Unknowns in photosynthesis and insights from kinetic experiments on leaves

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Contrary to isolated objects, leaves contain a complete functional photosynthetic machinery. Kinetic responses of leaf photosynthesis reveal information about the actual state and regulatory interactions between different components of the photosynthetic machinery.

An original system for kinetic investigation of leaf photosynthesis allows one to condition light, CO_2, O_2 and water vapor concentrations and to measure CO_2 , O_2 and H_2O exchange rates, Chl fluorescence and leaf transmittance at 810 and 950, 520 and 540 nm. Experimental procedures and data processing are computerized. Response times are 0.8 and 1.5 s for O_2 and CO_2 measurements respectively. Extensive studies have provided the following insights into the photosynthetic machinery.

Photosystem II. O_2 evolution from an individual single-turnover flash (STF) reveals PSII pools (1-2 μmol m^{-2}). O_2 evolution during intense ms-length light pulses (MTP) showed e^- transfer rates of 2000-3000 μmol m^{-2} s^{-1}. Only little P680 oxidation could be detected from fluorescence, but not at all from the 810 nm transmittance signal. Oxygen evolving turnover time is < 1 ms. Comparison of e^- transport rates (ETR) from fluorescence and O_2 evolution detected significant cycling of electrons around (inside) PSII.

Interphotosystem transport. Dark-light O_2 evolution transients show the pools of mobile carriers (20 to 30 e^- per PSII). Diffusion is not limiting interphotosystem transport. Cyt f, plastocyanin (PC) and P700 are close to redox equilibrium even during fast photosynthesis. Considering this, leaf transmittance measurements at 950 (or 810) nm reveal the pools PC and P700 in leaves and ETR through PSI. Interphotosystem linear ETR is controlled by Cyt b_6f complex. Turnover of the Q-cycle is controlled by proton flow through ATP synthase.

Cyclic electron transport (CET). CET was measured as the difference between electron flow for CO reduction (J_C) and electron flow through PSI (J_I), $J_{Cyc} = J_I - J_C$. CET is absent at light limitation, but forms about 30% of J_I in saturating light. Such fast CET cannot be H^+-coupled, since so much excess ATP is not needed. We propose CET mechanism is the reversal of the Q-cycle without the involvement of plastoquinone (Abstract by Eero Talts).

Alternative e^- pathways at PSI acceptor side (AET). Alternative e^- transport to NO_2- and oxaloacetate (OA) reduction were measured as difference between photosynthetic O_2 evolution and CO_2 uptake $A_O - A_C$. NO_2- reduction rate is about 1 μmol O_2 (or 4 μmol e^-) m^{-2} s^{-1}, saturating at PFD < 50 μmol quanta m^{-2} s^{-1}. OA reduction rate is similar, but saturates in parallel with photosynthesis. Mehler reaction is even slower. Since CET is uncoupled from H^+, AET compensates for ATP uses in excess of 3ATP/2NADPH ratio ensured by LET (Abstract by Hillar Eichelmann).

Stroma pH and lumenal proton pool. Changes in **s**tromal pH are indicated by shifts in the bicarbonate equilibrium $CO_2 + H_2O \leftrightarrow HCO_3^- + H_2$. CO_2 burst indicates that stroma is becoming acidic, CO_2 gulp – alkaline. CO_2 bursts recorded during the light-dark transients revealed pH decrease in stroma induced by H^+ returning from the lumen upon darkening. The lumenal H^+ pool is about 100 μmol m^{-2}. Stromal pH may increase from 7.8-8.0 in the dark to 8.3-8.5 in the light (Abstract by Vello Oja).

Rubisco activity and kinetics. The fast-response CO_2 measurement system allowed us to investigate Rubisco kinetics in intact leaves. The in vivo $K_m(CO_2)$ is 10 μM. Rubisco V_m exceeds the actual CO_2 saturated rate by about twice. RuBP and PGA compete for the active site with equal affinities. Rubisco activation state is variable, with k_{cat} between 1 to 5 s^{-1}, probably related to the capacity of the electron transport system via the conversion ADP ↔ATP on Rubisco activase.