Control of excitation energy distribution in photosynthetic systems in vivo

W. PATRICK WILLIAMS
Biophysics Department, Chelsea College, University of London, London SW3 6LX, U.K.

The occurrence of light-driven changes in the distribution of excitation energy between PSI and PSII in intact cells, commonly referred to as state 1/state 2 adaptation, was first reported by Bonaventura & Myers (1969) and Murata (1969). Over the years, different groups have shown that various aspects of these light-induced changes can be mimicked in experiments in vitro in a number of different ways. Homann (1969) first showed that similar changes in excitation energy distribution can be seen following the addition of Mg$^{2+}$ to broken chloroplasts, whilst Krause (1974) demonstrated that intact chloroplasts show sharply decreased PSI fluorescence, subsequently traced to energy-dependent proton-induced quenching (Briantais et al., 1979, 1980), on illumination. These ion-induced and energy-dependent quenching phenomena were, at one time or another, widely invoked in explanations of the light-induced state 1/state 2 changes seen in intact cells.

Parallel measurements of ATP-induced fluorescence quenching and phosphorylation of LHCP in pea chloroplasts (Horton et al., 1981; Telfer et al., 1983) and Chlorella thylakoids (Saito et al., 1983) have shown that these processes follow a similar time course to state 2 adaptation in vivo. Recent measurements of low temperature (77K) emission spectra in samples containing internal standards have confirmed earlier claims that the ATP-induced changes reflect a genuine redistribution of excitation energy between PSII and PSI rather than a preferential quenching of PSI by ATP (Krause & Behrend, 1983; Saito et al., 1983). These observations also show that ATP-induced changes are observed only under conditions in which the plastoquinone pool is over-reduced (Horton et al., 1981; Allen et al., 1981) strongly support the idea that state 1/state 2 changes are brought about by changes in LHCP phosphorylation. It is important to remember, however, that similar changes in excitation energy distribution and low temperature emission spectra are observed following changes in Mg$^{2+}$ concentration (Krause et al., 1983), that direct evidence of light-induced LHCP phosphorylation under conditions in vivo is not yet available and that whilst it is becoming increasingly clear that chlorophyll-protein complexes other than LHCP may be involved in the control of excitation energy distribution in green plants (Bennett, 1980), the state 1/state 2 adaptation in red and blue-green algae remains to be substantiated. With this in mind, the question of whether or not LHCP phosphorylation provides an adequate basis for explaining the changes seen under conditions in vivo is clearly not a trivial one.

The simplest and most convenient way to demonstrate state 1/state 2 changes in algae is to examine the effects of pre-illumination on the fluorescence yield of PSI. In general, this is determined by three factors: the fraction of open PSI traps, the extent of energy-dependent quenching and the extent of state 1/state 2 adaptation. Whilst these effects can be disentangled using modulated fluorescence techniques (Telfer & Barber, 1981), the simplest procedure is to measure the fluorescence yield shortly after the addition of DCMU. This leads to the closure of all PSII traps and the rapid relaxation of energy-dependent fluorescence (Williams et al., 1980). The fluorescence yield measured shortly after DCMU addition thus reflects the maximal fluorescence yield ($F_{m}$) of the pre-adapted state. Typical plots for Chlorella cells adapted to state 1 and state 2, by pre-illumination in red or far-red light, and for dark-adapted cells are shown in Fig. 1a. If the algae are left in light, the fluorescence yield of the state 2- and dark-adapted cells increases to that of the state 1-adapted cells. This is readily explained on the basis of the LHCP-phosphorylation model. DCMU blocks electron transport from PSI into the plastoquinone pool which consequently becomes oxidized leading to dephosphorylation of LHCP and a relaxation of the algae to state 1.

If, however, the poisoned algae are left in the dark, their fluorescence yield returns to that of the dark-adapted algae. The low fluorescence yield of such algae is more difficult to explain. Low temperature emission spectra (M. Catt, K. Saito & W. P. Williams, unpublished work) indicate that dark-adapted algae show a similar excitation energy distribution to state 2-adapted algae, indicating that their fluorescence yield is not yet available and that whilst it cannot be attributed to LHCP phosphorylation. Addition of uncouplers or ATPase inhibitors, however, abolishes the light-dependent relaxation of DCMU-poisoned algae to state 1 and also lowers the fluorescence yield of state 1- or state 2-adapted algae to that of dark-adapted samples (Figs. 1b and 1c). It is thus quite clear that the lowered fluorescence yield of these algae cannot be attributed to LHCP phosphorylation.

A further indication that at least two mechanisms of excitation energy distribution control are operative is provided by the results presented in Fig. 2. In these experiments, the algae have first been adapted to the dark state and then illuminated with different intensities of red or far-red light. DCMU has then been added and the value of $F_{m}$ measured as described above. Exposure to low light intensities leads to a sharp increase in $F_{m}$. This rise, which is DCMU-insensitive and wavelength-independent, appears to reflect a simple reversal of dark adaptation and leaves the algae in a state close to state 1. Higher intensities of red light tend to reverse this initial increase, lowering $F_{m}$ to its state 2 level. Exactly analogous results are obtained if far-red light or, as in Fig. 2, red light in the presence of DCMU is used to drive the algae towards state 1. Two separate control mechanisms appear to be in operation: one operating at very low light intensities which reflect a simple light/dark, change and the other operating at higher intensities reflecting the normal state 1/state 2 changes.

These observations can readily be explained in terms of the simple scheme involving a synergetic linkage between light-induced ion movements and LHCP phosphorylation set out in Fig. 3. In terms of this model, the low intensity DCMU-independent light/dark process reflects ion-induced changes in membrane organization of the type seen following changes in divalent ion concentration in broken chloroplasts (Barber, 1980). The driving force for these changes is probably the release of Mg$^{2+}$ bound to, or accumulated within, the thylakoids during dark adaptation. The inhibition of these changes by uncouplers is consistent with a linkage between these ion movements and light-induced proton fluxes. As such fluxes are a prerequisite for ATP formation under conditions in vivo, the changes associated with LHCP phosphorylation are necessarily superimposed on top of these ion-induced changes in intact cells. These fluxes are not, however, a requirement for LHCP phosphorylation per se. In the presence of exogenous ATP and the necessary reducing agents to activate the kinase system, LHCP is readily phosphorylated even in the dark or when uncouplers are present. Most ATP-induced fluorescence quenching experiments performed under...
Fig. 1. Fluoresent yield of Chlorella under different conditions
(a) Slow light-dependent increases in fluorescence yield ($F_m$) of Chlorella poisoned
with DCMU (6 $\mu$M) after pre-adaption to state 1, state 2 or Dark state. Initial
fluorescence yields are typical of the pre-adapted states. The dark-reversal of these
changes, measured at very low incident light intensities in the presence of NH$_2$OH
(250 $\mu$M) is shown in the inset. (b) and (c) show the effects of the uncoupler carbonyl-
cyanide m-chlorophenylhydrazone (CCCP) and ATPase inhibitor $N,N'$-dicyclo-
hexyl-carbodi-imide (DCCD) on these traces when added either (i) together with the
DCMU or (ii) after completion of the light-dependent fluorescence increase.

Fig. 2. Intensity dependencies of state I and state II adaptation
Algal samples were first dark adapted for 20 min then illuminated in different intensities of red light in the
presence or absence of DCMU (6 $\mu$M) for a further 15 min to drive them to state 2 or state 1 respectively. The value of $F_m$
at the end of this adaptation period was then measured as shown in Fig. 1a.

Fig. 3. Model illustrating the relationship between the ion-
duced changes associated with the wavelength-independent light/dark changes and the LHCP phosphorylation changes
associated with state I/state 2 changes
See text for further details.

This work was supported by S.E.R.C. grant GR/B98142.


Barber, J. (1980) FEBS Lett. 118, 1-10


Biochim. Biophys. Acta 548, 128-138

Biochim. Biophys. Acta 591, 198-202

218
