PHOTOPHOSPHORYLATION AND THE 518 NM ABSORBANCE CHANGE IN TIGHTLY COUPLED CHLOROPLASTS

Margareta BALTSCHEFFSKY and David O. HALL*

Bioenergetics Group, Departments of Plant Physiology and Biochemistry, University of Stockholm, Stockholm, Sweden

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1. Introduction

The carotenoid absorbance change of photosynthetic bacteria is an energy dependent change of the characteristic absorption spectrum of the endogenous carotenoids. It has been shown to occur in the dark, induced by energy rich phosphate compounds [1,2] or ionic gradients [3] as well as in the light [4]. The 518 nm absorbance change of plant chloroplasts is now recognized [5,6] to be similar to the light-induced bacterial carotenoid change. It has recently also been reported to be inducible in the dark, by ionic gradients [7]. However, this dark change may, at least partly, be due to changes in light scatering caused by shrinkage of the chloroplasts [8].

Several hypotheses concerning the mechanism underlying the carotenoid absorbance change have been suggested. The main considerations at present time are focused on two possibilities: a) the change in absorption spectrum is the result of a conformation change in the membrane close to the region where the carotenoid molecules are attached [9,12]; b) the change is due to an electrochromic effect due to the formation of a strong electrostatic field across the membrane to which the carotenoid molecules are attached [10,11].

* Permanent address: Department of Botany, King's College, London, S.E. 24, England.

Address correspondence to: M. Baltscheffsky, University of Stockholm, Arrhenius' laboratory, Biochemistry, S-104 05, Stockholm, Sweden.

In bacterial chromatophore preparations with high capacity for photophosphorylation the extent of the light-induced carotenoid absorbance change has been shown to be decreased under phosphorylating conditions [12]. In agreement with this observation, the addition of low concentrations of ADP to illuminated chromatophores causes a transient decrease in the extent of the carotenoid absorbance change [12]. This phenomenon can also be seen on the light-induced oxidation–reduction state of cytochromes participating in cyclic electron flow, reflecting the electron transport control of cyclic bacterial photophosphorylation. This regulatory property which is equivalent to the respiratory control in animal mitochondria is also well known to exist in non-cyclic electron transport in plant chloroplasts [13,14] and has been termed photosynthetic control.

It has been shown by Rumberg and Siggel [15] that the effect of phosphorylating conditions on a chloroplast suspension after a single flash is an increased rate of decay of the 518 nm absorbance change. This has been interpreted as evidence for the dissipation of an electrostatic field by the phosphorylating reactions. The dacay time of the 518 nm change under non-phosphorylating conditions is a function of the integrity of the chloroplast preparation, so that preparations with a high phosphorylating activity show a slow decay rate. Hauska et al. [16] have shown that in subchloroplast particles the occurrence of the 518 nm change but not the light-induced proton uptake, is dependent on the phosphorylating ability.

The present communication shows that the

515 nm absorbance change readily responds to the photosynthetic control phenomenon and emphasizes the close connection between this change and photophosphorylation in spinach chloroplasts.

2. Material and methods

Spinach chloroplasts were prepared according to Hall et al. [14] and used within four hours of preparation. Only preparations with a high ratio of photosynthetic control were used, the stimulation of non-cyclic electron transport by the addition of ADP was usually more than 5-fold.

The spectrophotometric measurements were performed in a dual beam spectrophotometer, designed and built at the Johnson Research Foundation, Philadelphia, USA. The optical path through the sample was 1 cm. Actinic illumination was obtained from a 40 W microscope lamp at 90° angle from the measuring beam. The actinic light was filtered either through three layers of Wratten 76 gelatin filter or through a 650 nm metal interference filter, 15 nm half band width, VEB Carl Zeiss, Jena. The light was adjusted to saturating intensity.

The ferredoxin was prepared from spinach according to Rao et al. [17]. All reagents used were of analytical grade.

3. Results and discussion

Fig. 1 shows the 518 nm absorbance change under steady state illumination with four different acceptor systems (including endogenous) for non-cyclic electron transport. In all four systems the presence of a phosphate acceptor, ADP, greatly decreases the extent of the 518 nm signal. The analogous result has earlier been obtained with the carotenoid absorbance change in bacterial chromatophores [12]. The presence of ADP in these chloroplasts causes, however, a more pronounced inhibition of the 518 signal than has been seen on the carotenoid change in chromatophores, where always more than 50% of the light induced absorbance change remains under phosphorylating conditions, even in highly active preparations.

Photophosphorylation is inhibited and the ADP

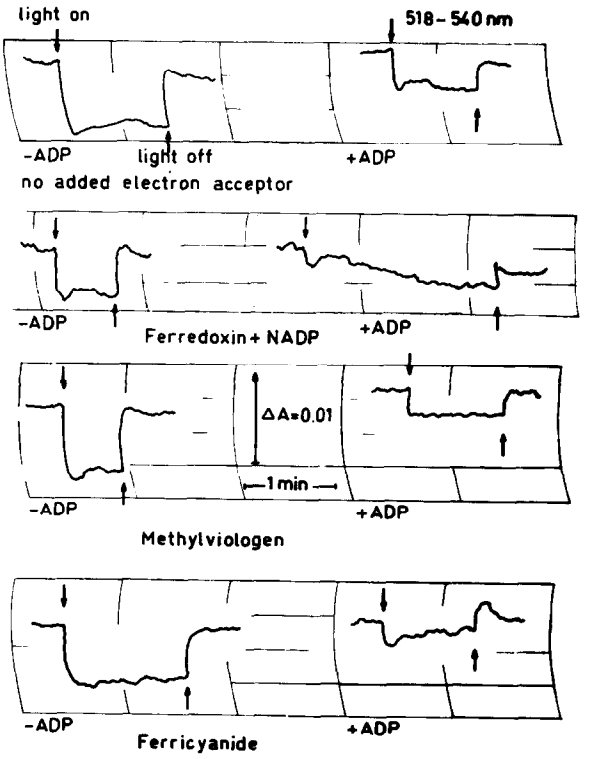

Fig. 1. The effect of ADP on the light-induced 518 nm absorbance change. The reaction medium contained in a total volume of 1.2 ml: chloroplasts equivalent to 0.06 mg chlorophyll in the resuspending medium of ref. [12]; 10 mM P_i and, where indicated, 0.16 mM ADP. Electron acceptor was 16.7 mM NADP and 12 μg ferredoxin or 0.2 mM methylviologen and 2 mM sodium azide or 2.5 mM ferricyanide. Actinic illumination from a 40 W microscope lamp adjusted to saturating intensity. The light was filtered through 2 cm of water and double layers of Wratten 76 gelatin filter.

induced increase in the rate of electron transport is abolished in this type of chloroplasts by the presence of excess EDTA [14]. Under such conditions the effect of added ADP on the 518 nm signal is abolished (fig. 2). The effect of EDTA could be either a chelation of Mg^{2+} necessary for phosphorylation, or a removal of coupling factor from the chloroplast membrane.

The experiments in figs. 1 and 2 were performed with an optimal concentration of ADP present in the chloroplast suspension before illumination. If instead a lower concentration of ADP is added under steady state illumination, i.e. low enough to be phosphorylated in a minute or two, the result is a character-

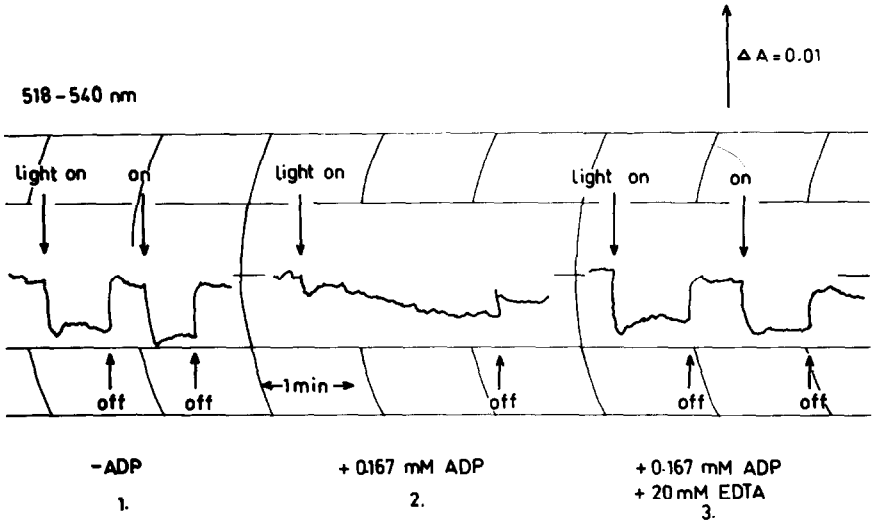

Fig. 2. Inhibition of ADP effect by EDTA. Reaction medium as in fig. 1. Electron acceptor was 16.7 mM NADP and 12 μg ferredoxin. Actinic illumination as in fig. 1.

istic ADP-cycle as shown in fig. 3. The dependence of the duration of the cycle on the concentration of added ADP is also clearly evident from this figure.

Finally, the spectra of both the light-induced change and the effect of ADP added under steady state illumination are shown in fig. 4. The spectra are essentially identical to other published spectra of the 518 nm change in chloroplasts [18] and clearly identify the ADP induced effects as a specific decrease of the 518 nm change.

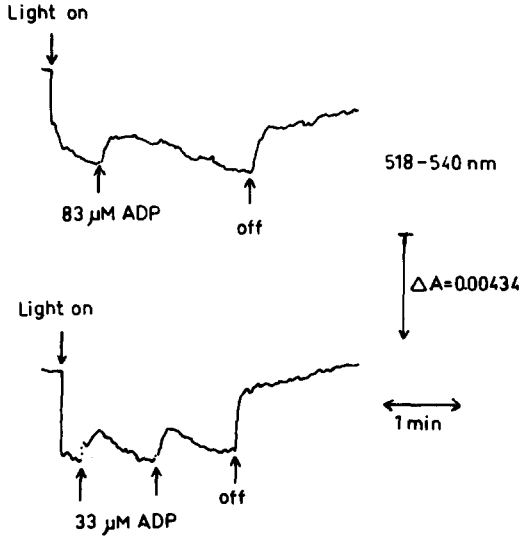

Fig. 3. 'ADP-cycles' on the 518 nm absorbance change. Reaction medium as in fig. 1. Electron acceptor was 2.5 mM ferricyanide. Actinic illumination as in fig. 1 except that a 650 nm interference filter was used instead of the Wratten 76 filter.

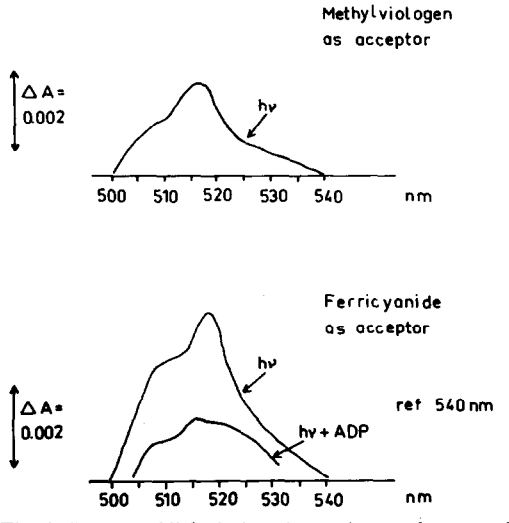

Fig. 4. Spectra of light-induced absorbance change and its reversal by ADP. Experimental conditions as in fig. 3. Electron acceptor in 4A was 0.2 mM methylviologen and 2 mM sodium azide. The light-induced spectra are measured as an absorbance increases at the measured wavelengths, whereas the ADP-induced spectrum consists of absorbance *decreases*.

Neumann et al. [19] studied the effect of ADP on the 518 nm change in chloroplasts and subchloroplast particles, however, using the cyclic PMS (phenazine methosulfate) system. They found that Mg^{2+} alone would cause a decreased signal under continuous illumination as well as an accelerated dark decay after single flashes. Since Mg^{2+} (5 mM) invariably was present in our controls, the effects of phosphorylating conditions in the non-cyclic system reported here are solely due to ADP.

Dilley and Vernon [20] showed that phosphorylating conditions had an inhibitory effect on light-induced conformational changes in chloroplasts. These conformational changes gave rise to light scattering effects which, however, kinetically were much slower than the changes reported here. This is particularly evident for the decay of the 518 nm change upon cessation of illumination.

Our results demonstrate the great similarity between the 518 nm absorbance change in chloroplasts and the carotenoid absorbance change in bacterial chromatophores and the usefulness of these changes as very rapidly responding and sensitive probes of the energetic state in photosynthetic membranes. Mechanistically it seems likely that both changes reflect the same phenomena and that what is known to be true for the change in one type of membrane can be extrapolated to the other type.

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