The size of the lumenal proton pool in leaves during induction and steady-state photosynthesis

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We applied a new method for the measurement of the lumenal proton pool in leaf chloroplasts, based on the shift of $CO_2 \leftrightarrow$ bicarbonate equilibrium due to pH change in chloroplast stroma, induced by protons returning from the lumen upon darkening. During the post-illumination proton transfer through ATPases stroma becomes more acidic, the $CO_2 + H_2O \leftrightarrow HCO_3^- + H_2$ equilibrium shifts towards CO_2, which evolves from the leaf as a CO_2 burst on the background of the post-illumination RuBP carboxylation. During the same process also 3-phosphoglyceric acid (PGA) is formed at the rate of 2 X CO_2 fixation, which also causes stroma acidification. The known PGA formation rate was used for the calibration of the CO_2 burst in the units of μmol H^+ m^{-2}. In Rubisco-deficient transgenic tobacco leaves the results showed that protons do not accumulate in the lumen to a significant extent while photosynthesis remains light-limited, but a large pool of about 100 μmol H^+ m^{-2} accumulates in the lumen as soon as photosynthesis becomes light-saturated. During the dark-light-dark transitions the stromal buffering system is depleted/reloaded by 100 (translocated into the lumen) + 200 (PGA carboxyl groups reduced) μmol H^+ m^{-2}, inducing alkalization or acidification in the stroma by up to 0.4 pH units. Stromal pH changes contribute to the transthylakoid ΔpH, but enzyme activities are little influenced since the pH changes occur in the alkaline range (7.8 to 8.2). The estimated amount of the protonated part of the stromal buffer is about 500 μmol m^{-2}. Since the estimate exceeds the possible pool of phosphate buffer in the chloroplast, we suggest that in the dark most protons are bound to the high-pK groups accessible in the thylakoid membranes from the stromal side, but in the light these protons are translocated to the low-pK groups accessible from the lumenal side.

In the course of the dark-light induction of photosynthesis protons accumulate in the lumen during the reduction of the PGA pool that was present in the dark. The accumulation of electrons in the reduced compounds of stroma (and cytosol) is the most natural cause for the primary accumulation of a coupled pool of protons in the lumen. During steady-state photosynthesis, even the 3ATP/2NADPH ratio ensured, additional losses of ATP for starch, protein and other secondary syntheses need to be compensated by continuous electron flow to alternative acceptors, such as dioxygen, nitrite and oxaloacetate.