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Effect of 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU) on Benzyladenine-induced Stimulation of Chlorophyll Formation in Etiolated Cucumber Cotyledons

By

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Abstract. 3-(3, 4-Dichlorophenyl)-1, 1-dimethylurea (DCMU) depresses the rate of chlorophyll (Chl) formation during the steady-state phase under continuous illumination in excised etiolated cotyledons of cucumber (*Cucumis sativus* L. cv. Aonagajibai) preincubated in darkness with either benzyladenine (BA) or water. The inhibition appears at a light fluence rate higher than 14 μmol m^{-2} s^{-1} and the extent of the inhibition increases with increasing fluence rate. Under 295 μmol m^{-2} s^{-1}, the extent of the inhibition is greater in cotyledons pretreated with BA for either a short (6 h) or a long (14 h) period compared to that in BA-untreated control; the extent of the stimulation by dark BA-pretreatment of the steady-state rate is greater under 295 μmol m^{-2} s^{-1} than under a lower fluence rate (43 μmol m^{-2} s^{-1}). Photosynthesis may contribute to the increase in the steady-state rate to a greater extent in BA-pretreated cotyledons than that in water controls under a high light fluence. DCMU scarcely suppresses 5-aminolevulinic acid accumulation in the presence of levulinic acid in both BA-pretreated and untreated cotyledons.

Introduction

Cytokinin stimulates Chl formation in etiolated plant tissues (Sugiura 1963, Banerji and Laloraya 1967, Fletcher and McCullagh 1971a, b, Buschmann and Lichtenthaler 1982). In etiolated cucumber cotyledons, BA eliminates the lag phase of Chl formation during a short (6 h) dark-pretreatment (the fast-appearing effect) and after a longer dark-pretreatment period (14 h) stimulates the steady-state rate of Chl formation (the late-appearing effect; Dei 1982, 1983).

However the effect of BA is affected by the light intensity (Dei 1984). Although the typical two-fold effect appears when the fluence rate is 20–100 μmol m^{-2} s^{-1} (1,500–8,500 lux) the BA-induced late-appearing effect is almost absent under a lower fluence rate ($<$1.4 μmol m^{-2} s^{-1}; 110 lux). Our previous investigations (Dei 1984) have suggested that the Chl-formation process which takes place upon transfer to continuous light consists of two components. One component, whose rate is saturated at 1.4 μmol m^{-2} s^{-1}, begins to operate rapidly after the onset of illumination (the fast-appearing component) and proceeds linearly with time for several h. The other component, which requires a higher light intensity to maximize its rate, begins to operate at the end of the lag phase (the late-appearing component). BA-induced

fast and late-appearing effects correspond to the stimulation of the rates of the fast and late-appearing components, respectively (Dei 1984). The rate-limiting steps of the Chl biosynthesis pathway affected by BA and light intensity have been investigated (Dei 1985).

Our previous data (Dei 1984) showed that under 295 μmol m^{-2} s^{-1} (21,000 lux), the extent of the stimulatory effect of BA on Chl accumulation between a 1.5 and 6 h light period is greater than that under 43 μmol m^{-2} s^{-1} (3,300 lux). This may be related to the fact that DCMU inhibits Chl and ALA synthesis only at a light intensity higher than 1,000 lux (Richard and Nigon 1973, Nigon et al. 1978). A related compound CMU also inhibits Chl formation in bean and mung bean leaves (Klein and Neuman 1966, Dodge et al. 1971). The extent of the inhibition by DCMU or CMU has been shown to be greater at the later stages of greening. Moreover, in greening radish seedlings PS–II herbicides such as bentazon or DCMU suppress the formation of 'sun-type' chloroplasts which are formed under a light intensity higher than 10,000 lux and differ in morphological and compositional characteristics and photosynthetic functions from the 'shade-type' chloroplasts formed under a lower light intensity (Lichtenthaler and Buschmann 1978, Lichtenthaler et al. 1981, Meier and Lichtenthaler 1981, Grumbach 1982).

In view of these results, we have supposed that photosynthesis may be involved in the greater effect of BA on Chl formation under 295 μmol m^{-2} s^{-1} than that under a lower light intensity. We used the PS–II herbicide DCMU to estimate the participation of photosynthesis in Chl formation in BA-pretreated and untreated etiolated cucumber cotyledons under various fluence rates. Our results suggest photosynthesis contribute to the increase in the steady-state rate of Chl formation to a greater extent in BA-pretreated cotyledons than in BA-untreated cotyledons. The characteristics of the action of DCMU and BA are discussed in relation to the results of our previous reports (Dei 1984, 1985).

Abbreviations: ALA, 5-aminolevulinic acid; BA, N^6-benzyladenine; Chl, chlorophyll; CMU, 3-(4-chlorophenyl)-l, 1-dimethylurea; DCMU, 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea; FW, fresh weight; LA, levulinic acid; PS–II, photosystem II.

Materials and Methods

Seeds of cucumber (*Cucumis sativus* L. cv. Aonagajibai) sown on wet vermiculite were germinated in darkness at 28°C. After about 2.25 days, the cotyledons were harvested and samples of 20 cotyledons were incubated with 1.4-ml water in 5-cm Petri dishes in the dark for 42–44 h prior to the onset of continuous illumination (Dei 1984). Pretreatments were begun by transferring the cotyledons to a Petri dish containing 1.4–1.6 ml of solutions of various chemicals. DCMU pretreatment was begun 14 h before the onset of illumination; BA (1 μM) pretreatment 6 or 14 h before the onset of illumination (Dei 1984); LA (100 mM unless otherwise stated, pH adjusted to 6.0 with NaOH) pretreatment 3 h before the onset of illumination (Dei 1985). Water-control cotyledons were also transferred to a Petri dish containing water 14 h (for Chl accumulation) or 14 and 3 h (for ALA accumulation) before the onset of illumination. All manipulations were carried out under dim green light (Dei 1985).

After the dark preincubation, continuous illumination was begun under various intensities

of warm white fluorescent light (FLR 80W/H, Mitsubishi Electric Co., Tokyo) at 28°C. The light quanta fluence rates were 7.0, 14.0, 43.0, 100, 295 μmol m^{-2} s^{-1} at 400–700 nm; illuminances were 530, 1,100, 3,300, 8,500, 21,000 lux, respectively. After illumination, each cotyledon sample was blotted, weighed, and stored at −20°C until used.

Chl extractions and determinations were carried out as described previously (Dei 1978) according to the method of Arnon (1949). In one experiment (Table 2), Chl was extracted and determined by the hydroxylamine method (Ogawa and Shibata 1965, Dei 1984). ALA extractions and determinations were performed as described previously (Dei 1985).

The rate of photosynthetic O_2 evolution in greening cotyledons was measured in a photosynthetic Warburg manometer. The greening cotyledons were incubated in the vessels (9–10 cotyledons in each vessel) with various test solutions using carbonate-bicarbonate buffer in the center well for maintaining CO_2 at 0.32%. The rates of O_2 consumption in the dark for 30 min and then in the light (360 μmol m^{-2} s^{-1} at 400–700 nm, provided by incandescent light) for the same period were measured. The difference in O_2 consumption rates between those in the dark and in the light, which were calculated from the changes in the gas volume during the latter 20 min of each measuring period was regarded as the rate of the photosynthetic O_2 evolution. After the gas-exchange measurement, the FW and the Chl content of the cotyledons were measured.

All determinations were made with 3 or 4 replicate samples and the mean value with the SE is shown. Chl or ALA contents of the cotyledons were expressed on a FW or on a cotyledon basis according to the criteria described previously (Dei 1984). DCMU treatment gave no influence on FW increase during a 6-h light period (data not shown).

Results

DCMU at concentrations higher than 1 μM suppressed Chl accumulation during 6-h illumination at 100 μmol m^{-2} s^{-1}, but did not affect Chl accumulation during 1.5-h illumination (Fig. 1). The photosynthetic O_2 evolution was also inhibited above 1 μM in both BA-pretreated and untreated cotyledons (Table 1). In the following experiments, the concentration of DCMU used was 5 μM. Under 100 μmol m^{-2} s^{-1}, DCMU depressed the rate of Chl formation only when the light period exceeded 2 h in water-control, 6-h or 14-h BA-pretreated cotyledons (data not shown).

The inhibitory effect of DCMU on Chl accumulation during 6-h illumination did not appear until the fluence rate exceeded 14 μmol m^{-2} s^{-1} (1,100 lux), and the extent of the inhibition increased with increasing fluence rate (Fig. 2). This was true in both 14-h BA-pretreated and untreated cotyledons except that the effect of DCMU levelled off at 100 μmol m^{-2} s^{-1} in BA-untreated cotyledons. In the presence of DCMU, the amounts of Chl accumulated under 295 μmol m^{-2} s^{-1} were reduced compared to those under 14 μmol m^{-2} s^{-1} to a similar degree in both BA-pretreated and untreated cotyledons. Under 100 μmol m^{-2} s^{-1} DCMU somewhat raised Chl a/b ratio after 6-h light period in cotyledons pretreated with BA for 14 h (Table 2).

Under 295 μmol m^{-2} s^{-1}, DCMU showed a greater effect on the steady-state rate of Chl formation in cotyledons pretreated with BA for both 6 and 14 h compared to that in BA-

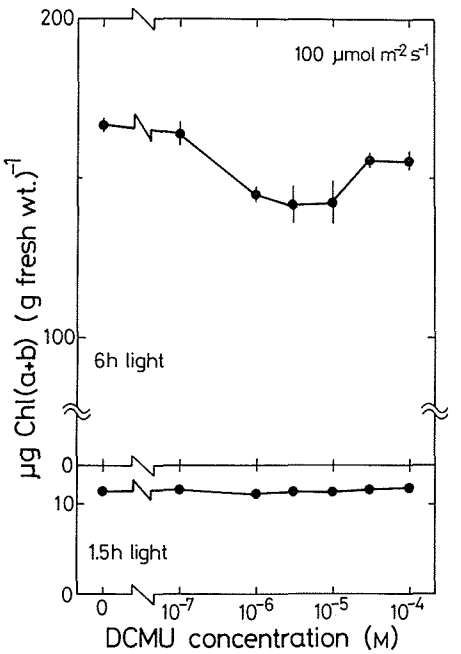

Fig. 1. Effect of concentration of DCMU on Chl a+b accumulation during 1.5 and 6 h of continuous illumination (100 μmol m^{-2} s^{-1}) in BA-untreated cotyledons. Etiolated cotyledons were harvested and preincubated as described in Materials and Methods. Vertical bar on the data point indicates SEM unless obscured by the width of the symbol.

Table 1. Effect of DCMU concentration on photosynthetic O_2 evolution in greening cucumber cotyledons

Pretreatment	Chl(a+b) content	Rate of photosynthetic O_2 evolution	
	mg Chl (g FW)$^{-1}$	ml O_2 (g FW)$^{-1}$ h^{-1}	ml O_2 (mg Chl a+b)$^{-1}$ h^{-1}
Expt. 1			
Water	0.23±0.01	0.44±0.04 (100)	1.97±0.13 (100)
0.1 μM DCMU	0.23±0.01	0.44±0.02 (99)	1.93±0.14 (98)
1 μM DCMU	0.20±0.01	0.21±0.04 (48)	1.05±0.16 (53)
10 μM DCMU	0.20±0.01	0.17±0.04 (37)	0.85±0.23 (43)
Expt. 2			
BA(14 h)	0.54±0.02	0.78±0.03 (100)	1.45±0.09 (100)
BA(14 h)+0.1 μM DCMU	0.53±0.01	0.79±0.05 (102)	1.51±0.11 (104)
BA(14 h)+1 μM DCMU	0.47±0.02	0.45±0.04 (58)	0.98±0.09 (68)
BA(14 h)+10 μM DCMU	0.44±0.02	0.23±0.04 (30)	0.53±0.06 (37)

Etiolated cotyledons were pretreated in the dark with various concentrations of DCMU in the presence or absence of 1 μM BA for 14 h. After 6-h illumination with 100 μmol m^{-2} s^{-1} light the rate of O_2 consumption of the cotyledons in the dark and then in the light were measured as described in Materials and Methods. The difference in the rates of O_2 consumption between in the dark and in the light was regarded as the rate of photosynthetic O_2 evolution. The rates of O_2 consumption in the dark were not affected by any of the DCMU concentrations used and their mean values in Expt. 1 and 2 were 1.01 and 1.09 ml O_2 (g FW)$^{-1}$ h^{-1}, respectively. Each mean value±SE is shown. Values in parentheses indicate percentage of DCMU-untreated control.

untreated cotyledons (Table 3). Accordingly, the two-fold effect of BA previously shown under 40–100 μmol m^{-2} s^{-1} (Dei 1982, 1983, 1984) may be modified under 295 μmol m^{-2} s^{-1}

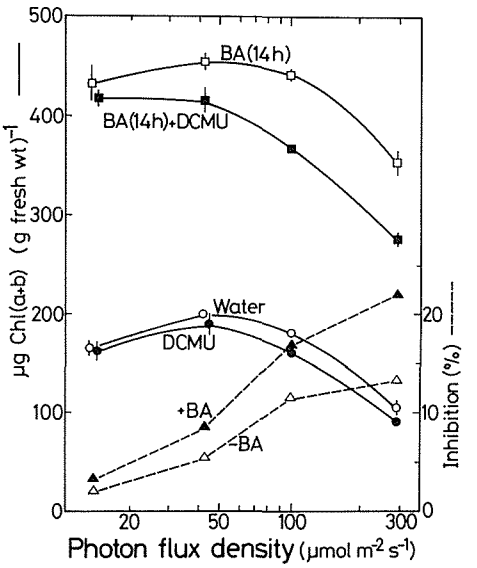

Fig. 2. Effect of DCMU on Chl a+b accumulation during 6 h of various photon flux densities of continuous white light in etiolated cucumber cotyledons. Cotyledons were pretreated with 1 μM BA for 14 h in darkness or incubated with water throughout the dark preincubation period. Percentage values of inhibition by DCMU are also shown (dotted line). Other notes as for Fig. 1.

Table 2. Effect of DCMU on Chl a/b ratio after 6-h illumination with 100 μmol m^{-2} s^{-1} light

Pretreatment	Water control	BA(6 h)	BA(14 h)
−DCMU	3.4±0.1	3.3±0.3	3.4±0.3
+DCMU	3.4±0.4	3.6±0.2	4.2±0.1

Cotyledons were pretreated as described in Materials and Methods. Chl a and b contents were determined by the hydroxylamine method of Ogawa and Shibata (1965). Each mean value± SE is shown.

Table 3. Effect of length of BA pretreatment on DCMU-inhibition of Chl formation under white light of 295 μmol m^{-2} s^{-1} in etiolated cucumber cotyledons

Pretreatment	Chl(a+b) content [μg Chl(a+b) cotyledon^{-1}]		Mean steady-state rate [μg Chl(a+b) cotyledon^{-1} h^{-1}] (% of control)
	2-h light	6-h light	
Water	0.28±0.01	3.35±0.14	0.77 (100)
DCMU	0.28±0.02	2.91±0.24	0.66 (86)
BA(6h)	1.23±0.04	8.61±0.17	1.85 (100)
BA(6 h)+DCMU	1.10±0.02	6.61±0.36	1.38 (75)
BA(14 h)	1.72±0.08	11.70±0.37	2.50 (100)
BA(14 h)+DCMU	1.74±0.13	8.46±0.28	1.68 (67)

Etiolated cotyledons were pretreated as described in Materials and Methods. Mean steady-state rate was calculated from the increase in Chl content between 2 and 6 h of illumination. Each mean value±SE is shown.

due to the greater participation of photosynthesis in raising the steady-state rate of Chl formation in BA-pretreated cotyledons compared to that in BA-untreated cotyledons.

This possibility was supported by the experiments in Table 4. Under 43 μmol m^{-2} s^{-1},

Table 4. BA-induced acceleration of the steady-state rate of Chl formation under white light of diffecrent photon flux densities in etiolated cucumber cotyledons

Treatment	Chl(a+b) content [μg Chl(a+b) cotyledon^{-1}]		Mean rate of Chl accumulation [μg Chl(a+b) cotyledon^{-1} h^{-1}]	
	2-h light	6-h light	during the lag phase	during the steady-state phase (% of control)
43 μmol m^{-2} s^{-1}				
Water	0.54±0.04	4.85±0.17	0.27	1.08 (100)
BA(6 h)	1.72±0.03	8.61±0.11	0.86	1.72 (160)
BA(14 h)	2.97±0.17	13.87±0.53	1.49	2.72 (253)
295 μmol m^{-2} s^{-1}				
Water	0.33±0.01	3.99±0.13	0.16	0.92 (100)
BA(6 h)	1.28±0.01	8.65±0.27	0.64	1.84 (201)
BA(14 h)	2.23±0.13	13.04±0.36	1.12	2.70 (293)

Etiolated cotyledons were pretreated as described in Materials and Methods. The rate of Chl accumulation during the lag phase was calculated from the increase in Chl content between 0 and 2 h of illumination. The Chl content at 0 h of illumination was regarded as zero. Other notes as for Table 3.

where the DCMU-inhibition is very small (Fig. 2), the rise in the steady-state rate of Chl formation caused by BA-pretreatment for 6 h almost equals the rise in the rate during the lag phase. The actual rates of Chl formation during the lag phase may be somewhat smaller than the values in Table 4, since small amounts of Chl formed rapidly after the onset of illumination are neglected in Table 4. But this approximation does not seriously influence the above interpretation. These results coincide with the hypothesis that the steady-state rate is the sum of the rates of the fast and the late-appearing components of Chl formation and that the rise in the steady-state rate by 6-h BA-pretreatment is caused by increasing the rate of the fast-appearing component of Chl formation, the latter rate which can be estimated from the rate of Chl formation during the lag phase (Dei 1984). However under 295 μmol m^{-2} s^{-1}, the rise in the steady-state rate caused by 6-h BA-pretreatment was much higher than the rise in the rate of Chl formation during the lag phase caused by the same pretreatment (Table 4). The stimulation of the steady-state rate by BA-pretreatment for 14 h was also more prominent than that under 43 μmol m^{-2} s^{-1}.

Under 7.0 μmol m^{-2} s^{-1}, DCMU did not inhibit Chl formation during 18-h illumination in both BA-pretreated and untreated cotyledons (data not shown). Thus it is unlikely that under a low fluence rate, DCMU-sensitivity appears at a later stage of greening than it does under a higher fluence rate.

Next, effect of DCMU on ALA accumulation in the presence of LA, a competitive inhibitor of ALA dehydratase (EC 4.2.1.24), was examined to estimate whether DCMU suppresses Chl formation through its effect on ALA production step or some other step(s) between ALA and Chl. Table 5 shows that at 295 μmol m^{-2} s^{-1}, DCMU did not inhibit ALA accumulation during 1.5-h illumination. On ALA accumulation during 6-h illumination, DCMU had no significant effect in BA-untreated cotyledons in agreement with the results of Beale and Castelfranco (1974) and inhibited slightly in 14-h BA-pretreated cotyledons.

Table 5. Effect of DCMU on ALA accumulation in LA-treated and Chl accumulation in LA-untreated etiolated cotyledons under white light of 295 μmol m^{-2} s^{-1}.

Pretreatment	ALA content [nmol ALA (cotyledon)$^{-1}$]			Chl(a+b) content [nmol ALA eq (cotyledon)$^{-1}$]
	0-h light	1.5-h light	6-h light	6-h light
Water	0.3±0.1	1.7±0.1	11.8±0.5 (100)	26.0±0.8 (100)
DCMU	0.3±0.1	1.7±0.2	11.3±0.7 (96)	20.6±0.9 (81)
BA(14 h)	0.7±0.1	10.0±0.7	45.8±2.5 (100)	106.8±4.4 (100)
BA(14 h)+DCMU	0.8±0.1	9.9±0.7	41.0±1.5 (89)	78.6±3.3 (74)

Etiolated cotyledons were pretreated as described in Materials and Methods. All Chl contents are expressed as ALA equivalent, taking into account that 8 molecules of ALA are required to form 1 molecule of Chl. "0-h light" indicates the cotyledons sampled at the end of the dark-incubation period. Values in parentheses indicate percent of DCMU-untreated control. Other notes as for Table 3.

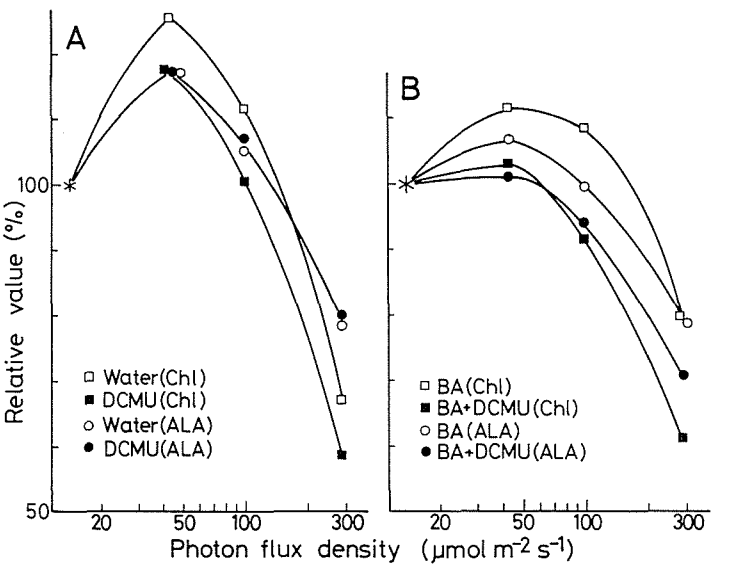

Fig. 3. Influence of DCMU on Chl a+b accumulation in LA-untreated cotyledons and ALA accumulation in LA-treated cotyledons during 6-h illumination under various photon flux densities. LA-untreated cotyledons were treated identically except that LA-containing solutions were replaced by solutions devoid of LA. ALA and Chl contents were calculated on a FW basis and each value is expressed as a percentage value against the value of the corresponding pretreatment illuminated under 14 μmol m^{-2} s^{-1}. (A) BA-untreated cotyledons; LA concentration was 100 mM. (B) 14-h BA-pretreated cotyledons; LA concentration was 120 mM. Other notes as for Fig. 1.

The effects of DCMU on Chl accumulation in LA-untreated cotyledons and ALA accumulation in LA-treated cotyledons were compared under various light fluences (Fig. 3). Similarly to our previous studies (Dei 1985), all ALA and Chl values were expressed as the relative values against the values of the corresponding pretreatment under 14 μmol m^{-2} s^{-1} where no significant effect of DCMU on Chl and ALA formation appeared.

Figure 3A shows the results using BA-untreated cotyledons. In DCMU-treated cotyledons, the relative values of Chl increased parallel to those of ALA from 14 to 43 μmol·m^{-2} s^{-1}, then decreased faster than that of ALA. The decrease in the amounts of ALA and Chl under high light fluences (>100 μmol m^{-2} s^{-1}) is probably due to the photooxidative destruction of Chl and impairment of the synthesis of ALA and Chl (Augustinussen and Madsen 1965). The destruction of accumulated Chl may result in a greater decrease in the amount of Chl than that of ALA. On the other hand, in DCMU-untreated cotyledons, the relative value of Chl was higher than that of ALA in DCMU-untreated cotyledons and also higher than that of Chl in DCMU-treated cotyledons at 43 μmol m^{-2} s^{-1}. The difference in the values of Chl between DCMU-treated and untreated cotyledons was maintained when the fluence rate increased further, whereas the values of ALA in both cotyledons were quite similar. Results using 14-h BA-pretreated cotyledons (Fig. 3B) were in principle similar to those using BA-untreated cotyledons except that the difference in ALA values between 14 and 43 μmol·m^{-2} s^{-1} was small regardless of the presence or absence of DCMU as described previously (Dei 1985).

Discussion

In both BA-pretreated and untreated cotyledons, DCMU depressed the steady-state rate of Chl formation only. It is consistent with our previous finding that the rate of Chl formation during the lag phase is saturated at a very low light intensity (Dei 1984) and with the results of some earlier reports (Klein and Neuman 1966, Richard and Nigon 1973, Gough 1978) which show that the inhibitory effect of DCMU or CMU on Chl and/or ALA formation increases at the later stages of greening. Our previous investigations (Dei 1984) suggested that the Chl-formation process upon transfer to continuous light consists of two components and BA stimulates the rates of the respective components after different periods of dark pretreatment. DCMU appears to inhibit a portion of the late-appearing component at a fluence rate higher than 14 μmol m^{-2} s^{-1} (Fig. 2), which can be referred to as the DCMU-sensitive Chl formation. Accordingly, the late-appearing component can be divided into DCMU-sensitive and resistant Chl formations if the activity of the latter one, which seems to be saturated at about 14 μmol·m^{-2} s^{-1} (Fig. 2), remains unchanged in the absence of DCMU (Nigon et al. 1978).

BA seems to stimulate the DCMU-sensitive Chl formation under a high light fluence (295 μmol m^{-2} s^{-1}) by a short (6 h) dark-pretreatment (Tables 3 and 4). Therefore, the effect of BA on Chl formation found under 40–100 μmol m^{-2} s^{-1} described previously (Dei 1982, 1983, 1984) appears to be modified under a higher fluence rate. Under 295 μmol m^{-2} s^{-1}, the rise in the steady-state rate of Chl formation caused by both 6 and 14-h BA-pretreatments is strengthened to a degree corresponding to the stimulation of the DCMU-sensitive Chl formation by BA (Tables 3 and 4).

Our results do not agree with the conclusion of Lichtenthaler and Buschmann (1978) and Buschmann and Lichtenthaler (1982). They showed using radish seedlings that the effect of BA or kinetin on a number of parameters relating to chloroplast and seedling development are less obvious under a high fluence rate (20,000 lux) compared to under a low fluence rate (2,000 lux), and concluded that cytokinin causes the modifications of these parameters in the

same way that high intensity illumination does. This discrepancy may be due to the differences in the experimental conditions. Our observations were confined to the effect of various treatments on Chl formation in excised cotyledons during a short period of illumination.

DCMU probably depressed Chl formation through its inhibition of photosynthetic electron transport (Table 1) although Fig. 1 suggests DCMU had some side effects which may appear above 10 μM. Fuesler et al. (1984) using isolated developing chloroplasts of cucumber showed the activities of some reactions of Chl biosynthesis pathway are inhibited by DCMU and this inhibition is reversed by adding ATP and NADPH. In all three types of cotyledons (after 6-h illumination), no indications were found of DCMU inducing the development of 'shade-type' chloroplasts (Table 2).

In various plant materials, DCMU inhibits Chl formation through inhibition of the provision of organic substrates for Chl (Klein and Neuman 1966, Dodge et al. 1971), inhibition of ALA synthesis in vivo (Richard and Nigon 1973, Gough 1978) and in isolated etio/chloroplasts (Kannangara and Gough 1977, Harel and Ne'eman 1983, Fuesler et al. 1984). Table 5 and Fig. 3 show that DCMU scarcely inhibited ALA accumulation in the presence of LA in both BA-pretreated and untreated cucumber cotyledons. Our previous studies suggested that ALA synthesis can be one of the rate-limiting steps of the late-appearing component of Chl formation in BA-untreated cotyledons between 1.4–43 μmol m^{-2} s^{-1} (Dei 1985). This apparent discrepancy can be solved by the results in Fig. 3, which suggest that the efficiency of the formation of Chl from ALA is improved at a light intensity higher than 14 μmol m^{-2} s^{-1} in DCMU-untreated cotyledons, though we did not detected this in our previous studies (Dei 1985). DCMU prevents this improvement of the efficiency. Increased production of NADPH and/or ATP by photosynthesis under high light fluences may stimulate some reaction(s) between ALA and Chl thereby diminishing the oxidative breakdown of some intermediates of Chl biosynthesis pathway (Gassman et al. 1978), which may lead to a high turnover rate of Chl (Grumbach and Lichtenthaler 1982). Modification of the composition of thylakoid membrane proteins by high light fluences and by PS II-herbicides has also been shown (Buschmann and Grumbach 1982).

BA-pretreatment may cause a greater degree of the improvement of the efficiency than water-pretreatment does under 295 μmol m^{-2} s^{-1} (Fig. 2 and Tables 3 and 4). Otherwise, a slight but significant inhibition of ALA accumulation by DCMU under 295 μmol m^{-2} s^{-1} in BA-pretreated cotyledons (Table 5, Fig. 3B) may be the cause of the greater inhibition of Chl formation by DCMU in BA-pretreated cotyledons than that in BA-untreated cotyledons.

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