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Modeling the light-induced electric potential difference (ΔΨ), the pH difference (ΔpH) and the proton motive force across the thylakoid membrane in C_3 leaves

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ABSTRACT

A model was constructed which includes electron transport (linear and cyclic and Mehler type reaction) coupled to proton translocation, counter ion movement, ATP synthesis, and Calvin-Benson cycle. The focus is on modeling of the light-induced total electric potential difference (ΔΨ) which in this model originates from the bulk phase electric potential difference ($\Delta\Psi_b$), the localized electric potential difference ($\Delta\Psi_c$), as well as the surface electric potential difference ($\Delta\Psi_s$). The measured dual wavelength transmittance signal (ΔA515–560 nm, electrochromic shift) was used as a proxy for experimental ΔΨ. The predictions for theoretical ΔΨ vary with assumed contribution of $\Delta\Psi_s$, which might imply that the measured ΔA515–560 nm trace on a long time scale reflects the interplay of the ΔΨ components. Simulations also show that partitioning of proton motive force (pmf) to $\Delta\Psi_b$ and ΔpH components is sensitive to the stoichiometric ratio of H^+/ATP, energy barrier for ATP synthesis, ionic strength, buffer capacity and light intensity. Our model shows that high buffer capacity promotes the establishment of $\Delta\Psi_b$, while the formation of pH_i minimum is not 'dissipated' but 'postponed' until it reaches the same level as that for low buffer capacity. Under physiologically optimal conditions, the output of the model shows that at steady state in light, the ΔpH component is the main contributor to pmf to drive ATP synthesis while a low $\Delta\Psi_b$ persists energizing the membrane. Our model predicts 11 mV as the resting electric potential difference across the thylakoid membrane in dark. We suggest that the model presented in this work can be integrated as a module into a more comprehensive model of oxygenic photosynthesis.

1. Introduction

In higher plants and algae, the light-dependent photosynthetic reactions are facilitated by pigment-protein complexes located in the thylakoid membrane of chloroplasts (for general reviews see, e.g., Govindjee, 1982; Eaton-Rye et al., 2012; Blankenship, 2014). Light is captured by light harvesting complexes (LHCs), which funnel excitons to photochemical reaction centers of photosystem I (PSI) and photosystem II (PSII). Special subsets of chlorophyll molecules in PSI (P700) and PSII (P680) are excited by the excitation energy transfer; this then leads to charge separation in P700 and P680. The electron separated from P700 is transported through PSI and then to ferredoxin (Fd), which, in turn, reduces $NADP^+$ to NADPH using ferredoxin-$NADP^+$-oxidoreductase (FNR). The electron separated from P680 is transported through PSII and then *via* the plastoquinone (PQ) pool, the cytochrome b_6f ($cytb_6f$) complex and plastocyanin (PC) to the oxidized

Abbreviations: ATP, adenosine triphosphate; BC, buffer capacity; b_H, b_L, the high and low potential hemes b of the cytochrome b_6f complex; CBC, Calvin-Benson cycle; CD, charge difference; CET, cyclic electron transport; CO_{sp}, coefficient which relates $\Delta\Psi_s$ to ΔpH; CS, current summing; $cytb_6f$, cytochrome b_6f; ΔA515-560, electrochromic shift measured by dual-wavelength technique; ΔpH, pH difference across the membrane; ΔΨ, (total) electric potential difference, i.e., voltage, across the membrane; ΔΨb, delocalized bulk phase electric potential difference across the membrane; ΔΨc, localized ("Coulombic") electric potential difference across the membrane; ΔΨs, surface electric potential difference across the membrane; E_C, electric capacity; ECS, P515, electrochromic shift; Fd, ferredoxin; FNR, ferredoxin-$NADP^+$-oxidoreductase; ΔG_{atp}, energy barrier for ATP synthesis; ΔG^0_{atp}, standard Gibbs free energy change of ATP formation; GHK, Goldman–Hodgkin–Katz; O_{atp}, fraction of "open" (active) ATPsynthases; LET, linear electron transport; LHCs, light harvesting complexes; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NPQ, non-photochemical quenching of chlorophyll fluorescecnce; OEC, oxygen evolving complex; OP, open probability; P680, P700, electron donor in photosystem II and in photosystem I – reaction center chlorophylls with absorption peaks at 680 nm and at 700 nm; PFD, photon flux density; PC, plastocyanin; pmf, proton motive force; PQ, plastoquinone; PSI, PSII, photosystem I, photosystem II; Q_A, Q_B, the first and the second plastoquinone electron acceptors in photosystem II; ST, single turnover

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P700 to reduce it. The oxidized P680 is reduced by electron coming from four-manganese cluster of the oxygen evolving complex (OEC). So that the above reactions can proceed continuously, oxidation of H_2O in the OEC provides the electrons upon releasing molecular oxygen and protons to lumen. The above-described linear electron transport (LET) from H_2O to NADPH is also coupled to translocation of protons across the thylakoid membrane (from stroma to lumen) by the PQ pool and the Q-cycle at $cytb_6f$. Protons in stroma are consumed by the Calvin-Benson cycle (CBC) function, namely during transition of 2,3-bisphosphoglyceric acid into glyceraldehyde 3-phosphate. All these proton-related reactions cause acidification of the lumen and alkalization of the stroma, generating a pH difference (ΔpH) across thylakoid membrane.

The ΔpH contributes to the proton motive force (pmf), which is the driving force for ATP synthesis facilitated by the CF_0-CF_1 type of ATP-synthase in chloroplasts. The pmf is defined as:

$$pmf = \Delta\Psi_{i-o} + \left(\frac{2.\ 3RT}{F}\right)\Delta pH_{o-i}, \quad (1)$$

where $\Delta\Psi_{i-o}$ and ΔpH_{o-i} represent the electric potential difference (i.e., voltage) across the thylakoid membrane and the pH difference, respectively, calculated as outside (stroma) minus inside (lumen) for ΔpH and inside (lumen) minus outside (stroma) for ΔΨ. R, T and F have their common physical meanings. According to the chemiosmotic hypothesis (Mitchell, 1966), the two pmf components are thermodynamically equivalent.

The formation of light-induced ΔΨ across the thylakoid membrane is multiphasic process (Junge, 1977; Witt, 1979; van Kooten et al., 1986; Cruz et al., 2001). (i) The electrons in the photosynthetic electron transport represent temporary fixed charges that generate localized electric potential difference ($\Delta\Psi_c$) due to the Coulomb's law. (ii) An increase in the positive charge in the lumen arises from the external H^+ uptake and internal H^+ release through the above-mentioned photolytic reactions. The increase is counterbalanced by movement K^+, Mg^{2+} and Cl^- through selective ion channels in the thylakoid membrane (Schönknecht et al., 1988; Tester and Blatt, 1989; Enz et al., 1993; Pottosin and Schönknecht, 1995, 1996; reviewed in Pfeil et al., 2014; Höhner et al., 2016; Pottosin and Shabala, 2016). The redistribution of protons and the other ions between aqueous bulk phases of stroma and lumen generates a delocalized electric potential difference ($\Delta\Psi_b$). (iii) The presence of protons and the ions in the bulk phases of stroma and lumen together with the presence of negative charges on the surfaces of the membrane and embeded proteins give rise to the formation of the surface electric potential difference ($\Delta\Psi_s$) across the membrane.

In addition to their roles in the driving force for ATP synthesis, ΔpH and ΔΨ have regulatory functions. Acidification of stroma decreases rate of PQH_2 oxidation at $cytb_6f$, (e.g, Kramer et al., 2003) and it enables activation of violaxanthin de-epoxidase and protonation of psbS protein, leading to onset of the energy dependent non-photochemical quenching (NPQ) (e.g., Johnson et al., 2009; Ruban et al., 2012, Demmig-Adams et al., 2014) of the excited state of chlorophyll a. On the other hand, alkalization of stroma enhances the turnover of CBC (e.g., Kuvykin et al., 2011). Artificial increase of ΔΨ by blockage of the K^+ channels decreases rate of O_2 evolution (Segalla et al., 2005), probably by charge recombination (Dau and Sauer, 1991) and deceleration of the photosynthetic efficacy. Silencing of genes for TPK3 K^+ channels has a similar effect (Carraretto et al., 2013).

The two components of pmf have been extensively studied. Early works indicate a significant pH difference across the thylakoid membrane (ΔpH > 3–3.5; Rottenberg et al., 1972; Rottenberg and Grünwald, 1972; Rottenberg, 1979). Subsequent works show moderate acidification of the thylakoid lumen (Rumberg and Siggel, 1969; Tikhonov et al., 1981; Nishio and Whitmarsh, 1993; Hope et al., 1994). Kramer and co-workers argued the pH_i should not drop below ~5.8 (Kramer et al., 1999). Tikhonov et al. (2008) fixed pH_o to 7.8 and measured biphasic transitions of pH_i between 5.4 and 5.7 in the phase of photosynthetic control, and pH_i between 5.7 and 6.0 in the phase of photophosphorylation, corresponding to ΔpH≈1.8–2.1.

ΔΨ is routinely probed by two approaches. The microelectrode method (Bulychev and Vredenberg, 1976; Vredenberg, 1981) shows a peak value of the light-induced ΔΨ of approx. 50–60 mV and the steady state level in the illumination is ~10 mV (Bulychev and Vredenberg, 1976). However, the low steady state ΔΨ may be an artifact, caused by the ion-conducting hole produced by the injection acting like a shunt (Witt, 1979) and by the high salt concentration in microelectrodes (Cruz et al., 2001). Alternatively, ΔΨ can be measured using the electrochromic shift (ECS; Witt, 1971, 1979; Joliot and Joliot, 1989; Vredenberg, 1981, 1997) of absorption maximum of pigments (chlorophylls and carotenoids) which linearly correlates with magnitude of ΔΨ across the thylakoid membrane. The maximal electrochromic changes take place at approximately 515 nm, thus the electrochromism is simply termed P515 (the terms P515 and ECS are used as synonymous in the text below). This technique might also be prone to artifacts. Thus, most of early studies were carried out using pulse technique to differentiate the P515 from slow non-specific absorption change (see, Klughammer et al., 2013). This slow non-specific band shift was mainly attributed to 'light-scattering' caused by the lumenal acidification (Heber, 1969). Measurements with diffuse incident light, to minimize the 'light scattering' effect caused by the sample, showed that approximately half of the maximal ECS persists in the light for several minutes (Kramer and Sacksteder, 1998). Kramer and co-workers concluded that ~ 50% of chloroplast pmf can be stored as ΔΨ under the steady state condition (Cruz et al., 2001, 2005a). But Johnson and Ruban (2014) suggested that no steady state ECS signal lasts in the light longer than ~ 60 s and the persisting ECS observed by Kramer and co-workers was due to the 'slow component' corresponding to qE-related absorbance change peaking at 535 nm.

ΔΨ is a fundamental cellular parameter determined by many factors, such as ionic activities in the lumen and stroma (Cruz et al., 2001), which, in turn, are influenced by ΔΨ due to its impact on transmembrane ion fluxes. These complex interrelationships make any computational modeling valuable in order to gain an integral understanding of experimental results. Numerous models were built to study the photosynthesis on different levels, such as comprehensive C_3 photosynthesis (Laisk et al., 2006; Zhu et al., 2013), electron transport (Laisk and Walker, 1989; Lebedeva et al., 2002; Vredenberg, 2011), carbon metabolism (Laisk et al., 1986, 1989; Pettersson and Ryde-Pettersson, 1988; Pettersson, 1997; Poolman and Fell, 2000), chlorophyll fluorescence induction (Stirbet et al., 1998; Tomek et al., 2001; Lazár, 2003, 2009, 2013; Sušila et al., 2004; Lazár et al., 2005; Zhu et al., 2005), non-photochemical quenching (Ebenhöh et al., 2011; Zaks et al., 2012) or the state transitions (Ebenhöh et al., 2014; Strirber and Govindjee, 2016). For reviews of modeling of light-dependent photosynthetic reactions, such as the electron transport in thylakoid membrane (for the purpose of modeling of the chlorophyll fluorescence induction) see Lazár and Schansker (2009), Stirbet and Govindjee (2011, 2012), Stirbet et al. (2014) and Tikhonov and Vershubskii (2014), whereas for reviews of the CBC models see Arnold and Nikoloski (2011) and Jablonsky at al. (2011). For a review of the modeling of photosynthetic processes generally, from molecules to ecosystems, see Laisk et al. (2009).

In this work, we have focused on modeling of the light-induced ΔΨ, as well as the pmf partitioning to ΔΨ and ΔpH components under various scenarios. The model presented in this work provides a fundamental computational platform consisting of the photosynthetically indispensable segments. We suggest the current model can function as a starting point for the more comprehensive model of oxygenic photosynthesis in the future.