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Interaction of Chloroplasts with Inhibitors

LOCATION OF CAROTENOID SYNTHESIS AND INHIBITION DURING CHLOROPLAST DEVELOPMENT

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ABSTRACT

The inhibitor SAN 6706 [4-chloro-5-(dimethylamino)-2-(α,α,α,-trifluoro-*m*-tolyl-3(2H)-pyridazinone] has been used to study the synthesis of carotenes and xanthophylls during the conversion of etioplasts to chloroplasts in developing barley (*Hordeum vulgare*) shoots. SAN 6706 inhibits carotenoid synthesis and causes an accumulation of phytoene, but it is also a potent inhibitor of chloroplast electron transport. When developing barley is treated with SAN 6706, carotenoid synthesis is inhibited but total photosynthesis is unaffected. The ability of SAN 6706 to inhibit carotenoid synthesis becomes progressively less if etiolated shoots are illuminated for increasing lengths of time before treatment. During the greening of treated barley shoots only light-induced β-carotene synthesis is immediately inhibited; xanthophyll synthesis is not affected until after about 8 hours. The hypothesis that SAN 6706 cannot enter the chloroplast but inhibits carotenoid synthesis from the cytoplasm is discussed, and the question as to whether there are not two separate groups of enzymes for the synthesis of carotenes and xanthophylls is considered.

Inhibitors that adversely affect chloroplast development may do so through interaction with components either inside or outside the plastid because the chloroplast is dependent on substrates and enzymes (or subunits of enzymes) formed in the cytoplasm as well as those formed in the chloroplast. For example, the synthesis of carotenoids is dependent on nuclear DNA (27) and it is known specifically from studies with carotenoid mutants that many of the biosynthetic steps, such as those from phytoene to β-carotene, are under nuclear control (15). Yet the complete synthesis of carotenoids is believed to take place within the chloroplast (4, 11, 32) which implies that at least some of the enzymes are translated in the cytoplasm and then move into the chloroplast to effect carotenoid synthesis. This process has been studied using antibiotics that inhibit transcription and translation in the chloroplast or in the nuclear-cytoplasmic system (25, 27) but has not been studied using more specific enzyme inhibitors of carotenoid synthesis, which can be conveniently done during chloroplast development.

The illumination of dark-grown barley seedlings triggers the conversion of etioplasts into active chloroplasts capable of fixing CO_2 within about 2 hr (5, 9, 21, 28). Further illumination amplifies the synthesis of components and related activities developed in that initial period. Carotenoids are present in etioplasts mainly as xanthophylls and there is relatively little β-carotene present, but large increases in the synthesis of β-carotene and xanthophylls occur when dark-grown shoots are illuminated [reviewed by Britton (6)].

The inhibitor SAN 6706[1] blocks desaturation reactions in carotenoid synthesis causing an accumulation principally of phytoene (2, 3), but it is also capable of inhibiting chloroplast electron transport (12). We have used this dual activity to establish the location of both the synthesis and inhibition of carotenoids during chloroplast development.

MATERIALS AND METHODS

Plant Material. Barley (*Hordeum vulgare* L. var. Julia) was grown in trays (36 × 22 cm) of moist Vermiculite in the dark at 23 C for 6 days.

Treatment of Plants with Inhibitors. Six-day etiolated barley was sprayed with 100 ml per tray of inhibitor solution (concentrations indicated under "Results") containing 0.01% Tween 20 and 1% ethanol. Control plants were sprayed with the same solution without the inhibitor. Spraying was carried out under a green safelight (Ilford Bright Green 909) and the plants left for a further 12 hr in the dark before illumination at a moderate light intensity of 2,400 lux. Where the complete prevention of pigment photodestruction was of particular importance, shoots were illuminated at a low light intensity of approximately 15 lux. Samples were taken at intervals by harvesting the top 7 cm of shoot; approximately 1 g of plant material was used per sample for pigment analysis.

Chl Estimation. Total Chl was estimated by the method of Arnon (1). Where more accurate Chl *b* values were required, two 1-cm sections were cut (the 2nd and 3rd cm below the tip) from 20 barley shoots per sample and ground to homogeneity in a TenBroeck type tissue grinder with absolute methanol. Chl *a* and Chl *b* were estimated by the hydroxylamine technique of Ogawa and Shibata (19).

Carotenoid Fractionation and Estimation. Carotenoids were fractionated into carotenes and xanthophylls as previously described (24) by a procedure based on that of Davies (8). The concentration of total xanthophylls in ether was determined from spectra using an absorption coefficient of 142 mM^{-1} cm^{-1} at 443 nm [based on lutein (16)]. The concentration of carotenes was determined directly from the spectra in hexane. Absorption coefficients used were 137 mM^{-1} cm^{-1} at 450 nm for β-carotene, 86 mM^{-1} cm^{-1} at 347 nm for phytofluene, and 41 mM^{-1} cm^{-1} at 286 nm for phytoene.

Photosynthetic Activity. Rates of exchange of O_2 were measured manometrically in a photosynthetic Warburg apparatus at 20 C using the conventional "CO_2 buffer" (36). A constant CO_2 tension of 0.4% was maintained. Illumination by the apparatus gave 12,000 lux at the base of the sample flasks. Barley shoots were harvested at intervals during greening and the second and third cm sections below the tip were transferred to 70-mm Warburg

[1] Abbreviations: I_{50}: concentration producing 50% inhibition; norflurazon: 4-chloro-5-(methylamino)-2-(α,α,α,-trifluoro-*m*-tolyl)-3(2H)-pyridazinone; SAN 6706: 4-chloro-5-(dimethylamino)-2-(α,α,α,-trifluoro-*m*-tolyl)-3(2H)-pyridazinone.

reaction flasks containing 15 ml water; 40 shoots per sample were used. Dark respiration was recorded for 1 hr and then the flasks illuminated for sufficient time to obtain a linear rate of O_2 evolution (1.5 to 2 hr). Periods up to 5 hr were employed where the progress of photosynthetic development was monitored directly (such as in preillumination experiments).

Isolation of the P700 - Chl *a* Protein. The technique using a hydroxyapatite column (60 mm high × 27 mm diameter) was essentially that reported by Shiozawa *et al.* (26).

Determination of P700. P700 concentration was estimated on an Aminco DW-2 dual wavelength, double beam recording spectrophotometer from the reversible light-induced absorbance change at 697 nm, using an isosbestic point of 725 nm as a reference (26). An extinction coefficient for P700 at 697 nm of 64 mM^{-1} cm^{-1} was used (14).

Isolation of Barley Chloroplasts. Barley chloroplasts were isolated from 8 g for Hill reaction assays essentially as described by Ridley (24) but with the addition of 0.1% BSA to the grinding medium. Chloroplasts were resuspended in grinding medium without the sorbitol osmoticum.

PSII Activity by Isolated Chloroplasts. O_2 evolution was recorded directly using a Rank electrode as previously described (24), but the reaction mixture (3 ml) contained 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 5 mM potassium ferricyanide, 3 mM ammonium chloride, and chloroplasts containing 100 μg Chl.

RESULTS

Effects of SAN 6706 on Carotenoid Synthesis. When barley seedlings are grown in the dark for 6 days xanthophylls accumulate in the shoots but there is relatively little β-carotene (as shown by the levels of these carotenoids at zero time in Fig. 1, C and D). There is a large light-induced synthesis of β-carotene when etiolated shoots are greened. In shoots treated with SAN 6706 β-carotene synthesis is completely halted and the small amount accumulated during dark growth begins to fall after 8 to 9 hr greening, almost reaching zero after 26 hr illumination (Fig. 1C). Treatment of etiolated shoots with different concentrations of SAN 6706 reveals an I_{50} of 14 μM for the inhibition of light-induced β-carotene synthesis following 10 hr illumination at 2,400 lux. Treated shoots show a build-up of phytoene, and a much smaller build-up of phytofluene (Fig. 1, A and B). Much of the phytoene accumulation occurs in the 12-hr dark period after treatment and before the start of illumination. The amount of phytoene present at zero time indicates that this accumulation in the dark is at the expense of both xanthophyll and carotene synthesis (but mainly the former).

Unlike β-carotene synthesis, the light-induced synthesis of xanthophylls is not inhibited until after 8 hr illumination (Fig. 1D) at which time the level of phytoene rises further (Fig. 1A). This clearly demonstrates a difference in effect of SAN 6706 on the two carotenoid types.

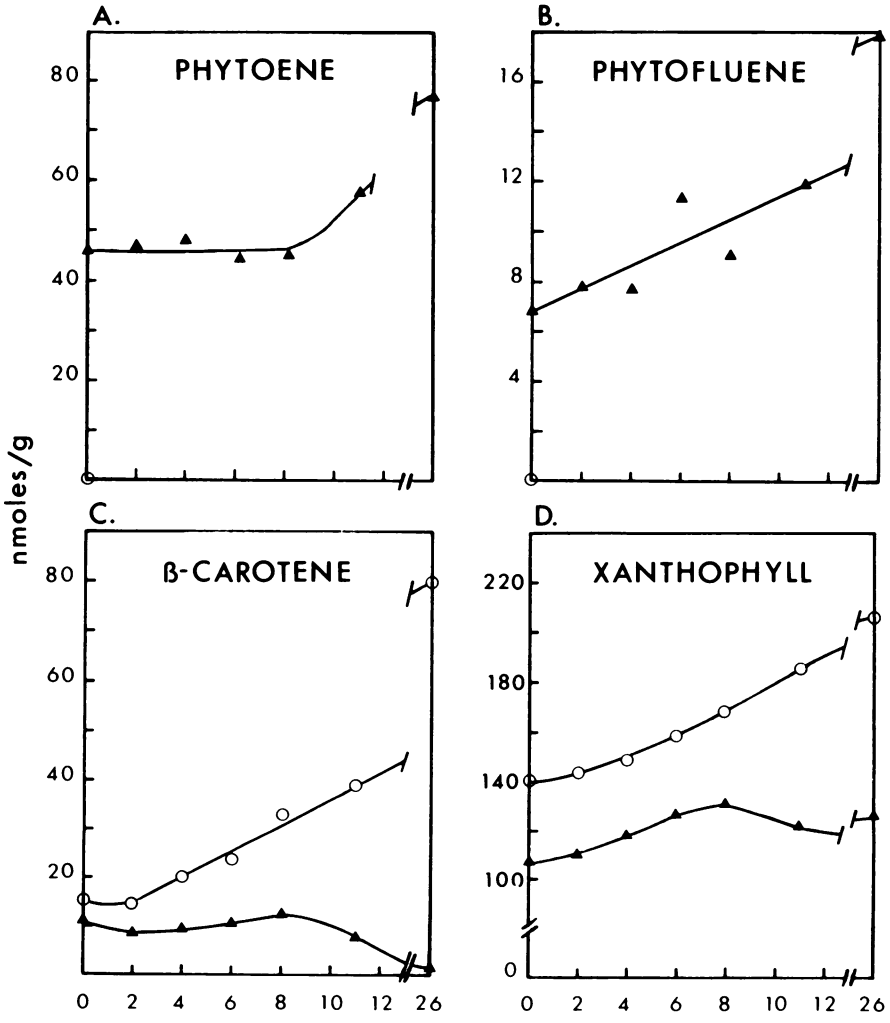

FIG. 1. Accumulation of carotenoids in barley shoots during greening at 2,400 lux following 6-days dark growth. Twelve hr before the start of illumination plants were treated with 100 μM SAN 6706. (○): controls; (▲): treated.

The ability of SAN 6706 to inhibit β-carotene synthesis becomes progressively less when etiolated shoots are illuminated for increasing lengths of time before treatment (Fig. 2); with about 9 hr preillumination there is only about 10% inhibition after a subsequent 5-hr period of illumination. The rates of photosynthesis at the points indicated (Fig. 2) were the same in control and treated shoots.

Effects of SAN 6706 on Chl Synthesis. Total Chl synthesis is slightly inhibited (about 17%) after 26 hr greening of SAN 6706-treated shoots at 2,400 lux (Fig. 3), but there is no such inhibition when greened at 15 lux. In the first few hr of illumination the synthesis of Chl *b* is delayed; Chl *b* first appears in the control shoots after 2 hr but not until after 4 hr greening in the treated shoots (Fig. 4). The Chl *a*/*b* ratio in the control and treated shoots is the same after about 5 hr (Fig. 4).

The slightly lower level of Chl in the treated shoots during illumination (Fig. 3) implies either that 2,400 lux is of sufficient intensity to induce some Chl photodestruction when the level of β-carotene is lowered, or that there is a direct effect on Chl synthesis not observed under low light because of slower Chl turnover, as suggested by Lichtenthaler and Kleudgen (17).

Effects of San 6706 on the Development of Photosynthetic Activity. Development of photosynthetic activity during illumination (2,400 lux) of dark-grown shoots takes about 7 hr to reach a maximum when the rates are recorded manometrically at intervals using a measuring light intensity of 12,000 lux (Fig. 5). The shoots treated with SAN 6706 take approximately the same time, but development is initially delayed so that photosynthetic rates in control and treated shoots do not become the same until after 5 hr illumination.

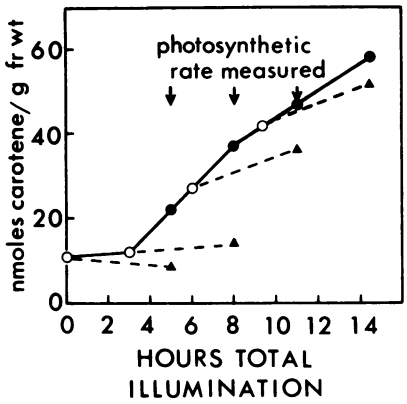

FIG. 2. Influence of preillumination on the ability of SAN 6706 to inhibit β-carotene synthesis. Etiolated shoots were illuminated at 2,400 lux for either 0, 3, 6, or 9.5 hr before spraying with 100 μM SAN 6706 or formulation (controls). The plants were returned to the dark for 12 hr after spraying to allow adequate time for inhibitor uptake, then given a further 5-hr illumination after which time the β-carotene levels were determined. β-carotene levels: (○), hr illumination before treatment; (●), hr pre- plus 5-hr postillumination controls; (▲), hr pre- plus 5-hr postillumination treated.

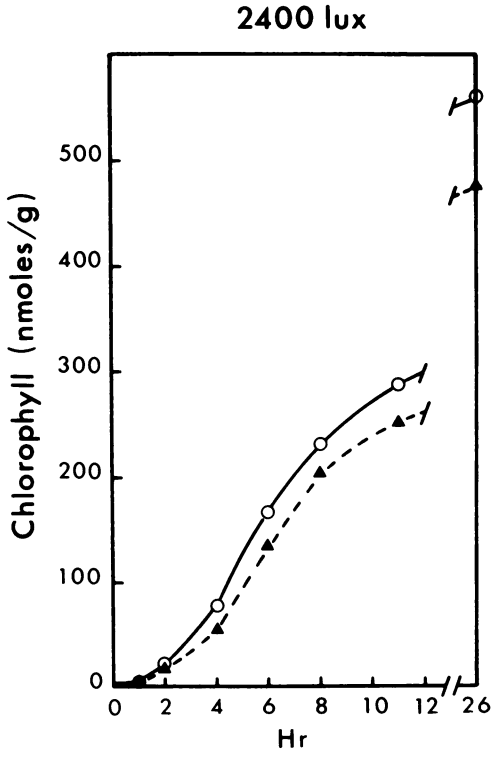

FIG. 3. Synthesis of total Chl in barley shoots during 26-hr greening at 2,400 lux with and without 100 μM SAN 6706 treatment. (○): control; (▲): treated.

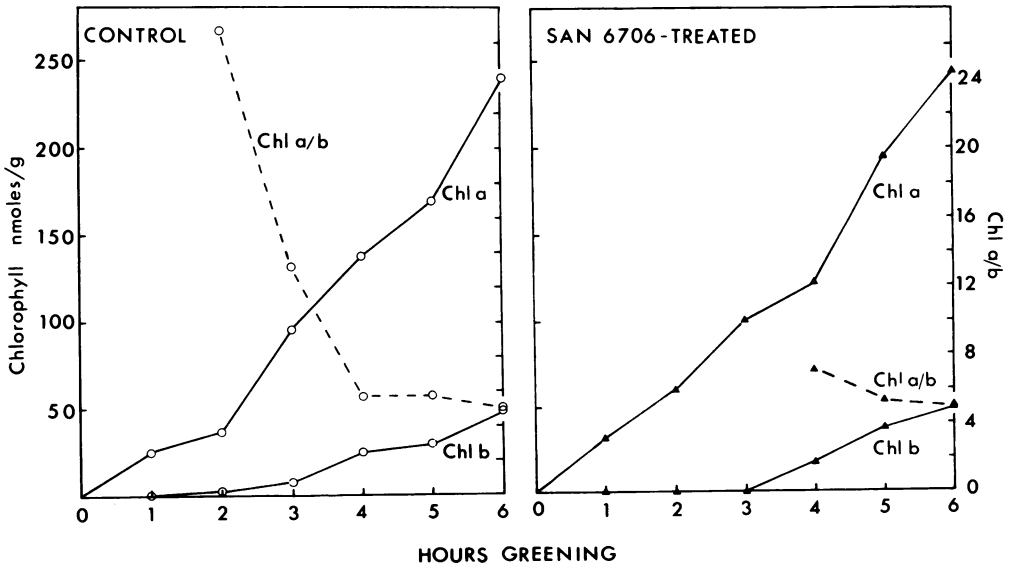

FIG. 4. Synthesis of Chl *a* and Chl *b* during the first 6 hr of greening at 2,400 lux of control and 100 μM SAN 6706-treated shoots. Chls were estimated in methanol by the method of Ogawa and Shibata (19).

This delay is also revealed in an experiment in which etiolated shoots are illuminated for varying lengths of time before treatment, and the subsequent development in excised tissue allowed to continue in Warburg flasks so that photosynthetic activity can be recorded directly and continuously. With no preillumination in control shoots the ability to evolve O_2 takes about 1.5 hr illumination to develop (Fig. 6), but the SAN 6706-treated shoots do not develop this capacity even after 5 hr illumination in the Warburg flasks. With increasing periods of preillumination the effect of SAN 6706 becomes progressively less until after 7 hr the photosynthetic activity in control and treated shoots is the same (Fig. 6); the same type of experiment conducted with 0.7 μM DCMU shows complete inhibition of photosynthesis at all of the periods of preillumination (data not shown). Halving the light intensity (to 6,000 lux) for measuring photosynthesis in samples preilluminated for 7 hr has no effect on control shoots but reduces the rate of photosynthesis in treated shoots by 22%.

One of the major components of the chloroplast with which β-carotene is known to be associated is the P700-Chl *a*-protein complex (7, 24, 34). We examined this to see whether the delay in photosynthetic development might be reflected in changes of activity when light-induced β-carotene synthesis is inhibited. Preparations of the P700-Chl *a*-protein complex taken at intervals during development under low light (to ensure no photodestruction of pigments) produce a biphasic pattern in the ratios of β-carotene to P700 with no difference between control and treated material. The ratio falls from 25:1 at 2 hr to 3:1 at 10 hr. Thereafter the ratio remains constant at this value which is close to the ratios reported in preparations from mature chloroplasts of several species (26) but lower than that in mature pea chloroplasts (24).

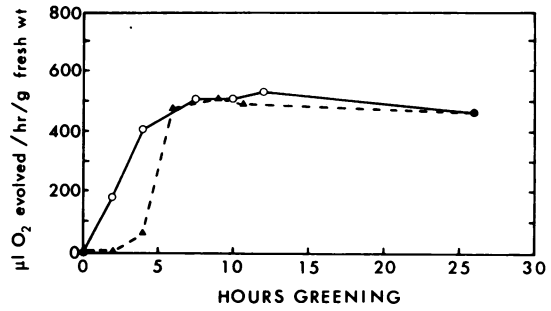

FIG. 5. Rates of photosynthesis during greening of barley shoots under 2,400 lux illumination. (▲): Plants treated with 100 μM SAN 6706; (○): untreated controls.

Direct Effects of SAN 6706 on Electron Transport System. The inhibition of electron transport in isolated chloroplasts from barley grown in the dark for 6 days and then illuminated for 44 hr yields an I_{50} of 1.6 μM SAN 6706. Clearly this potential inhibitory action is not being expressed during chloroplast development *in vivo* even when plants are sprayed with 100 μM. If chloroplasts are isolated from barley shoots which have been treated with different concentrations of SAN 6706 and greened for 12 hr, the Hill reaction is seen to be inhibited (Table I) where total photosynthesis in whole shoots (whether expressed on a Chl or fresh weight basis) is unaffected but β-carotene synthesis blocked. This confirms that SAN 6706 is not expressing its ability to inhibit electron transport until the chloroplasts are isolated, during which time there is some mechanical disruption of the plastids.

Comparison of Effects of Chloroplast Protein Synthesis Inhibitors with SAN 6706 on Development. The antibiotics D-*threo*-chloramphenicol and lincomycin inhibit 70S ribosomes and hence block the translation of proteins inside the chloroplast. The effects of these antibiotics have been studied for comparison with those caused by SAN 6706 which, as the evidence suggests, probably exerts its inhibitory action in the cytoplasm. The antibiotics inhibit β-carotene synthesis (lincomycin being more active than chloramphenicol) but without the accumulation of any detectable intermediates. They also exhibit a similar progressive inability to inhibit with increasing periods of preillumination as SAN 6706 (Fig. 7); similar patterns are obtained using 1 mM chloramphenicol

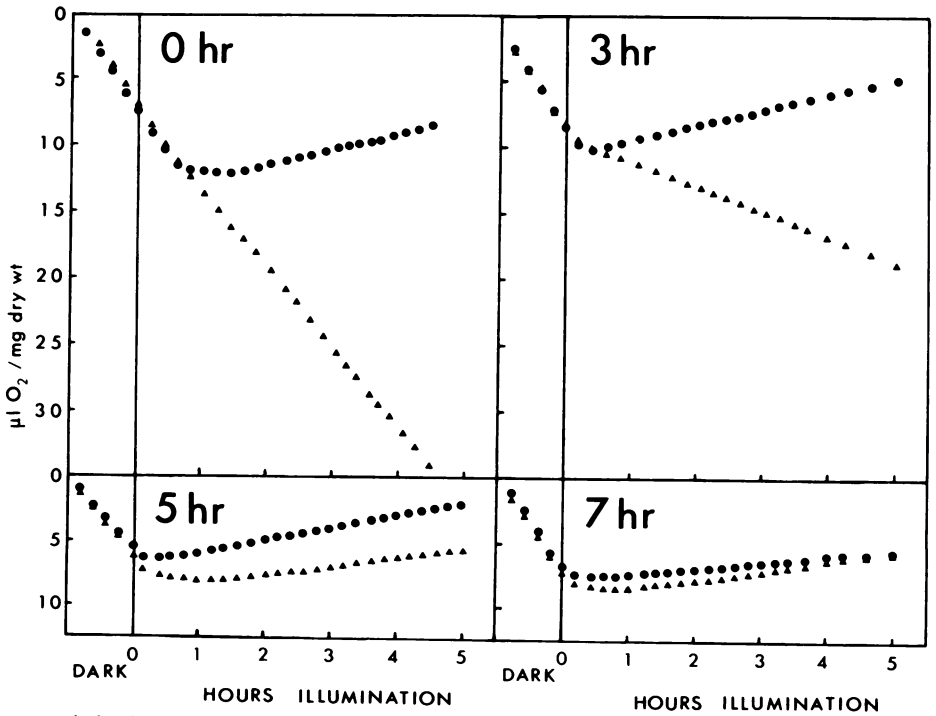

FIG. 6. Effect of different periods of preillumination of etiolated barley shoots on the ability of SAN 6706 to influence photosynthetic activity during a subsequent 5-hr illumination. Etiolated shoots were illuminated at 2,400 lux for 0, 3, 5, and 7 hr before spraying with 100 μM SAN 6706 or formulation (controls). The plants were returned to the dark for 12 hr to allow adequate time for inhibitor uptake. Subsequent photosynthetic activity was monitored directly by taking manometric readings at regular intervals over 5 hr. The zero on the ordinate is the starting point of the measurements when the Warburg flasks were closed to the atmosphere after equilibrating at 20C. (●): control; (▲): SAN 6706-treated.

Table I. Comparative effects of different concentrations of SAN 6706 on oxygen evolution in leaf segments and in isolated chloroplasts, and on carotenoid synthesis, from shoots greened for 12 hr.

Six-day etiolated barley was sprayed with SAN 6706, left 12 hr in the dark, then illuminated for 12 hr at 2400 lux before harvesting. Leaf segments were taken from 40 shoots per sample for measurement of photosynthesis (containing between 0.43 and 0.55mg Chl/g); chloroplasts were isolated from 8g of leaves per sample (and assayed in duplicate), and pigments extracted from 1g of leaves per sample.

SAN 6706	Chl	Photosynthesis in leaf segments	Hill reaction in isolated plastids		Light-induced β-carotene synthesis
μM	μg/g fresh weight	μmoles O_2 evolved /hr/mg Chl	μmoles O_2 evolved /hr/mg Chl	Percentage inhibition	Percentage inhibition
None	548	48	28	0	0
10	481	54	16	43	39
50	465	58	11	61	96
100	428	61	9	68	100

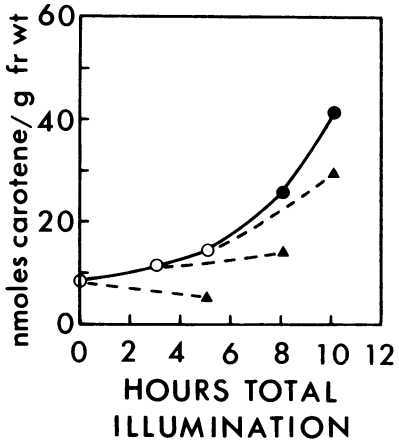

FIG. 7. Influence of preillumination on the ability of D-*threo*-chloramphenicol to inhibit β-carotene synthesis. Conditions as in Figure 2. β-carotene levels: (○), hr illumination before treatment; (●), hr pre- plus 5-hr post illumination controls; (▲), hr pre- plus 5-hr postillumination D-*threo*-chloramphenicol-treated (1 mM).

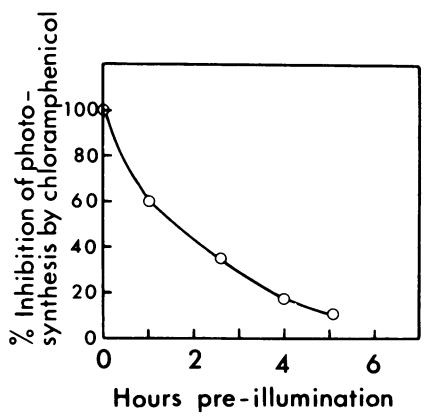

FIG. 8. Effect of preillumination on the ability of D-*threo*-chloramphenicol to influence the rate of photosynthesis. Etiolated barley shoots were illuminated for 0, 1, 2, 5, and 5.2 hr before spraying with 1 mM chloramphenicol or formulation (controls). The plants were returned to the dark for 12 hr to allow adequate time for inhibitor uptake and the subsequent rates of photosynthesis determined by manometry.

or 0.1 mM lincomycin. There is a further similarity in that the protein synthesis inhibitors delay or inhibit photosynthetic development and there is a progressively diminishing effect as shoots are illuminated for increasing lengths of time before treatment such that there is no inhibitory effect after 7 hr preillumination (Fig. 8). This closely matches the effects of SAN 6706 in a similar experiment (Fig. 6).

DISCUSSION

Using the light-induced differentiation of 6-day etiolated barley shoots to study carotenoid synthesis with the inhibitor SAN 6706 avoids the secondary effects that will occur if plants are grown for the same length of time following seed treatment because the effects can be confined to those processes that are triggered by light. When moderate or low light intensities are also used, little or no photodestruction will be superimposed on direct inhibition by SAN 6706. Potential inhibition of the desaturation of linoleic acid as an additional mode of action (13) which might have complicated the interpretation of our results also becomes unimportant in these experiments since there is a considerable lag in linolenic acid formation when etiolated barley shoots are illuminated (18). Thus we can focus our attention on the two effects of SAN 6706 as an inhibitor of carotenoid synthesis and of chloroplast electron transport which enables this compound to be used as a tool for studying the location of carotenoid synthesis and inhibition.

When there is complete and sustained inhibition of light-induced β-carotene synthesis by SAN 6706 during 26 hr illumination (Fig. 1c) the development of photosynthetic activity is not inhibited but only delayed (Fig. 5). This separation of activities can be obtained in shoots treated with 100 μM SAN 6706 even though the compound is a potent inhibitor of both processes with an I_{50} for inhibition of light-induced β-carotene synthesis of 14 μM and an I_{50} for inhibition of the Hill reaction in isolated barley plastids of 1.6 μM. For comparison, a low dose of DCMU (0.7 μM) under similar experimental conditions maintains complete inhibition of photosynthesis during 26-hr greening of etiolated barley shoots. It has been proposed (30, 31) that SAN 6706 has to demethylate to norflurazon to inhibit carotenoid synthesis but this does not detract from our finding that only one of the two major activities (carotenoid inhibition) is expressed; the duration of our experiments is short by comparison with published rates of breakdown (31) and norflurazon is also a Hill reaction inhibitor. Treated plants showing no inhibition of total photosynthesis reveal that inhibition may be induced when the chloroplasts are isolated from these plants (Table I). The action of disrupting the leaf cells probably allows the SAN 6706 accumulated in the cytoplasm to become accessible to the chloroplasts broken during the isolation procedure.

These data provide evidence that SAN 6706 cannot enter the developing chloroplast of barley to inhibit photosynthesis and is therefore likely to exert its inhibitory effect on carotenoid synthesis from the cytoplasm. This hypothesis is supported by differences in effects on carotenes and xanthophylls; only light-induced β-carotene synthesis is immediately inhibited while synthesis of xanthophylls during illumination is unaffected for the first 8 hr of greening (Fig. 1). A reasonable explanation for this is that the

enzyme involved in carotene synthesis which SAN 6706 inhibits are made *de novo* in the cytoplasm at the start of illumination of the etiolated shoots. However, the etioplast already contains some of these enzymes which are inaccessible to SAN 6706 inhibition and whose activity is essentially related to xanthophyll synthesis. Hence they are not affected until the enzymes turn over and have to be resynthesized in the cytoplasm (after about 8 hr illumination). This raises the question as to whether there are not two separate groups of enzymes for the synthesis of carotenes and xanthophylls. The question is a reasonable one since the two groups of carotenoids have different distributions within the lamellae (35). This suggestion does not preclude the conversion of carotenes to xanthophylls (and *vice versa*) within the separate groups. The data in Figure 1 also reveal that under our experimental conditions (2,400 lux at 23 C) the turnover of β-carotene is about once every 8 hr; the same figure applies to total xanthophylls although we have not assessed the turnover of individual xanthophylls.

Further evidence for the concept of inaccessibility of SAN 6706 to the inside of the chloroplast comes from the experiments in which etiolated shoots are illuminated for different lengths of time before treatment when progressively less inhibition of light-induced β-carotene synthesis is found (Fig. 2). Our interpretation again is that one of the enzymes which SAN 6706 inhibits and which results in an accumulation of phytoene is produced in the cytoplasm and becomes progressively less susceptible to inhibitor attack as sufficient of the enzyme accumulates in the developing chloroplast to sustain β-carotene synthesis. This also provides evidence that the actual desaturation of phytoene takes place within the plastid and not in the cytoplasm. Our conclusion that SAN 6706 does not enter the developing chloroplast to inhibit photosynthesis differs from that of others who have used different species, techniques of inhibitor application, and light intensities for growth (12, 22). Thus Hilton *et al.* (12) used root treatment in four different species and a light intensity of about 30,000 lux, whereas Porter and Bartels (22) studied the direct effects on single leaf cells from soybean and cotton. This points to a difference in the permeability of the envelope of these plastids to SAN 6706 compared with our experiments.

Both the delay in photosynthetic development (Fig. 5) and the progressively diminishing effect on photosynthetic activity in SAN 6706-treated shoots (Fig. 6) are probably secondary effects of the inhibitor action. Preliminary observations with the electron microscope show a delay in grana stacking which can account for this, but Chl *b* formation is also delayed (Fig. 4) suggesting a lag in the assembly of the light-harvesting Chl *a*/*b* protein. The expected consequence of this is a higher light requirement for saturating the photosynthesis of treated plants during the first few hr of greening. Evidence that this is so was found by halving the light intensity (12,000 lux) used in measuring the rate of photosynthesis in shoots greened for 7 hr; a reduction in the rate was found only in the treated shoots. The explanation as to why the inhibition of light-induced β-carotene synthesis should delay Chl *b* formation must await further experimentation.

Apart from this initial delay, the specific absence of light-induced β-carotene (and hence the bulk of the carotene) appears to have had no major adverse effect on the conversion of the prolamellar body into an actively photosynthesizing lamellar system. The light intensity for greening must be maintained at a fairly low level otherwise Chl synthesis is also affected; a slight loss can be seen even under 2,400 lux (Fig. 3). The role of the bulk of β-carotene is therefore principally confined to that of protection; it does not directly affect Chl synthesis and is not involved in structural assembly of the lamellar membranes [although carotenoid mutants of maize lack grana in the mesophyll chloroplasts (10)]. This is in agreement with the findings of Pardo and Schiff (20) who studied the effects of carotenoid inhibition during the greening of etiolated bean seedlings. A different situation exists in algae where carotenoids are directly involved both in regulating Chl synthesis and in controlling structural assembly of the membrane (37).

A considerable proportion of the total β-carotene is associated with PSI in mature chloroplasts, yet no detrimental effect on the activity of the P700-Chl *a* complex is found during development over 18 hr when light-induced β-carotene synthesis is inhibited and the ratio of β-carotene to P700 in the complex from treated and control shoots is the same. The small amount of β-carotene present in the dark must therefore be adequate for association with P700 during at least 18 hr greening even when subsequent light-induced β-carotene synthesis is inhibited.

The antibiotics D-*threo*-chloramphenicol and lincomycin act inside the chloroplast to inhibit protein synthesis, and yet they also inhibit light-induced β-carotene synthesis and show the same progressively diminishing action with increasing time of preillumination as SAN 6706 which we believe to act outside the plastid (Fig. 7). Since the diminishing effects in the experiment in Figure 7 are produced by both antibiotics, the possibility that those caused by chloramphenicol are due to detoxification, as found in developing *Euglena* (38), is unlikely. The proper assembly of the lamellae may regulate the rate of synthesis of carotenoids, yet it seems that the converse is not true. Schnarrenberger and Mohr (25) studied the effects of chloramphenicol on carotenoid synthesis in mustard seedlings and concluded that inhibition of chloroplast protein synthesis affects carotenoid synthesis indirectly through impairment of the effect of phytochrome and ultimately lamellar membrane integrity. Rademacher and Feierabend (23) found a deficiency (but not complete absence) of carotenoid formation in 32 C-grown rye seedlings which lacked 70S ribosomes and concluded that a limitation of acceptor membranes in the chloroplast prevented carotenoid accumulation.

Grana formation is inhibited by chloramphenicol and lincomycin (29, 33) and the interpretation has been that chloroplast ribosomes synthesize a lamellar component necessary for grana assembly. But the effect could also be due to the indirect inhibition of carotenoid synthesis. Plants seed-treated with SAN 6706 also lack grana (2).

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