THE EFFECT OF ATP ON PHOTOCONVERSION OF PROTOCHLOROPHYLLIDE INTO CHLOROPHYLLIDE IN ISOLATED ETHIOPLASTS

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1. Introduction

The light-induced synthesis of chlorophyll during the development of chloroplasts from etioplasts in greening etiolated leaves is characterised by several spectral shifts [1–5]. These shifts have been interpreted as reflecting not only chemical changes but also alterations in the orientation and association of pigment molecules (see [5] for a review). The gross ultrastructural changes occurring in the plastid are closely associated with these spectral shifts [3, 6, 7].

Physical changes such as alternate freezing and thawing or elevation of temperature have been shown to affect the first of these shifts, the conversion of protochlorophyllide (PChl) into chlorophyllide (Chl) [3, 7, 8], but there is no information about the factors which may control the shifts during etioplast development. Since intact etioplasts can now be isolated in sufficient quantities for biochemical investigation [9] and can be maintained in culture for periods of up to 10 hr (P. Horton, unpublished data) it is possible to examine the factors which modify the photoconvertibility of PChl. This paper presents evidence for an ATP effect on this photoconvertibility in isolated etioplasts.

2. Materials and methods

Seedlings of Zea mays (var. Kelvedon Glory) were grown in total darkness at 27° for 8 days. The etioplasts were isolated by the method of Leese et al. [9]. The plastids were resuspended in a medium containing 0.5 M sucrose, 0.4% bovine serum albumin (Cohn Fraction V, Koch Light), 2.5% Ficoll, 5 mM cysteine, 1.0 mM $MgCl_2$, 1.0 mM EDTA, 50 mM $KHCO_3$, 0.5 mM sodium acetate, 1 mM glutamic acid, in 67 mM Na_2HPO_4–KH_2PO_4 buffer adjusted to pH 7.5. The suspension was incubated at 20° in darkness under constant slow rotation as described by Ridley and Leech [10]. The absorption spectra of the etioplasts before and after 5 hr of incubation were measured at room temperature on a Shimadzu MPS 50L multipurpose spectrophotometer [11]. Complete photoconversion was achieved by 20 sec of white light from a tungsten filament lamp (Mazda 150W) filtered through 17 cm of water giving an intensity of 11.6×10^3 ergs $cm^{-1} sec^{-1}$ at the cuvette surface. ATP was added where indicated as the disodium salt (Sigma).

3. Results and discussion

When dark grown plants are illuminated the first change to be recorded is the conversion of PChl to Chl involving a spectral shift from approx. 650 nm to approx. 680 nm [1–5]. Non-convertible PChl absorbing around 630 nm remains; the proportion of non-convertible ($PChl_{630}$) to convertible ($PChl_{650}$) varies with different species and ages of plants [4, 12, 13]. The spectral shifts recorded for suspensions of isolated etioplasts are shown in fig. 1. The absorption spectrum before illumination had the main PChl peak at 650 nm with a shoulder between 630–635 nm. On illumination the 650 nm peak disappears and the Chl absorption band appears (680 nm). If the etioplast suspension is incubated in darkness for 2 hr before illumination a different result is obtained (fig. 1). Before illumination a plateau appears from

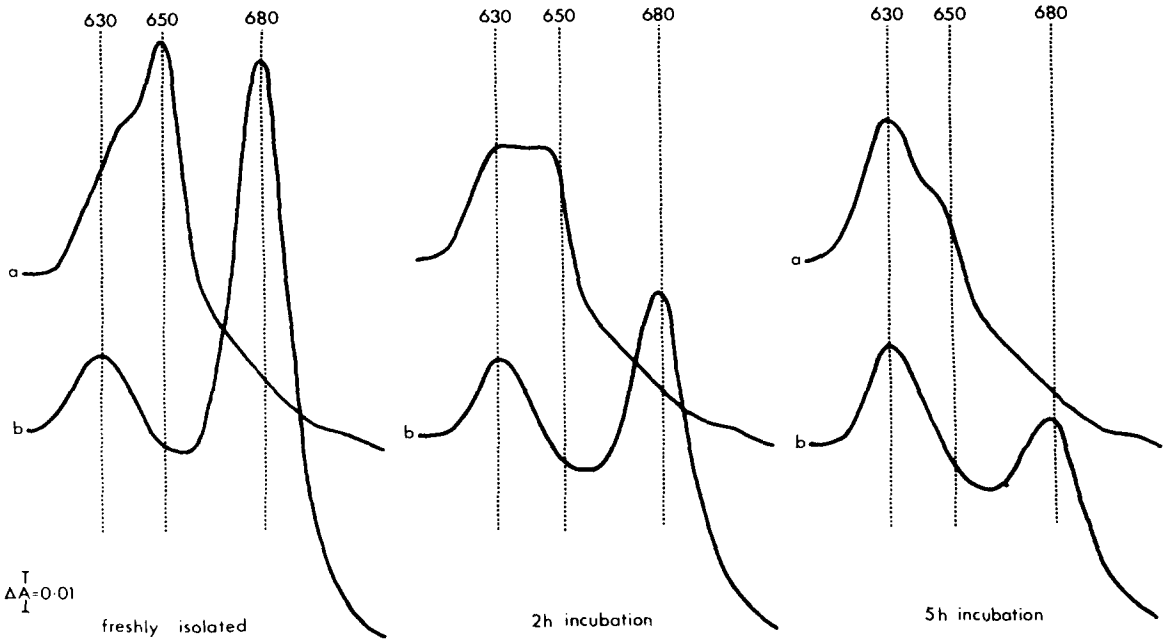

Fig. 1. The absorption spectra of etioplasts 0 hr and 5 hr after isolation. The absorption was measured while the etioplasts were suspended in 66% glycerol to reduce scattering; a) spectra of etioplasts before illumination; b) specta of etioplasts after 20 sec illumination as described in the text.

from 630–650 nm interpreted as showing a decrease in the proportion of $PChl_{650}$ to $PChl_{630}$. On illumination chlorophyll formation is substantially reduced (to 50%) compared with the value for freshly isolated etioplasts. If the pre-illumination dark incubation is continued for 5 hr these differences are further accentuated.

It seemed possible that the photoconvertibility of PChl may depend on the presence of some plastidic or extraplastidic cofactor(s) which are lost during extended incubation. A variety of compounds were added to 1 ml samples of the etioplast incubation and their effect on subsequent photoconvertibility measured. In μmoles NAD, 3; NADP, 3; phosphoenolpyruvate, 4; GSH, 5; dithiothreitol, 5; polyvinylpyrrolidone (1%); and ATP, 5, were added separately but only ATP had a marked and reproducible effect. After incubation in the presence of ATP for 5 hr 75% of the initial photoconvertibility remains and the $PChl_{650}$:$PChl_{630}$ ratio is 1.6 (table 1). This compares with figures of 33% photoconvertibility and a ratio of

Table 1
The effect of ATP on the photoconvertibility of PChl.

Treatment	% Photoconvertibility	$\frac{A_{650}}{A_{630}}$
Intact etioplasts		
a) 0 hr	100	1.95
5 hr	33	1.02
5 hr + ATP	75	1.56
Etioplasts without envelopes		
b) 0 hr	100	1.71
5 hr	37	0.78
5 hr + ATP	76	1.27

a) The effect of the addition of 1.5 mM ATP (final conc.) to intact etioplasts; b) the effect of the addition of 1.5 mM ATP to etioplasts prepared without envelopes. Incubation conditions as described in the text. The degree of photoconvertibility of PChl was measured by expressing the Chl peak height at 680 nm produced by illumination as a % of that obtained by illuminating freshly isolated etioplasts.

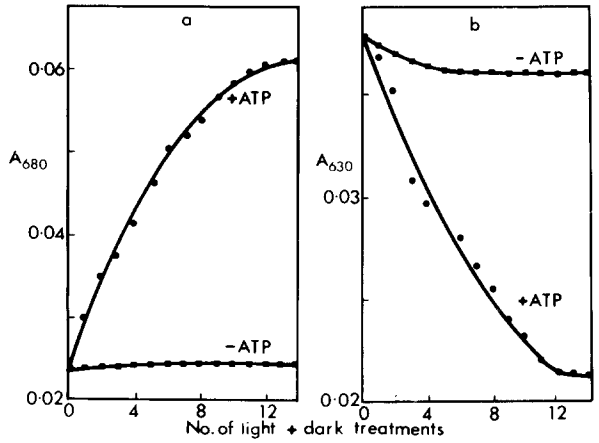

Fig. 2. The ATP-induced synthesis of Chl_{680} from $PChl_{630}$ by light + dark treatments. a) Increase in absorbance at 680 nm; b) decrease in absorbance at 630 nm, Light/dark treatments were 5 sec illumination (as described in text) + 6 min dark. ATP (final conc. 1.5 mM) was added to etioplasts at the end of 5 hr of incubation in darkness with no ATP. Two samples (one with and one without ATP) were given the usual 20 sec illumination to convert $PChl_{650}$ to Chl before being treated with the light + dark regime. The absorption spectra were recorded after each treatment; A_{680} and A_{630} were measured from the same spectrum. Etioplasts were not suspended in glycerol for these measurements.

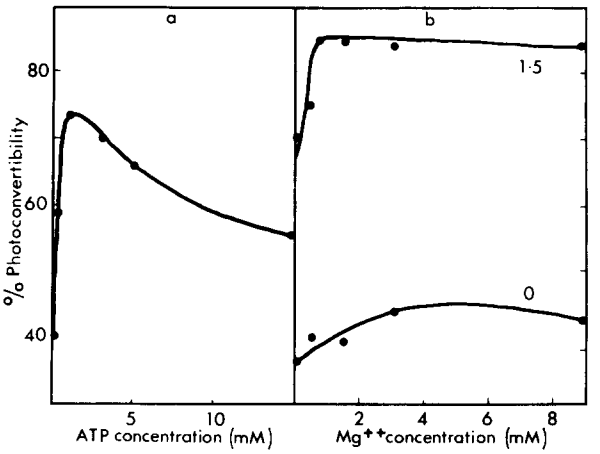

Fig. 3. The effect of varying the ATP and Mg^{2+} concentrations in the etioplast incubation medium on the % photoconvertibility after 5 hr incubation in darkness. a) Different ATP concentrations in the presence of 0.5 mM Mg^{2+}. b) Different Mg^{2+} concentrations at 0 and 1.5 mM ATP levels. % Photoconvertibility was measured as described under table 1. Mg^{2+} was added as $MgCl_2$ in the presence of equimolar Na_2-EDTA. All concentrations are final concentrations in the incubation medium.

$PChl_{650}$:$PChl_{630}$ of 1.0 in the control without ATP. Since there is no difference in total PChl it seems that this increase in the proportion of convertible PChl is at the expense of the non-convertible form. Similar results were obtained when envelope-free etioplasts (prepared by osmotic shock) were used. A demonstration of the ATP-induced photoconversion of non-convertible PChl is shown in fig. 2. Etioplasts were incubated in darkness with no added ATP for 5 hr so that the photoconvertibility had decayed to 21%. The addition of ATP combined with a series of light + dark treatments caused considerable chlorophyll formation (fig. 2a). The absorbance at 680 nm increases from 0.024 after the normal 20 sec illumination to 0.061 after a series of 12 light + dark treatments in the presence of ATP: without ATP no increase above 0.024 at 680 nm is obtained. In the absence of ATP the peak of non-convertible PChl at 630 nm is unaffected by further light + dark treatments, but with added ATP a decrease in absorbance at 630 nm from 0.038 to 0.021 parallels the increases in Chl formation (fig. 2b). Transformation of $PChl_{630}$ is never complete but reaches saturation after 12 light treatments. This level is the same as the level of non-convertible PChl in freshly isolated etioplasts. Thus the $PChl_{630}$ which is activated by ATP appears to be different from the $PChl_{630}$ present initially.

The ATP effect on photoconvertibility has been characterised and shows the expected optimum dependent on the Mg^{2+} concentration (1.0 mM in the presence of 0.5 mM Mg^{2+}, fig. 3a). Fig. 3b shows the effect of altering the Mg^{2+} concentration in the presence of 1.5 mM ATP. ATP in excess of the Mg^{2+} concentration appears to be inhibitory; with 0.5 mM Mg^{2+} only 75% photoconvertibility was obtained whereas at 1.0 mM 85% was obtained.

Fig. 3a and b show that the maximum effect is obtained at a minimum Mg:ATP ratio of 0.5. The reason for this being less than 1 is probably due to either the presence of endogenous Mg^{2+} or the breakdown of ATP during the incubation period. ATP decreases from 1.5 mM to 0.1 mM during the 5 hr incubation. AMP (1.5 mM) reduces the subsequent photoconvertibility after incubation. ADP gives some stimulation of photoconvertibility but is not yet clear whether this effect is due to ADP per se.

The effect of ATP on the photoconvertibility of PChl into Chl is dramatic and represents the first demonstration of an identified biochemical effect on this reaction. The photoconversion itself is dependent on radiant energy. This effect can be correlated with the ATP requirement for the synthesis of PChl for which an ATP induced positioning at a specific membrane site has been suggested [14]. Since ATP can act on envelope-free etioplasts (table 1) and since PChl is located in the prolamellar body [15, 16], the effect of ATP on photoconvertibility appears to be localised at sites within the prolamellar body. The association between PChl and a specific protein unit, the holochrome, is required for both the photoconversion of PChl and its characteristic absorption spectrum [17, 18]. The change in the degree of photoconvertibility and also the absorption maximum on addition of ATP suggests that ATP is acting on the pigment–holochrome association. In the absence of ATP we would suggest that the complex becomes altered so that the PChl becomes non-convertible; addition of ATP stabilises the complex so maintaining a high proportion of convertible $PChl_{650}$. The conversion of $PChl_{630}$ into Chl by light + dark treatments indicates that ATP can indeed activate non-convertible PChl (absorbance changes at 650 nm representing the transistory formation of convertible PChl would be expected to be very small and were not detected). In leaves fed with δ-aminolaevulinic acid a conversion of $PChl_{630-5}$ into $PChl_{650}$ has previously been demonstrated [11, 19–21] and there is some evidence for such a conversion in leaves not fed with δ-aminolaevulinic acid [21, 4]. The ability of ATP to rapidly determine the convertibility of PChl by affecting its binding to the holochrome and thereby altering the equilibrium between convertible and non-convertible PChl could be a point of control in Chl formation. The similarity of the optimal effective ATP concentration (approx. 1.0 mM) and the internal ATP concentration in freshly isolated etioplasts (1.2 mM, assuming an etioplast volume of 3×10^{-11} ml) adds credence to this suggestion.

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