserving the pH gradient. According to Kramer and colleagues, in the steady state, the inward proton flux is balanced by the ATP synthase which becomes activated and a large fraction of pmf might be stored prevalently as \(\Delta \Psi\). The partial dissipation of \(\Delta \Psi\) by counterion movements via ion channels would allow the development of significant \(\Delta \mathrm{pH}\) across the thylakoid membrane (and acidification of the lumen) and would thus lead to activation of qE at a given pmf. Overall pmf increases upon illumination. Thus, differential partitioning of the thylakoid pmf into \(\Delta \Psi/\Delta \mathrm{pH}\) could contribute to optimization of the regulation of energy transduction, and under stress conditions, to triggering of the photoprotective mechanism qE. In this scenario, ion channels would play a regulatory role even at constant total pmf.

The electrical compensations of light-driven H\(^+\) uptake into thylakoids have been proposed already long time ago to be achieved by concomitant fluxes of Cl\(^–\), K\(^+\) and Mg\(^2+\) [11–14] but the molecular entities responsible for these fluxes are being determined only nowadays. Fig. 1 illustrates the ion channels identified from molecular point of view and among them the ones shown to regulate photosynthesis in cyanobacteria and chloroplasts.

Cyanobacterial ion channels

Thanks to the complete genome sequencing of various cyanobacteria species, several putative ion channels have been identified based on sequence similarity to ion channels from higher organisms and/or on...
the presence of typical amino acid sequences, such as the selectivity filter sequences[15]. Several of these putative channels have been expressed in heterologous systems in order to study their functional electrophysiological properties. Among these channels are the first prokaryotic glutamate receptor from Synechocystis which was found to be potassium-selective and represents an evolutionary link between two transmembrane containing potassium channels and glutamate receptors of higher organisms[16] and a proton-gated ion channel from the nicotinic acetylcholine receptor family of Gloeobacter violaceus, named GLIC[17]. Both channels have been successfully crystallized and gave important insight into the function of their eukaryotic counterparts[18,19]. In addition, a 6 TM potassium channel named SynK was identified[20] as well as the cyanobacterial homolog of the CLC chloride channel family, which has been recently crystallized and proved to function as a slow transport-rate chloride–proton antiporter[21].

Only a few reports deal with determination of the function of cyanobacterial ion channels in cyanobacteria themselves. These studies take advantage of the fact that homoplasmic knock-out mutants can be obtained in cyanobacteria by homologous recombination. Nazarenko and colleagues provided evidence that the Synechocystis sp. PCC 6803 large mechanosensitive channel of the plasmamembrane (MsCl) operates as a verapamil/amiloride-sensitive outward Ca²⁺ channel that is involved in the plasma-membrane depolarization-induced Ca²⁺ release from the cells under stress conditions[22]. In a recent study, a K⁺–dependent slight growth defect was observed when the cyanobacterial homolog of KirBac6.1 (inward rectifying K⁺ channel) was deleted from Synechocystis, suggesting that KirBac might contribute to low affinity uptake of K⁺[23]. Our group has recently identified a calcium-dependent potassium channel as well, whose deletion results in a photosynthesis-unrelated phenotype and confers increased resistance to zinc[24].

Cyanobacterial channels/transporters and photosynthesis

As mentioned above, we have recently identified a putative, 6 transmembrane-domain K⁺ channel, SynK in the genome of Synechocystis whose amino acid sequence contains the typical selectivity filter of potassium channels (amino acid sequence TMTTVGYGD). SynK mediated K⁺ transport when it was expressed in Escherichia coli (E. coli) mutant strain LB2003 lacking endogenous K⁺ channels and gave rise to an outwardly rectifying voltage-dependent potassium selective activity when expressed in Chinese hamster ovary cells (CHO). Using membrane fractionation and Western blot as well as immunogold electron microscopy, SynK was located to Synechocystis thylakoid and to plasma membrane[20]. To our knowledge, SynK is the only by far identified bona fide channel in cyanobacteria thylakoid. In order to understand its physiological role, a SynK-less mutant was obtained, which displayed a clear photosynthetic phenotype: the SynK-less mutant showed clear photosensitivity already at moderate light intensity and was unable to efficiently build up a proton gradient upon illumination[25]. SynK was suggested to modulate the balance between the osmotic (ΔΨm) and electric (ΔΨr) components of the transthylakoid proton gradient. It has been proposed that proton pumping into the lumen results in the building of a large ΔΨ component, which activates proton efflux through the ATP synthase[26], preventing the accumulation of high proton concentrations in the lumen. Movements of thylakoid membrane-permeable counter ions, such as efflux of cations or influx of anions through ion channels would partially dissipate the ΔΨ, thereby allowing establishment of significant ΔpH (Fig. 2). Thus, the ionic composition, as well as the activity of ion-flux pathways, in thylakoids is expected to determine the degree to which the movement of ions can dissipate the ΔΨ[4]. In accordance with this proposal, in ΔδSynK, we found an increased ΔΨ and a decreased ΔpH as evaluated by measuring the turnover of the cytochrome bf complex under single-turnover flashes regime[26] and acridine orange fluorescence for pH changes[3]. It is to note that in cyanobacteria the photoprotective mechanism NPQ does not seem to take place, therefore the change in the ΔpH/ΔΨ ratio leads to photosensitive phenotype most probably by NPQ-independent mechanisms.

While this review is focused on ion channels, it has to be briefly mentioned that several transporters have been shown to affect efficiency of photosynthesis, including PM-located aquaporin[27], thylakoid-located Na⁺/H⁺ antiporter NhaS3transporter[28], and the copper-transporting ATPase[29].
potassium, and divalent cation selective ion channels [36–50] and light-induced currents [51–54] have been recorded from all three chloroplast membranes (outer, inner envelope and thylakoid). All ion channels and transporters of chloroplasts are encoded by the nuclear genome, and the protein products are imported into the chloroplast during its biogenesis after their synthesis in the cytoplasm. Even though bioinformatics algorithms exist for prediction of chloroplast localization of nucleus-encoded proteins, the validity of the prediction for localization has to be proved by biochemical evidence, or alternatively, by studying targeting in vivo, using proteins in fusion with fluorescent proteins. Mass spectrometry (MS) might also be a valid alternative for protein targeting has to be proved by biochemical evidence, or alternatively, by study- ing targeting in vivo, using proteins in fusion with fluorescent proteins. Mass spectrometry (MS) might also be a valid alternative for protein localization, although this option is mostly limited in the case of ion channels because of their very low abundance and high hydrophobicity. Indeed, while numerous transporters of chloroplasts have been identified by MS (e.g. [55,56]), only very few bona fide channels were revealed by this technique (see e.g. [57]). Thus, since a high-throughput approach is not available for the molecular identification of the channels residing in chloroplast membranes, this task remains challenging. In the few cases in which molecular identification was successfully achieved (see also next paragraph), precious information could be obtained on the physiological roles of these channels by using knock-out Arabidopsis plants. For example, MscS (small mechanosensitive channel)-like Arabidopsis homolog AtMSL3 was shown to rescue the osmotic-shock sensitivity of a bacterial mutant lacking MS-ion-channel activity, suggesting that it functions as a mechanosensitive ion channel [58] and was later studied by electrophysiology [59]. Interestingly, two homologs, MSL2 and MSL3, located in the envelope, have been shown to control plastid size and shape [58], to protect plastids from hypoosmotic stress [60] and were identified as components of the chloroplast division machinery [61]. Expression of a truncated version of a Chlamydomonas homolog of MscS (MSC1) in E. coli gave rise to mechanosensitive currents and knock-down of the full-length protein caused abnormal localization of chlorophyll [62]. Unfortunately, in none of these works were the photosynthetic parameters determined. It is to note, that paradoxically, knowledge about the physiological role is available on some channel proteins whose ion-conducting properties in native membranes or in heterologous systems are still not proved (no electrophysiological studies on Arabidopsis chloroplast membranes are available to our knowledge).

Chloroplast ion channels and photosynthesis

As mentioned above, chloride, magnesium [63] and potassium [41] have been proposed to act as dominant counterions in order to counterbalance proton entry into the thylakoid lumen. The relative contribution of these three ions is still unclear. In higher plants, a K⁺ flux out of the thylakoid was measured upon illumination [64,65] and tetraethylammonium⁺ (TEA⁺), a potassium channel inhibitor, was shown to reduce photosynthetic electron transport rate (ETR) [66]. The authors proposed that restriction of K⁺ efflux in the light would lead to an increased membrane potential (the lumen becoming more positive).
across the thylakoid concomitantly with light-induced proton pumping. The buildup of positive charge within the lumen would increase the electrical gradient against which proton pumping must occur, thus imposing an increased restraint on electron transport. However, 10 mM TEACl has also been observed to increase ETR in other experiments because of its action as uncoupler [67,68]. Instead, another general potassium channel blocker, Cesium, significantly decreased ETR (100 mM) [67]. A channel activity, inhibited by 500 mM TEA+, was recorded with thylakoid vesicles incorporated into artificial membrane [41]. The conductance of all cation-permeable channels recorded in thylakoids is large enough to account for an efficient counterbalance through these channels as elegantly calculated by Pottosin [46]. At least one potassium channel protein is indeed present in thylakoids, given that a thylakoid protein of 33 kDa, recognized by an antibody specific for the pore region in K+ channels, was found in spinach [66]. We have recently reported detection of the putative two-pore potassium channel 3 from WT Arabidopsis thaliana (AtTPK3) in thylakoid membrane using a specific monoclonal antibody which specifically recognizes the 51 kDa protein [20]. This localization was further confirmed by other methods (Carraresco et al, unpublished result) but the channel activity of TPK3 remains to be established. Work is under way in our laboratory to determine the role, if any, of TPK3 in the regulation of photosynthesis, in light of the observation that in cyanobacteria a potassium channel regulates photosynthesis as described above.

Chloride channel activities in the thylakoid membrane have been reported by Schönknecht et al. [36] in higher plant, and in an alga [44]. The chloride channel (ClC) family comprises seven members in Arabidopsis, present in various membrane compartments [69]. Interestingly, AtClCe sequence is highly similar to cyanobacterial CLCs [70] and prokaryotic CLCs were shown to function as H+/Cl− antiporters [71,72]. AtClCe was proposed to function in maintaining the H+ gradient across the thylakoid membrane [73], however no proof in favor of such hypothesis has been obtained. The clce mutant displayed slight alterations in the kinetics of fluorescence changes upon transfer of dark-adapted leaves to light. Based on increased nitrite content in the cytosol of clce mutant plants, it has been proposed that CLCe transports NO2− to compensate for excess positive charge in the thylakoid lumen [74]. It is to note, that the closest homolog of ClCe in functional organisms has allowed to prove by genetic means an important contribution of ion channel activities to the regulation of photosynthesis, either directly or indirectly. For mammalian mitochondria a compendium of proteins with proven mitochondrial localization (either mass spectrometry or targeting of proteins fused to GFP), named MitroCarta is available [88]. Excellent mass spectrometry studies became available on chloroplast sub-membranes as well [89,90] and hopefully will help the molecular identification of further channels and understanding of their physiological roles.

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