QUANTITATIVE ANALYSIS OF INTRATHYLAKOID pH AND XANTHOPHYLL CYCLE EFFECTS ON PSII FLUORESCENCE LIFETIME DISTRIBUTIONS AND INTENSITY.

[1]Gilmore AM, [2]Shinkarev, VP, [2]Hazlett TL and [2]Govindjee
[1]PBE, ANU/RSBS, Canberra 0200 ACT, Australia and [2]UIUC, Urbana, IL 61801 USA.

1. Introduction

Dissipation of excess absorbed light energy as heat in the photosynthetic apparatus of higher plants is feedback regulated by limitations in the photosynthetic capacity (1-3). Although, the energy dissipation process depends on both intrathylakoid acidification and xanthophyll cycle deepoxidation (2-4) these relationships have not yet been quantified. Here we summarize a kinetic model, derived from a global analysis, that quantifies the relationships between the intrathylakoid pH, the level of xanthophyll cycle deepoxidation and the PSII chlorophyll (Chl) *a* fluorescence lifetime distributions and intensity. Supporting experimental results precede the model derivation and application. Details of this summary are in press elsewhere (5).

2. Materials and Methods

All procedures used in this study have been described in detail by Gilmore et al. (5) and in references cited therein.

3. Results and Discussion

3.1. PSII Chl a fluorescence and intrathylakoid pH data
Figure 1 shows the results of experiments with spinach on PSII Chl *a* fluorescence, measured by using both Pulse Amplitude Modulation (Fig. 1A) and ps-time-resolved fluorescence methods (Fig. 1B). Figure 1A shows a typical recording of nonphotochemical quenching measurements during fluorescence and uncoupler-induced reversal of this fluorescence quenching. Most importantly, Fig. 1A shows the level of fluorescence under three intrathylakoid pH conditions: under light saturating conditions at
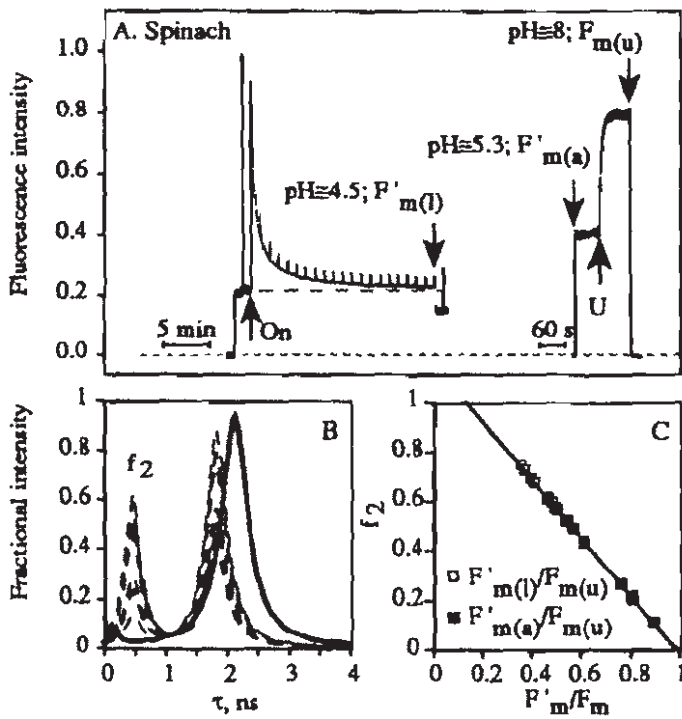
Figure 1. Chlorophyll a Fluorescence intensity and lifetime distributions in spinach thylakoids. In (A) On indicates actinic light and U indicates uncoupler, see ref. (5) and text for details.

pH$\cong$4.5 (F'$_{m(l)}$), under ATPase mediated pH conditions at pH$\cong$5.2 (F'$_{m(a)}$) and after uncoupling at pH$\cong$8 (F$_{m(u)}$). The pH was estimated from quenching of 9-aminoacridine fluorescence (5). Figure 1B shows the bimodal PSII Chl a fluorescence lifetime distributions under the uncoupled conditions (dark solid line) and under the ATPase (pH$\cong$5.2) conditions (dashed lines). The fractional intensity of the 0.5 ns lifetime distribution f$_2$ correlates with increasing concentrations of the deepoxidized xanthophylls zeaxanthin [Z] and antheraxanthin [A]; dithiothreitol addition was used to vary the [Z+A] (see below) Figure 1C shows the results of a global analysis where the f$_2$ component is fit to a model where increasing levels of f$_2$ correlate linearly with decreasing F'$_m$/F$_m$. Here F($'$)$_m$ is defined as the maximum fluorescence when all PSII traps are closed and the thylakoid membrane is (energized) or deenergized. Similar profiles are reported for *Arabidopsis*, barley WT and *chlorina* (*clof104*) (5).
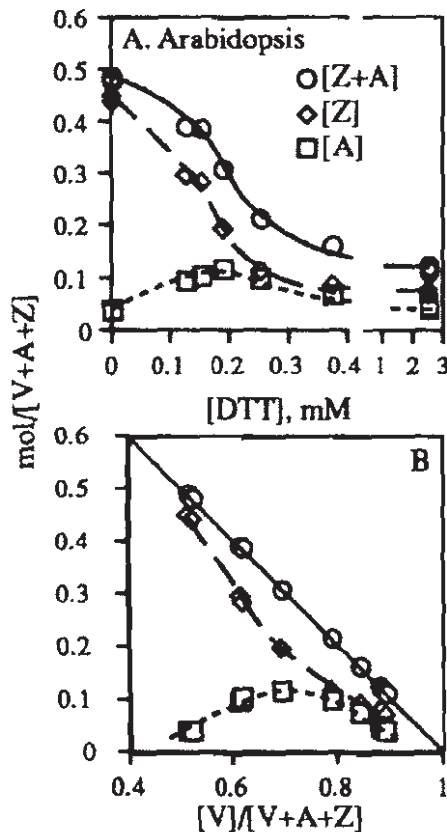
3.2. Xanthophyll cycle pigment concentration data

Figure 2 shows the effects of increasing dithiothreitol concentrations [DTT] on the relative concentrations of the xanthophyll cycle pigments measured in light induction experiments similar to those shown in Fig. 1 for WT *Arabidopsis*. The plots show the concentration of violaxanthin [V] relative to [Z] and [A] and their sum [Z+A]. Obviously increasing [DTT] decreases the amount of [Z]. However, increasing [DTT] effects on [A] are more complex with the [A] first increasing then decreasing as earlier reported by Gilmore and Yamamoto (4). It is clear that beyond 0.25 mM [DTT], the [A] pool constituted about 50% of the total pool of [Z+A]. In no sample was [A] much less than 10% of [Z+A]. Similar results are reported for thylakoids of spinach, WT and *clof104* barley (5).
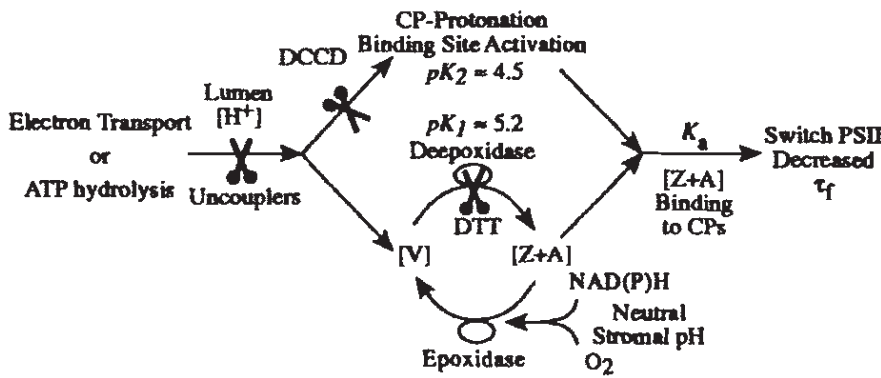

Figure 2. Effects of dithiothreitol on the xanthophyll cycle conversion state in *Arabidopsis* thylakoids (5).

Figure 3. Schematic summary of the xanthophyll cycle-dependent energy dissipation mechanism in thylakoids (5).

3.3. Model derivation and description

Figure 3 summarizes our current model (5) of how the intrathylakoid pH and xanthophyll cycle pigment concentrations interact to influence the PSII Chl a fluorescence yield. Intrathylakoid acidification [H$^+$] plays two roles: first, deepoxidase activation ($pK_1 \cong 5.2$) converting [V] to [Z+A] (6) and, second, possible protonation of glutamate carboxyl groups ($pK_2 \cong 4.5$) of the CP26 and CP29 complexes PSII unit (1, 7). We suggest CP protonation causes a conformational change that activates a binding site for either Z or A. The binding of Z or A (6), defined by the equilibrium constant K_a, causes the PSII unit to *switch* to a decreased fluorescence lifetime, τ_f (8, 9). Although uncouplers inhibit and reverse both pH dependent steps uncouplers do not alter the [Z+A]unless both NAD(P)H and bovine-serum albumin are added to activate the epoxidase located on the stromal side of the thylakoids with an optimal pH$\cong$7 (9). Dithiothreitol, DTT, inhibits deepoxidation (6) but not the light- or ATPase proton pumping. The carboxyl-labeling reagent dicyclohexylcarbodiimide, DCCD, inhibits the CP protonation and hence the energy dissipation (1).

From these assumptions, we derived our simple pH-dependent binding model. The protonation step(s) are accounted for by the Henderson-Hasselbach equation pH = pK_2 + log[PSII$^-$]·[PSII$^+$]$^{-1}$, where [PSII$^-$] is the concentration of PSII units with an inactive binding site and [PSII$^+$] is the concentration of PSII units with an active binding site. The simple binding equation K_a = [PSII$^+$-Z]·([PSII$^+$]·[Z])$^{-1}$ defines the association constant, where [PSII$^+$-Z] is the concentration of PSII units with bound Z or A and [Z] is the Z+A concentration per PSII. After, combining the pK_2 and K_a equations and other steps (see ref. 5) the final equation is:

$$f_2 = \frac{[PSII^+ - Z]}{[PSII_{tot}]} = \frac{K_a[Z]10^{pK_2-pH}}{1+10^{pK_2-pH}(1+K_a[Z])} \qquad (1).$$

3.4. Model applications and implications

Figure 4 presents the correlations between the [Z] and [A] and the fluorescence lifetime distribution f2 under the three intrathylakoid pH conditions. Figure 4A shows the correlations depend on incorporating both [A] and [Z]; the optimal K_a for [A] was 1.26 times [Z], meaning that [A] binds and quenches as efficiently as [Z]; this confirms other recent studies (4, 8, 11). Figures 4A and B show K_a is erroneous without [A]; when x=0 the variance in K_a increases by over ten fold. Figure 4C shows the predictions (lines) for the three intrathylakoid pH levels (open squares, pH≅4.5;closed pH≅5.2; circles pH≅8); the dashed lines represent the standard deviation in the calculated K_a value. Low levels of [Z] and [A] correlate with substantial values of f2. In our model only 1 mol Z or A per PSII actively engages in energy dissipation at any one instant. Note also the f2 nearly saturates under low pH conditions when the [Z]+[A] exceeds 5 or 6 mols per PSII. No energy dissipation was predicted or observed to correlate with the [Z+A] under the uncoupled pH=8 conditions. Also the level of energy dissipation was stimulated in the LHCIIb deficient barley *clof104* mutant with a 50% reduced Chl *a/b* antenna size compared to the WT (not shown) (8).
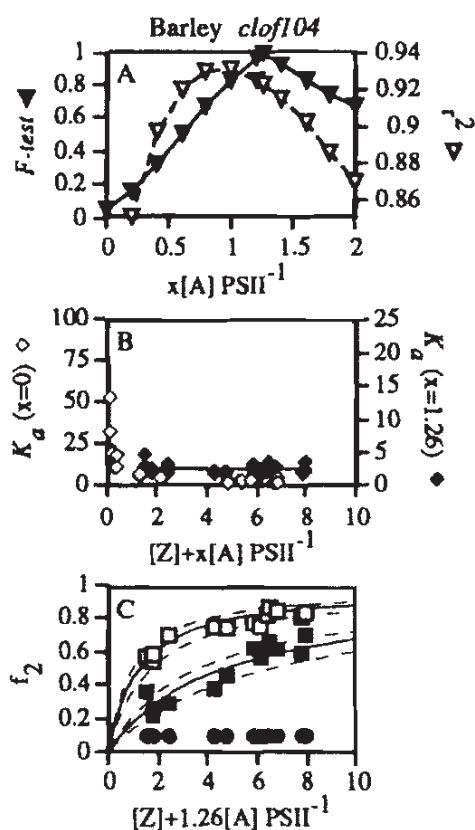
Figure 4. Analysis of the effects of the intrathylakoid pH and xanthophyll cycle pigments on the PSII fluorescence lifetime distributions in *clo104* thylakoids (5).

References

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