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Changes in the pools of carotenoids and protochlorophyll(ide) in etiolated cucumber (*Cucumis sativus*) cotyledons treated with norflurazon and KC 6361

S. JUNG*,***, J.S. KIM*, K.Y. CHO*, and B.G. KANG**

*Screening Research Division, Korea Research Institute of Chemical Technology, P. O. Box 107, Yusung, Taejon, 305-600, Korea**
*Department of Biology, Yonsei University, Seoul, 120-749, Korea***

Abstract

Changes in the pools of carotenoids and protochlorophyll(ide) were investigated in etiolated cucumber cotyledons treated with norflurazon (NF) and an experimental herbicide KC 6361 (KC). Both the NF- and the KC-treated tissues considerably accumulated the colourless carotenes phytoene and phytofluene with a concomitant depletion of the coloured carotenoids lutein and β-carotene in darkness. However, the profiles of changes in chlorophylls (Chls) and carotenoids were different for the two herbicides. The plants were also influenced by the photosynthetic photon flux densities (PPFD's), with a more pronounced decline of Chl under high PPFD than under low PPFD. The ratios of protochlorophyll (PChl)/protochlorophyllide (PChlide) were greatly altered due to a decrease and an increase of PChl in the NF- and the KC-treated etiolated tissues, respectively, whereas the PChlide content was not significantly influenced by the inhibitors. Large increase of PChls in the KC-treated tissues seems to derive from the binding of accumulated geranylgeraniol (GG) equivalents, through carotenogenic inhibition, to PChlide. Therefore, the alterations of PChl and PChlide occurring under disturbed carotenogenesis may suggest an interaction between the biosynthetic pathways of Chls and carotenoids. In addition, the great proportion of PChl GG and PChl dihydro-GG in the KC-treated tissues implies that PChl formation is regulated at the level of hydrogenation.

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***Author for correspondence; fax: 82-42-861-4913, e-mail: syjung@pado.krict.re.kr
Abbreviations: ALA - 5-aminolevulinic acid; Chlide - chlorophyllide; DHGG - dihydrogeranylgeraniol; GG - geranylgeraniol; KC - KC 6361 [3-(*N,N'*-diethylcarbamoyl)-phenyl-4-nitrophenylether]; NF - norflurazon [4-chloro-5-(methylamino)-2-(3-trifluoromethyl)phenyl-3(2*H*)-pyridazinone]; PChl - protochlorophyll; PChlide - protochlorophyllide; PChl(ide) - mixture of protochlorophyll and protochlorophyllide; PPFD - photosynthetic photon flux density; THGG - tetrahydrogeranylgeraniol.
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Additional key words: 5-aminolevulinic acid; β-carotene; carotenogenesis; irradiance; photosynthetic photon flux density; phytol; protochlorophyll; xanthophylls.

Introduction

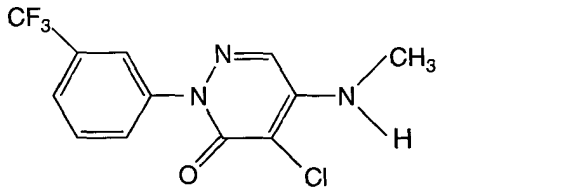
In higher plants Chls are formed from a multistep biosynthetic pathway with two major points of control. One is at the 5-aminolevulinic acid (ALA) synthesis, the first committed step of Chl biosynthesis. The other is at the reduction of PChlide to chlorophyllide (Chlide) (Beale and Weinstein 1990). Etioplasts from dark-grown leaves are well characterized concerning their ultrastructure, PChl and PChlide contents, and the occurrence of PChlide oxidoreductase, which is the key enzyme in the phototransformation of PChlide to Chlide (Mc Ewen *et al.* 1994).

Etiolated tissues of higher plants accumulate the Chl-precursor pigment PChlide and its esterified form, PChl. Considerable experimental evidence shows that these pigments are not chemically homogeneous. PChlide consists of monovinyl (MV)- and divinyl (DV)-derivatives, and PChl comprises at least four species esterified with different alcohols (Belanger and Rebeiz 1980, Shioi and Sasa 1983a). The phototransformable unesterified PChlide is mainly localized in prolamellar bodies, whereas the relative content of the esterified pigment, PChl, is higher in prothylakoids (Böddi *et al.* 1989). After light-induced reduction of PChlide to Chlide, in etiolated tissues, Chlide *a* is esterified and subsequently Chl *a* geranylgeraniol (GG), its dihydro (DH)- and tetrahydro (TH)-derivatives, and Chl *a* phytol are formed (Rüdiger and Schoch 1988). A similar pathway has been reported in the formation of PChl phytol (Shioi and Sasa 1983a). PChls are esterified with four different alcohols in analogy to the alcohols found in the esterification sequence of Chl *a* biosynthesis.

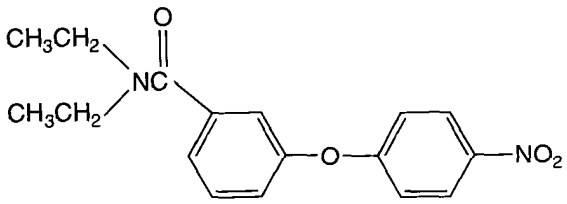
Carotenoid biosynthesis is essential for the formation of thylakoids, and its regulation is closely related to that of Chls. Carotenoids not only function as accessory pigments in photosynthesis but also protect plants from photooxidative damage by scavenging active oxygen species. When carotenogenesis is inhibited, endogenous concentration of protective carotenoids becomes low and thus Chls are not protected from photodestruction (Bramley 1993). Phytoene desaturase, which catalyzes a rate-limiting step in carotenoid biosynthesis (Chamovitz *et al.* 1993), is a target site of various bleaching herbicides such as NF and fluridone (Bramley 1993).

With its carotenogenic inhibition, NF causes either slight decline of PChl(ide) contents in etiolated bean seedlings (Pardo and Schiff 1980) or no effect in etiolated barley seedlings (Bolychevtseva *et al.* 1995). Other carotenoid biosynthesis inhibitors such as amitrole and clomazone influence the PChl(ide) concentration and the phytylation of Chlide, respectively (Duke and Kenyon 1986, Rascio *et al.* 1996). However, exact action modes of the chemicals on PChl formation have not been elucidated in detail. The early stages of the carotenoid biosynthetic pathway are common to all isoprenoids. The C_{20} geranylgeranyl pyrophosphate (GGPP), which is accumulated after NF treatment (Bramley 1993), gives rise to the colourless carotene phytoene and to the diterpenes including phytol, which provides the isoprenoid side chain of the Chls (Britton 1993).

During the course of herbicide screening, we have observed that an experimental herbicide KC 6361 (KC) (Fig. 1) causes whitening of the treated tissues (Cho *et al.* 1998), similarly to the typical symptom of carotenogenic inhibitors. To examine if the whitening effect of KC is due to the inhibition of carotenoid biosynthesis, the concentrations of carotenoid biosynthetic intermediates were measured in the KC-treated tissues under dark and light and compared to those in the NF-treated tissues. Changes in PChl and PChlide concentrations caused by the inhibitors were separately determined and the composition of PChls esterified with different alcohols was also examined in order to investigate how carotenogenesis relates to Chl biosynthesis.



NF



KC

Fig. 1. Chemical structures of the carotenoid biosynthesis inhibitors used in this study.

Materials and methods

Plants: Cucumber (*Cucumis sativus* L.) seeds were germinated directly in moist vermiculite in darkness at 25 °C for 6 d. The experiments were triplicated each with three determinations.

Chemicals: Technical grade NF (80.3 % purity) was generously provided by *Novartis Korea Inc.* (Seoul, Korea). KC (>95 % purity) was synthesized in Korea Research Institute of Chemical Technology. ALA and phytol were purchased from *Sigma Chemical Co.* (St. Louis, MO, USA). Other chemicals used were of the highest quality commercially available.

Formation of Chl and carotenoids under dark and irradiation: For dark experiments, the etiolated cucumber cotyledons were incubated with various concentrations of either NF or KC at 30 °C in darkness for 24 h. The reason for using the etiolated

tissues was to ascertain that the inhibitors affect the formation of carotenoids rather than the photooxidation of pigments. For irradiation experiments, the etiolated cotyledons in 10 mM potassium phosphate buffer (pH 6.5) containing either 0.05 mM NF or 0.5 mM KC were exposed to "white light" of 15 or 150 μmol m^{-2} s^{-1} (PPFD) at 30 °C for 8 h following 24 h dark incubation. The irradiation period gave a better distinction of pigment accumulation between the NF and KC treatments without causing severe photoinhibitory damage of the tissues.

Formation of PChl and PChlide in darkness: The etiolated cotyledons were incubated in 10 mM potassium phosphate buffer (pH 6.5) containing 0.05 mM NF, 0.5 mM KC, 1 mM ALA, or 1 mM phytol at 30 °C in complete darkness for 24 h.

Determination of Chl and carotenoids: After the incubations of the etiolated cotyledons either with NF or with KC for 24 h, the treated tissues were freeze-dried overnight. Chl concentration was determined spectrophotometrically according to the method of Arnon (1949). For extracting carotenoids, 20 cm^3 of methanol containing 6 % KOH was added to about 0.1 g dry ground cotyledon tissues and heated at 60 °C for 20 min. The methanol extract was poured into a separatory funnel containing 10 cm^3 of 10 % diethyl ether in petrol (b.p. 30 to 60 °C), and then 3 cm^3 of a saturated NaCl solution was added. The upper layer was collected and the lower one reextracted with another 10 cm^3 of diethyl ether in petrol. All procedures were conducted under dim laboratory light. Total carotenoid content in the combined upper layers was determined spectrophotometrically at 445 and 550 nm according to Sandmann (1993a). After the total carotenoid determination, the solution was evaporated to dryness at room temperature and resuspended in acetone. Carotenoids were separated on a reversed phase column 25-cm *Spherisorb ODS-1* 5-μm (*Alltech*, Deerfield, IL, USA) eluted with an isocratic run of acetonitrile : methanol : 2-propanol (85 : 10 : 5, v/v/v) at a flow rate of 16.7 mm^3 s^{-1} created by two *Waters 510* pumps (*Millipore*, Milford, MA, USA) (Sandmann 1993a). Two HPLC runs were carried out, one at 445 nm for xanthophylls and β-carotene, and at 285 nm for phytoene, and the other at 347 nm for phytofluene.

Determination of PChl and PChlide: All extractions for HPLC were made under a dim, green safe radiation source. Thirty pairs (approximately 1.5 g FM) of etiolated cucumber cotyledons were homogenized for 45 s in 7 cm^3 of HPLC-grade acetone : 0.1 M NH_4OH (9 : 1, v/v) using a *Brinkmann Polytron* (*Brinkmann*, Littau, Switzerland). The homogenate was centrifuged at 39 000×*g* for 10 min and the resulting supernatant was saved. The pigments in the acetone extract were partitioned into hexane and hexane-extracted acetone residue. The pigments in the hexane-extracted acetone residue were transferred to ether with saturated NaCl and 0.37 M KH_2PO_4 (pH 7.0). The aliquots of the hexane and ether layers containing PChl and PChlide, respectively, were separately evaporated under a stream of nitrogen gas. The pigments were then dissolved in acetone. Samples were stored in light-tight vials at −70 °C until analysis by HPLC.

HPLC determinations were made with the same HPLC system and column, as described in the above section, equipped with a *Waters 474* Scanning Fluorescence Detector (*Millipore*). The solvent used was 100 % methanol in an isocratic run at a

flow rate of 16.7 mm^3 s^{-1}. Pigments were quantified by extracting PChl and PChlide from etiolated tissues, determining the concentration spectrophotometrically acording to Brouers and Michel-Wolwertz (1983), and injecting spectrophotometrically-assayed PChl and PChlide into the HPLC for the calibration of the fluorescence detector. Pigments were detected using the excitation and emission wavelength settings of 437 and 638 nm, respectively, for PChl; and 440 and 630 nm, respectively, for PChlide.

Results

Inhibitory effects of NF and KC on carotenoid biosynthesis in darkness: Both NF and KC decreased contents of coloured carotenoids of etiolated cucumber cotyledons in a concentration-dependent manner (Table 1). NF greatly decreased total carotenoid contents with the I_{50} value of 23.4 μM. KC was much less inhibitory in darkness (Table 1) in contrast to a marked inhibition of carotenogenesis in light (Table 2).

Table 1. Total carotenoids [mg kg^{-1}(DM)] in etiolated cucumber cotyledons treated either with NF or with KC [μM] as determined by spectrophotometric analysis. The 6-d-old cotyledons were incubated in potassium phosphate buffer (pH 6.5) either with NF or with KC, in complete darkness at 30 °C for 24 h. Means ± SE from 3 replicates.

NF	Carotenoids	KC	Carotenoids
0	25.2 ± 0.07	0	25.4 ± 1.05
5	16.7 ± 0.16	10	24.3 ± 1.64
12.5	13.6 ± 0.47	100	23.2 ± 2.58
25	12.0 ± 0.60	500	20.0 ± 1.34
50	11.4 ± 0.03	1000	19.6 ± 1.95

Contents of phytoene and phytofluene, the intermediates of the carotenoid biosynthetic pathway, increased sharply in both the NF- and the KC-treated tissues compared to the untreated control (Fig. 2*A,C*). Phytoene content increased rapidly at low NF concentrations, but the increase slowed down with further increasing NF concentrations. On the other hand, the KC-treated tissues showed a continual increase of phytoene content upon increasing concentrations, but the increase was much lower than that caused by NF. Phytofluene content increased continually with increasing KC concentrations, but the increase was transient, followed by a decrease at increasing NF concentrations. Both NF and KC decreased the concentrations of lutein and β-carotene in a concentration-dependent manner (Fig. 2*B,D*). However, the NF-treated tissues showed a more pronounced decrease in lutein and β-carotene, which was mainly responsible for the decrease in total carotenoids, compared to the KC-treated tissues.

Effects of irradiation on contents of Chls and carotenoids: The pigment alterations in the NF- and the KC-treated etiolated cucumber cotyledons were examined upon

exposure of the tissues to various PPFD's to compare their bleaching effect. Effects of NF and KC on the Chl and carotenoid contents were significantly changed by the irradiance of the treated tissues, with lesser amounts of Chl and carotenoids in the NF-treated tissues under all PPFD's (Fig. 3). Under low PPFD, Chl accumulated drastically in all treatments (Fig. 3*A*). High PPFD gave a sharp decline in Chl content compared to low PPFD in the NF- and KC-treated cotyledons. In the untreated controls, however, high PPFD did not cause photooxidative damage, since the Chl content was slightly higher under high PPFD than under low PPFD. Carotenoid content in the control tissues increased upon irradiation, especially more carotenoids accumulated in high PPFD than in low PPFD (Fig. 3*B*). Contrary to the control tissues, the carotenoid contents in the NF- and in the KC-treated tissues declined under irradiation, more under high PPFD.

Table 2. Effect of different PPFD's on the carotenoid composition [mmol kg^{-1}(DM)] in etiolated cucumber cotyledons treated either with NF or KC. The tissues were subjected to the same treatments as in Fig. 3. Treatment notations indicate the control (C), the dark (D), the low (LI) or high (HI) irradiance. ND, not detected. Means ± SE from 3 replicates.

	Phytoene	Phytofluene	Lutein	β-carotene	Antherax.	Violaxanth.	Neoxanth.
C-D	1.91±0.31	0.37±0.01	3.91±0.04	0.75±0.05	0.22±0.02	0.11±0.01	0.15±0.01
C-LI	1.73±0.25	0.51±0.03	5.31±0.17	1.64±0.15	0.39±0.01	0.25±0.02	0.32±0.03
C-HI	1.54±0.00	0.66±0.02	6.74±0.75	2.57±0.15	0.61±0.06	0.30±0.06	0.34±0.07
NF-D	39.78±1.06	0.83±0.04	2.05±0.23	0.19±0.01	0.18±0.01	0.01±0.01	ND
NF-LI	52.72±2.56	0.49±0.02	2.20±0.19	0.09±0.05	0.18±0.01	0.06±0.01	ND
NF-HI	32.94±1.75	0.39±0.02	1.47±0.17	ND	0.12±0.06	ND	ND
KC-D	13.38±0.94	1.72±0.10	3.18±0.22	0.61±0.08	0.18±0.03	0.11±0.02	0.13±0.01
KC-LI	28.52±0.79	1.29±0.01	3.21±0.00	0.18±0.01	0.27±0.01	0.12±0.00	0.13±0.00
KC-HI	18.91±0.46	0.38±0.01	2.70±0.39	0.07±0.05	0.12±0.03	0.04±0.01	0.11±0.01

In the untreated controls, contents of all the pigments except phytoene increased with higher PPFD (Table 2). The NF-treated tissues showed a greater decrease and increase in coloured carotenoids and in phytoene, respectively, compared to the KC-treated tissues (Table 2). Phytoene contents were higher in the NF- and KC-treated tissues in low PPFD than in the untreated control, but were lower in both treated tissues under high PPFD. Phytofluene in the treated tissues was accumulated maximally in darkness but declined with higher PPFD. The KC-treated tissues accumulated more phytofluene than the NF-treated ones under dark and low PPFD. The changes in contents of lutein and β-carotene appeared to be responsible for the main part of decline in coloured carotenoids under both dark and PPFD, and drastic decline in β-carotene was prominent under high PPFD.

Changes in concentrations of PChl and PChlide were investigated in the NF- and the KC-treated tissues to find their effect on the relation of carotenogenesis with Chl biosynthesis. Unlike other studies, the PChl and PChlide contents were separately

determined. In comparison with the untreated control, PChl content was increased by 65 % in the KC-treated tissues, but PChlide content was slightly decreased (Fig. 4). However, the NF-treated tissues exhibited a decline in PChl without any significant change in PChlide level. ALA feeding to the tissues resulted in a considerable increase in PChlide content. Exogenous supply of phytol decreased PChlide but increased PChl considerably. The PChl/PChlide ratios were increased by 81 and 177 % in the KC- and phytol-treated tissues, respectively, compared to the untreated control (Fig. 4). The NF- and ALA-treated tissues, however, exhibited a considerable decrease in the PChl/PChlide ratio.

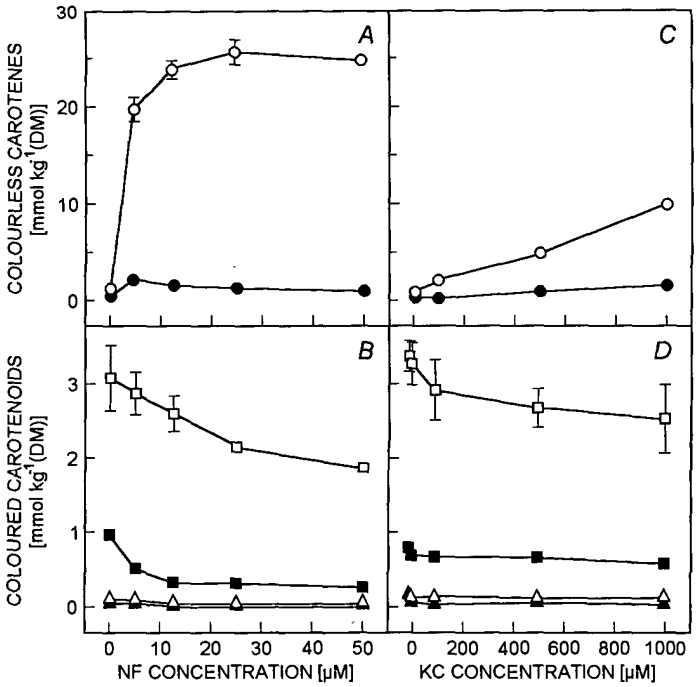


Fig. 2. Carotenoid composition in etiolated cucumber cotyledons treated either with NF (*A*, *B*) or with KC (*C*, *D*) as determined by HPLC. The cotyledons were subjected to the same treatments as in Table 1. ○, phytoene; ●, phytofluene; □, lutein; ■, β-carotene; Δ, antheraxanthin; ▲, violaxanthin; Δ, neoxanthin. Means ± SE from 3 replicates. In some cases the error bar is obscured by the symbol.

Composition of PChls esterified with alcohols: The treatment with NF, KC, ALA, or phytol influenced the composition of PChls esterified with alcohols in the etiolated cucumber cotyledons (Table 3). In the untreated control, the major components of PChls were PChl THGG, PChl DHGG, and PChl GG (in this order), but PChl phytol was negligible in the mixture. This trend was similar for both the NF- and the ALA-treated tissues, with increased proportion of PChl THGG and overall decrease of the four different PChls in the NF-treated tissues. The KC-treated tissues, however, showed a greatly increased proportion of PChl GG and PChl DHGG and a great decline in PChl THGG. In contrast to KC, exogenous phytol tended to increase the

hydrogenated esterifying alcohols, resulting in a great accumulation of PChl phytol as well as total PChls, but decline in the other components of alcohols.

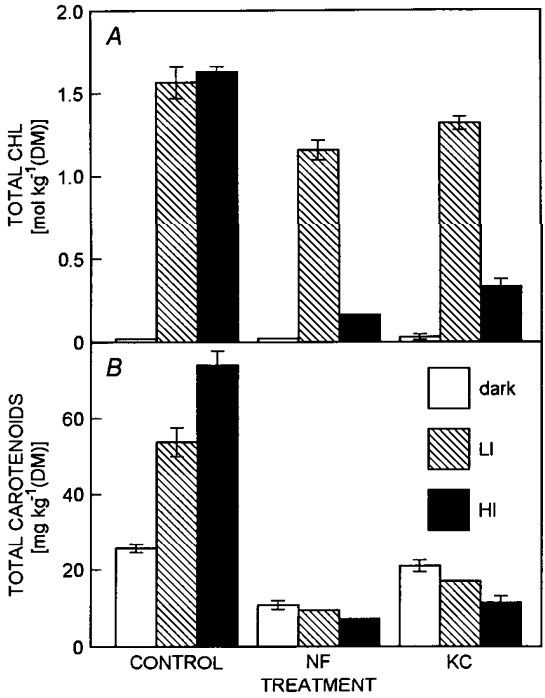


Fig. 3. Effects of different irradiances (PPFD) on the contents of total chlorophyll, Chl (*A*) and total carotenoids (*B*) in etiolated cucumber cotyledons. The cotyledons, incubated in potassium phosphate buffer (pH 6.5) either with 0.05 mM NF or with 0.5 mM KC at 30 °C in darkness for 24 h, were subsequently irradiated either with 15 (LI) or 150 μmol m^{-2} s^{-1} PPFD (HI) for 8 h. Means ± SE from 3 replicates. In some cases the error bar is obscured by the symbol.

Discussion

In both the NF- and the KC-treated etiolated cucumber cotyledons, the colourless carotenes phytoene and phytofluene were markedly accumulated in darkness with a concomitant decrease of coloured carotenoids (Fig. 2). These observations are consistent with results of NF treatment in the alga *Scenedesmus acutus* and dark-grown wheat (Dahlin and Ryberg 1986, Sandmann 1993b) and suggest that KC also inhibits carotenoid biosynthesis. KC bleaches plants presumably by a similar mechanism as NF, however, their profiles of inhibitory effect on Chl and carotenoids were different and also influenced by the doses of PPFD to the treated tissues (Fig. 3 and Table 2). Thus, the two inhibitors might exert their respective effects on Chl biosynthesis by interfering differently with carotenogenesis.

No significant photooxidative damage on Chl was found in the untreated controls even under high PPFD (Fig. 3). On the contrary, the photoprotectant β-carotene,

which is highly susceptible to oxidative species (Young *et al.* 1989), was further decreased by photooxidative PPFD in the treated tissues, with a drastic decline in KC (Table 2). Thus, both the treated tissues became more sensitive to photoinhibitory stress and the decline of Chl was obvious (Fig. 3). Under excess PPFD, generally, both photooxidative and photoadaptive events can occur (Foyer *et al.* 1994, Jung and Steffen 1997). Transgenic tobacco plants with a genetically manipulated carotenoid biosynthetic pathway possess increased biosynthetic activities for β-carotene and are resistant to NF (Misawa *et al.* 1993). Total decreases in β-carotene and xanthophylls were much lower than the corresponding increases in phytoene and phytofluene (Table 2), which were probably not only accumulated following the inhibition of carotenogenesis but also synthesized *de novo* from an earlier step of the biosynthesis. With this accumulation of the carotenoid biosynthetic intermediate, we speculate that the primary effect of KC is through inhibition of phytoene desaturase. To substantiate our speculation, a direct effect on this enzyme should be evaluated in the near future. KC inhibited carotenoid biosynthesis in the irradiated tissues nearly as strongly as in NF whereas it had a smaller effect in darkness (Table 2). The content of carotenoids formed in both NF- and KC-treated etiolated tissues during darkness might not be sufficient to effectively dissipate excessive radiant energy upon irradiation, and thus the inhibitory effects on carotenoid biosynthesis were similar in the light.

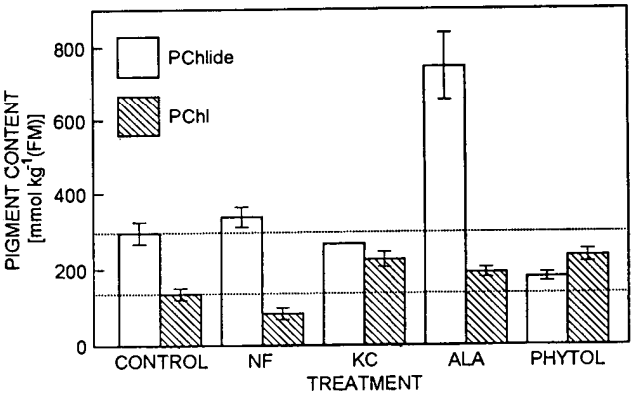


Fig. 4. Changes in the concentrations of protochlorophyll (PChl) and protochlorophyllide (PChlide) in etiolated cucumber cotyledons. The cotyledons were incubated in potassium phosphate buffer (pH 6.5) with 0.05 mM NF, 0.5 mM KC, 1 mM ALA, or 1 mM phytol in darkness at 30 °C for 24 h. Means ± SE from 3 replicates. In some cases the error bar is obscured by the symbol.

The relative amounts of PChl and PChlide vary with plant species and age (Shioi and Sasa 1983a, Mc Ewen and Lindsten 1992). We found that the amount of PChlide was 2-fold greater than that of PChl in the untreated control of etiolated cucumber cotyledons (Fig. 4). Administering the precursor ALA to the etiolated tissues caused an increase of the contents of both PChl and PChlide (Fig. 4, Vezitskiĭ and Shcherbakov 1988), whereas exogenous phytol led to esterification of an additional amount of aphytolic pigment (Fig. 4). The existence of putative PChl synthetase along with Chl synthetase has been suggested by Vezitskiĭ and Shcherbakov (1988).

The PChlide level was not changed greatly in the NF- and the KC-treated tissues, similar to what was observed in the NF-treated etiolated bean and barley seedlings (Pardo and Schiff 1980, Bolychevtseva *et al.* 1995). On the other hand, the substantial change in PChl/PChlide ratio can be ascribed to a decrease and an increase of PChl content in the NF- and the KC-treated tissues, respectively. Some carotenoid-deficient *tigrina* mutants of barley, *p*-nitrosubstituted diphenyl ether- and amitrole-treated etiolated barley accumulated high amounts of PChl(ide) (Nielsen 1974, Kouji *et al.* 1989, Rascio *et al.* 1996). The superoptimal MV- and DV-PChl(ide) accumulation may be the most important reason for photodynamic cell destruction in ALA-treated plants (Rebeiz *et al.* 1988, Chakraborty and Tripathy 1992, Toneva *et al.* 1997). However, the mechanism of PChl and PChlide alterations occurring in concomitance with the disturbed carotenogenesis needs further clarification.

Table 3. Changes in the composition of protochlorophylls, PChls [mmol kg^{-1}(FM)] esterified with four different alcohols in etiolated cucumber cotyledons treated with NF, KC, ALA, or phytol. The cotyledons were subjected to the same treatments as in Fig. 4. ND, not detected. Means ± SE from 3 replicates.

Treatment	PChl GG	PChl DHGG	PChl THGG	PChl phytol
Control	11.4 ± 0.9	49.1 ± 3.7	76.7 ± 5.3	2.0 ± 0.4
NF	3.5 ± 1.4	20.1 ± 3.0	58.3 ± 9.8	1.8 ± 0.5
KC	53.3 ± 4.9	124.5 ± 9.0	52.3 ± 3.8	ND
ALA	15.7 ± 1.0	57.6 ± 3.9	113.3 ± 6.4	6.7 ± 0.9
Phytol	2.9 ± 0.5	12.4 ± 0.8	26.5 ± 2.9	195.2 ± 11.9

The changes in composition of PChls esterified with four different alcohols (Table 3) confirm that PChlide is esterified by GG with its subsequent hydrogenation to phytol. The reason for PChl decline in the NF-treated tissues is as yet unknown, and most PChl GG and PChl DHGG accumulations by KC are probably esterified *de novo* rather than dehydrogenated from PChl THGG. The effect of KC on increase in PChl concentration having a high proportion of GG or DHGG is probably not primary, similarly to the effect of amitriazole and *s*-ethyl dipropylthiocarbamate which inhibits the conversion of GGPP to phytol (Rüdiger and Benz 1979, Wilkinson 1985). Accumulation of PChl GG was a much faster reaction than the following hydrogenation steps in the *Scenedesmus* mutant (Knaust and Senger 1994). Hydrogenation of PChl esters was stepwise inhibited with increasing age of the etiolated seedlings (Shioi and Sasa 1983a). The biosynthesis of the alcohols corresponding to C_{20} and earlier precursors of phytol occurs if certain photosynthetic bacteria strains are unable to generate sufficient reducing power (Ellsworth and Nowak 1974). Exogenous phytol induced accumulation of a great proportion of PChl phytol following PChlide decrease (Table 3 and Fig. 4). Besides the direct phytylation, the indirect conversion through intermediate stages to phytol may also be responsible for the considerable increase in PChl phytol. This might be due to the

result of transesterification of PChl GG, PChl DHGG, and PChl THGG to PChl phytol as suggested by Shioi and Sasa (1983b).

Taken together, the disruption of carotenogenesis by the two inhibitors seems to result in the alteration of Chl biosynthesis, especially in PChl content. In contrast to a PChl decrease induced by NF, KC caused the treated tissues to accumulate PChls esterified with earlier precursors of phytol. Large increase of PChls in the KC-treated tissues is likely to be due to an increased concentration of available GG equivalents which might result from the inhibition of carotenoid biosynthesis. Our results support that GGPP is channeled towards the biosynthesis of carotenoids or Chls (Dogbo *et al.* 1984). A hydrogenation of esterifying alcohols was lacking in KC-treated tissues, implying that the PChl formation is probably caused by inactivation of specific hydrogenase(s). The apparent difference between NF and KC effects on PChl formation could be utilized for further defining the interaction of Chl biosynthesis with carotenogenesis.

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