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Light-Stimulated Carotenoid Biosynthesis during Transformation of Maize Etioplasts Is Regulated by Increased Activity of Isopentenyl Pyrophosphate Isomerase[1]

Manuela Albrecht and Gerhard Sandmann*

Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, P.O. Box 5560, D-78434 Konstanz, Germany

Light-stimulated carotenoid biosynthesis associated with the transformation of etioplasts to chloroplasts was investigated after dark-grown maize (*Zea mays*) seedlings were transferred into light. These studies focused on the enzymes of the pathway to detect those enzyme activities that were stimulated in the light and thus that were responsible for increased biosynthesis of carotenoids. In preliminary experiments, norflurazon, an inhibitor of phytoene desaturase, was used to prevent phytoene being further metabolized to carotenoids. Light-dependent stimulation of phytoene accumulation indicated that the light-regulated steps are located in the pathway leading to phytoene synthesis. The use of the ^{14}C-labeled precursors mevalonic acid, isopentenyl pyrophosphate, and farnesyl pyrophosphate pointed to increased activity of an enzyme involved in the biosynthetic steps between isopentenyl pyrophosphate and farnesyl pyrophosphate. Determination of the activities of all five enzymes of the pathway involved in the sequence from mevalonic acid to phytoene revealed that the only enzyme activity stimulated by light was isopentenyl pyrophosphate isomerase. Over a 3-h period of illumination, this enzyme activity, like carotenoid biosynthesis, was stimulated 2.8-fold.

Light is essential for plant life. It serves not only as an energy source in photosynthesis but also influences plant growth and development. During leaf formation, the maturation of proplastids into chloroplasts requires light. In nonilluminated plants etioplasts are formed instead, which can be recognized by their crystal-like structure (Boardman, 1977); after illumination, however, etioplasts are converted very rapidly into fully functioning chloroplasts. During this photomorphogenic event, massive structural and biochemical modifications occur. The most prominent changes are in leaf pigmentation. Light stimulates Chl biosynthesis by stimulating the reduction of Pchlide but, more particularly, by stimulating the synthesis of several early regulatory enzymes of this pathway (Mohr et al., 1984; Rüdiger and Schoch, 1988).

Not only Chl but also the carotenoid content is increased during light-dependent transition from etioplasts to chloroplasts. Carotenoids are present in etioplasts but in a lower amount than in the corresponding chloroplasts (Grumbach, 1981; Barry et al., 1991). However, upon transition in the light to chloroplasts, the formation of carotenoids is also stimulated in parallel to the biosynthesis of Chl (Cohen and Goodwin, 1962; Virgin, 1967; Frosch and Mohr, 1980).

It is generally assumed that photomorphogenesis and photoinduction of metabolism proceed via light-mediated gene expression, followed by enhanced protein synthesis including increased concentrations of appropriate enzymes leading to enhanced biosynthetic capacities (Hoober, 1987). This model may also apply for photoregulation of carotenogenesis (Tobin and Silverthorne, 1985).

In the present investigation, work was focused on the last step in this chain of events: the formation of enzymes of the carotenoid biosynthetic pathway and the resulting accumulation of carotenoids during light-stimulated conversion of etioplasts into chloroplasts. In vitro analyses of segments of the biosynthetic pathway and assays of single enzymes involved in the conversion of MVA to phytoene were performed to find out which enzymes of the carotenoid pathway were stimulated during light-induced chloroplast formation to account for increased carotenoid biosynthesis.

MATERIALS AND METHODS

Growth Conditions

Maize seeds (*Zea mays* cv Pyton) were soaked overnight in tap water and sown the next day on vermiculite. The seedlings were grown for 10 to 11 d at 24°C in complete darkness. All manipulations such as watering and harvesting were carried out under green light. *Fusarium moniliforme* SG4 was cultivated for 3 to 4 d as previously described (Sandmann, 1993) and freeze dried after harvesting.

Treatment of Maize Leaves Prior to Carotenoid Determination

Etiolated seedlings were placed in white light (150 $\mu E\ m^{-2}\ s^{-1}$) and leaves were cut 0, 3, 5, and 8 h after illumination. In norflurazon treatments, etiolated seedlings were cut in the dark and immediately placed in a solution containing 50 mM

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* Corresponding author; fax 49–7531–883042.

Abbreviations: DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate; MVA, mevalonic acid; MVAP, mevalonic acid phosphate; MVAPP, mevalonic acid pyrophosphate; pcv, packed chloroplast volume.

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K phosphate buffer (pH 8.0) and 50 μM norflurazon, an inhibitor of phytoene desaturase (Sandmann and Böger, 1989), and left in darkness for a further 2 h to ensure uptake of the herbicide. After this pretreatment, half the plants were placed in white light (15 $\mu E\ m^{-2}\ s^{-1}$) for 3, 5, or 8 h, and the others were kept in the dark for the same periods. The plants were then collected, frozen, and lyophilized. Dry weights, Chl, and colored carotenoid concentrations were estimated, and phytoene content was determined by HPLC. Norflurazon was obtained from Sandoz AG (Basel, Switzerland).

Incorporation of Labeled Precursors into Excised Maize Leaves

Etiolated seedlings were illuminated with white light (150 $\mu E\ m^{-2}\ s^{-1}$) at 28°C and cut after 0, 3, 5, and 8 h, and placed in 50 mM K phosphate buffer (pH 8.0) containing 0.25 μCi of R-[2-^{14}C]MVA, [1-^{14}C]IPP, or [2-^{14}C]FPP. The samples were incubated in the dark for 2 h. After this treatment the leaves were frozen and lyophilized, the dry weight was estimated, the radioactive-labeled carotenoids were extracted, and the incorporated radioactivity was determined after HPLC separation. The radioactive substrates used were all bought from Amersham-Buchler (Braunschweig, Germany) with a specific radioactivity of about 50 mCi/mmol. MVA was purchased as lactone and converted into the sodium salt by addition of 10 mM NaOH.

Isolation of Chloroplasts and Preparation of Soluble Chloroplast Proteins

For chloroplast preparation, the leaves from seedlings were illuminated for 0, 3, 5, or 8 h with 150 $\mu E\ m^{-2}\ s^{-1}$ at 28°C and finally cut, mixed with 3.5 mL/g tissue of ice-cold homogenization buffer (0.5 M sorbitol, 50 mM Mes, 1 mM $MgSO_4$, 1 mM $MnCl_2$, 3 mM $Na_4P_2O_7$, 2 mM EDTA, 1 mM DTT, 0.15% BSA, pH 6.2), and homogenized in a Sorvall blender (Dietz and Bogorad, 1987). The homogenate was filtered through three layers of cheesecloth and centrifuged for 5 min at 3000*g*. The pellet was resuspended in homogenization buffer and again centrifuged for another 5 min at 500*g* to pellet cell fragments. Then the resulting supernatant was layered on a 15% Percoll solution in homogenization buffer and centrifuged for 5 min at 8700*g*. The chloroplast pellet was collected, diluted with homogenization buffer, and finally pelleted by centrifugation for 15 min at 3000*g*. The chloroplasts were then resuspended in ice-cold buffer containing 0.5 M sorbitol, 50 mM Hepes, 1 mM $MgSO_4$, 1 mM $MnCl_2$, 5 mM $Na_4P_2O_7$, 1 mM DTT (pH 7.6) and stored at −20°C. The pcv and the carotenoid content were determined and a soluble protein extract was prepared in a Potter-Elveghem homogenizer on ice for 2 to 3 min and centrifuged for 15 min at 16,000*g*. The supernatant contained the soluble chloroplast proteins and was further used in all the in vitro enzyme assays. The protein content was determined according to Bradford (1976) using the dye concentrate of Bio-Rad.

Enzymic Assays

Activities of IPP isomerase and FPP synthase were assayed by determining the amount of radioactivity incorporated into acid-labile reaction products (Fujisaki et al., 1986). In the case of GGPP synthase the reaction product was separated by HPLC. The incubation medium was basically the same for the three assays and contained 20 mM ATP, 1 mM KF, 6 mM $MnCl_2$, 4 mM $MgSO_4$, 280 mM Tris (pH 8.0), 3.5 mM DTT, and 0.3 to 0.4 mg of soluble chloroplast proteins in a total volume of 0.5 mL. The reaction was started by adding the appropriate radioactive-labeled substrate. Incubation was carried out in the dark at 30°C for 2 h. Then the reaction was stopped with HCl (final concentration 1.5 M) and acid hydrolysis of the pyrophosphates proceeded for 30 min at room temperature (Holloway, 1972). The pH was adjusted to 8 with NaOH and the reaction products were extracted into diethylether in the case of IPP isomerase or into 10% diethylether in petroleum spirit (boiling point 35–80°C) in the case of FPP and GGPP synthases. The organic phases were evaporated under a stream of nitrogen, the residues were dissolved in scintillation fluid (Ultima Gold, Canberra Packard, Australia), and the radioactivity was determined by liquid scintillation counting.

For the GGPP synthase assay, however, the dried reaction products were taken up in acetone and subjected to HPLC separation. This assay also revealed the absence (or presence) of synthesized squalene as an indicator for the purity of the chloroplast preparation. In the IPP isomerase assay, radioactivity was corrected for background radioactivity of about 1500 dpm per assay derived from contamination of free alcohol in the commercial IPP; this value was determined in a control incubation with boiled enzyme. Substrate for IPP isomerase was [1-^{14}C]IPP (0.25 μCi, 4.7 nmol) and for FPP synthase it was [1-^{14}C]IPP (0.25 μCi, 4.7 nmol) together with the allylic cosubstrate DMAPP (2 μmol). The enzyme extract used to determine FPP synthase was preincubated for 30 min with 1.5 mM iodoacetamide to inactivate IPP isomerase completely (Spurgeon et al., 1984). The substrates added to the GGPP synthase assay were unlabeled IPP (2 μmol) and [2-^{14}C]FPP (0.25 μCi, 4.9 nmol).

The activity of phytoene synthase was determined in a coupled enzymic system with a GGPP-generating extract of the fungal mutant *Fusarium* SG4 and the chloroplast supernatant. The assay conditions and the preparation of the extract were recently described in detail (Sandmann, 1993). The reaction mixture contained in 0.5 mL 20 mM ATP, 15 mM NAD, 6 mM $MnCl_2$, 4 mM $MgSO_4$, 200 mM Tris-HCl (pH 8.0), 0.4 mg of soluble chloroplast proteins, and 100 μL of the *Fusarium* SG4 extract. After incubation for 2 h at 30°C in the dark with continuous shaking, methanol containing 6% KOH was added and the samples were heated for 20 min at 60°C. Then the carotenoids were extracted into 10% diethylether in petroleum spirit (boiling point 35–80°C) and separated by HPLC.

The in vitro reactions for MVA kinase, MVAP kinase, and MVAPP anhydrodecarboxylase were carried out in 0.5 mL containing 6 mM ATP, 10 mM KF, 6 mM $MnCl_2$, 4 mM $MgSO_4$, together with the substrates (MVA, MVAP, or MVAPP, all 0.25 μCi, 5 nmol) and chloroplast supernatant equivalent to 0.4 mg of protein in 0.1 M Tris-HCl buffer, pH 8. After incubation for 1 h, the reaction products were separated from the nonconverted substrate by ion-exchange chromatography on Dowex 1x8 microcolumns using different concentrations

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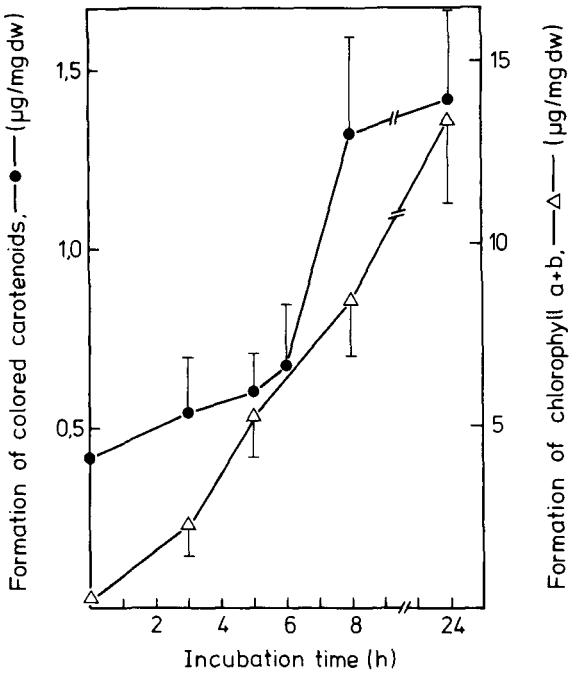

Figure 1. Carotenoid (●) and Chl (△) content of chloroplasts from 11-d-old etiolated maize leaves after illumination with white light (150 $\mu E\ m^{-2}\ s^{-1}$).

of formic acid followed by counting of the incorporated radioactivity. Details of the separation are described elsewhere (Sandmann and Albrecht, 1994).

Extraction and Determination of Pigments

Powdered lyophilized leaves and isolated chloroplasts were extracted either with methanol for determination of Chl estimation or with methanol containing 6% KOH for carotenoids by heating for 25 min at 60°C. The Chl samples were centrifuged and the clear supernatant was used for spectrophotometric determination of the Chl content (Porra, 1991). The carotenoids were extracted into 10% diethylether in petroleum spirit (boiling point 35–80°C) and the upper phase was either used for spectrophotometric estimation of total colored carotenoids (Davies, 1976) or evaporated in a stream of nitrogen and redissolved in acetone. Then the carotenoids were separated by HPLC.

HPLC Systems

All HPLC separations were carried out on a Spherisorb ODS-1 5-µm, 250-mm column at a flow of 1 mL/min. In the GGPP synthase assay, the reaction product was separated using a step gradient of water:methanol (10:90, v/v) for 10 min with a subsequent change to acetonitrile:methanol:2-propanol (85:10:5, v/v/v). In the phytoene synthase assay, the products were eluted isocratically with acetonitrile:methanol:2-propanol (85:10:5, v/v/v). Radioactivity was determined online with the radioactivity monitor RAMONA LS (Raytest, Straubenhardt, Germany).

HPLC separation of unlabeled carotenoids was carried out with acetonitrile:methanol:2-propanol (80:13:7, v/v/v) as eluant. The spectra of the carotenoids were recorded from the eluant peaks online with a Waters 994 programmable photodiode array detector. The prenyl alcohols and the carotenoids were identified by comparison with standards of known concentration (Ernst and Sandmann, 1988; Fraser et al., 1993).

RESULTS

With the maize cultivar Pyton used in this investigation, a rapid response of etiolated seedlings to light leading to changes in pigmentation was observed. In less than 10 h, Chl was drastically increased to values of around 10 µg/mg dry weight (Fig. 1). In parallel, the amount of colored carotenoids formed during this illumination period was about 1 to 1.5 µg/mg dry weight compared to less than 0.5 µg/mg dry weight in etiolated leaves of plants grown in complete darkness. Stimulation factors for pigment formation are listed in Table I. They are derived from determinations using either whole leaves that are related to dry weight of leaf powder or from isolated chloroplasts when the pigment content is related to the pcv. The carotenoid concentration determined with chloroplasts was about 3-fold higher after 5 h of illumination and about 6-fold higher after 8 h of illumination (Table I). The corresponding values calculated from carotenoid analysis of leaves were 1.4- and 3.4-fold, respectively. Under the same experimental conditions, the Chl content determined from chloroplasts increased 46-fold after 5 h and 84-fold after 8 h of illumination. The corresponding stimulations by light were for the leaf values 21- and 52-fold.

Using the phytoene desaturase inhibitor norflurazon, it is possible to inhibit the carotenoid biosynthesis pathway at the level of colorless phytoene (Sandmann and Böger, 1989). Thus, measuring phytoene accumulation in etiolated maize before and after illumination makes it possible to determine whether the light-activated step of carotenoid biosynthesis occurs before or after formation of phytoene. Figure 2 shows the kinetics of phytoene accumulation in the presence of 50 µM norflurazon in darkness or in the light. In illuminated plants phytoene accumulation was observed, but levels were almost constant in leaves from plants kept in darkness over 8 h.

Table I. *The stimulation factor for carotenoid and Chl biosynthesis in etiolated 11-d-old maize seedlings during 5 or 8 h of illumination (150 $\mu E\ m^{-2}\ s^{-1}$)*

Pigment content before illumination was 25 ± 8 µg carotenoids/mL pcv and 46 ± 4 µg Chl/mL pcv as determined from chloroplasts, and 422 ± 71 µg carotenoids/g dry weight and 285 ± 76 µg Chl/g dry weight as determined from leaves.

	Pigments Determined from			
	Chloroplasts		Leaves	
	5 h	8 h	5 h	8 h
	-fold			
Colored carotenoids	2.9	5.9	1.4	3.4
Chl *a* + *b*	46	84	21	52

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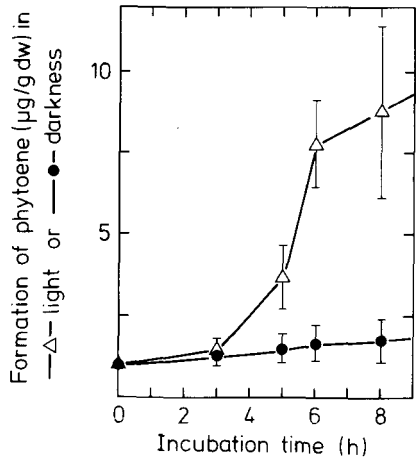

Figure 2. Formation of phytoene in etiolated maize leaves. Leaves were excised and preincubated for 2 h with 50 µM norflurazon and subjected to subsequent illumination (△) or dark treatment (●).

Another method of locating light-regulated biosynthetic steps in carotenoid formation is to determine the light-dependent incorporation of different radioactive-labeled intermediates of the terpenoid biosynthetic pathway (which are carotenoid precursors) into carotenoids. This was carried out with preilluminated leaves excised before metabolite application (Table II). Incubation followed then for 2 h in darkness. The precursors employed were [^{14}C]MVA, [^{14}C]IPP, and [^{14}C]-FPP. Radioactivity was determined mainly in phytoene, zeta-carotene, and β-carotene. Light-stimulated incorporation of radioactivity into carotenoids over the 8-h period was observed in the range of 5-fold with MVA and IPP only. In contrast, application of FPP resulted in a very small increase of radiolabeled carotenoids.

In the next stage of this investigation, activities of three enzymes catalyzing the steps from MVA to IPP were determined from chloroplast extracts (Table III). The chloroplasts were isolated from etiolated maize leaves illuminated for 0, 3, or 5 h. Purity of the chloroplasts was checked by lack of

Table II. *Incorporation of radioactive-labeled precursors into carotenoids by excised maize leaves during a 2-h incubation period in the dark*

The seedlings used were grown for 11 d in darkness, then preilluminated as indicated before they were excised and incubated with the radioactive compounds. Radioactivity the substrates offered was 0.125 µCi and uptake by three leaves per assay (average dry weight of 30–40 mg) was typically 70 to 80%. The radioactive carotenoids formed during the incubation were mainly phytoene, zeta-carotene, and β-carotene.

Preillumination	Conversion into Carotenoid (dpm mg^{-1} dry weight h^{-1}) of		
	[^{14}C]MVA	[^{14}C]IPP	[^{14}C]FPP
0	180 ± 40	175 ± 33	106 ± 5
3	524 ± 115	677 ± 198	170 ± 19
5	1064 ± 191	856 ± 173	169 ± 28
8	876 ± 265	891 ± 31	139 ± 21

Table III. *Enzyme activities given as conversion rate (%) of early reactions of the terpenoid biosynthetic pathway with chloroplast preparations from etiolated leaves preilluminated as indicated*

Enzyme	Illumination for		
	0 h	3 h	5 h
MVA kinase	22	28	23
MVAP kinase	58	62	50
MVAPP anhydrodecarboxylase	84	79	86

squalene synthase activity, which is substantial in cytoplasmic/ER fractions. The absence of this enzyme is a recommended criterion for the purity of the chloroplast preparation (Kleinig, 1989). The activities of MVA kinase, MVAP kinase, and MVAPP decarboxylase were expressed as percent conversion of the substrates. All three enzymes exhibited considerable activity; however, light treatment of the plants did not increase these activities.

The carotenoid biosynthetic pathway from IPP to phytoene is catalyzed by the enzymes IPP isomerase (Poulter and Rilling, 1981), a prenyl transferase with FPP and GGPP synthase activity (Camara et al., 1989), and phytoene synthase (Chamovitz et al., 1992). Again, their enzymic activities were determined in preparations of plastids isolated from maize leaves kept in darkness or treated with light for 3 or 5 h (Table IV). When prenyl transferase was assayed, both its activities, which are either FPP synthesis from DMAPP or GGPP synthesis from FPP, were measured. There was no significant increase in the enzyme activity for FPP/GGPP synthase nor for phytoene synthase. However, IPP isomerase was stimulated by a factor of 1.8 and 2.8 when the seedlings were preilluminated for 3 or 5 h, respectively. These increases were significant at the 0.1% level as indicated by a *t* test.

DISCUSSION

Detailed studies of light regulation on carotenogenesis have been carried out mainly with fungi (Bramley and Mackenzie, 1988) and bacteria (Hodgson and Murillo, 1993). In higher plants, light-stimulated biosynthesis of carotenoids is associated with the conversion of etioplast to chloroplast. To understand light stimulation of carotenoid biosynthesis, determination of the level of enzyme activities, before and after greening of maize leaves, was very useful. In contrast to radish and barley (Lichtenthaler, 1975; Lichtenthaler and Becker, 1975), the kinetics of light-dependent increase in carotenoid formation is very fast in maize (Fig. 1; Table I). This minimizes superimposing effects caused by an increasing number of plastids rather than by their stimulated carotenoid-synthesizing activities after illumination. For this reason, most of the values were related to pcv. After 8 h in the light, the maize leaves contain considerably more fibrous material than leaves kept in the dark. This indicates an increase of leaf dry weight and would explain the different stimulation factors for Chl and carotenoid biosynthesis calculated on a dry weight or pcv basis (Table I).

The use of the phytoene desaturase inhibitor norflurazon made it possible to interrupt the biosynthesis at the stage of phytoene and prevent its further metabolism by subsequent

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reaction steps. Phytoene accumulation in the presence of the inhibitor exhibited the same degree of light stimulation as formation of colored carotenoids in untreated plants (Fig. 2). This result demonstrates that light regulation of carotenoid biosynthesis is due to increased activities leading to phytoene synthesis rather than its subsequent metabolism. A closer investigation of the reactions involved in stimulated carotenogenesis was achieved by incorporation of radioactive precursors. Radioactivity from MVA and also from IPP was more readily accumulated in phytoene and carotenes of preilluminated leaves than in the dark control. When both substrates were used the stimulation factors were very similar. Thus, the light-regulation step of this pathway involves IPP conversion.

This conclusion may be challenged by the controversy about the permeability of chloroplasts to MVA; possibly, only IPP can be taken up by the chloroplast (Charlton et al., 1967; Green et al., 1975; Beyer et al., 1980). If MVA has to be converted first to IPP in the cytoplasm before it can enter the plastid, the ^{14}C feeding experiments exclude the plastidic steps of IPP synthesis. Therefore, activities of the enzymes responsible for the steps from MVA to IPP in the plastid (MVA kinase, MVAP kinase, and MVAPP anhydrodecarboxylase) were assayed individually in plastid preparations from illuminated and dark-grown seedlings. The results in Table III demonstrate that all three enzymes were unaffected by light.

Heintze et al. (1990) concluded from their investigations that during leaf development there is a shift from an autonomous sterol biosynthesis in the chloroplast to a synthesis that relies on an external supply of precursors. The conversion of the incorporated exogenously applied precursors MVA and IPP after light stimulation can be calculated to be in the range of 30 to 40% (Table II). This rate is very high, especially since a transfer of the precursors into the chloroplast where carotenogenesis is located has to occur. Obviously, the light-stimulated chloroplasts used in our investigations resemble an intermediate state in which plastidic as well as extraplastidic provision of MVA and/or IPP is operative.

All the enzymes involved in the reaction sequence from IPP to phytoene were assayed for enhanced activities in parallel to increased carotenoid synthesis during illumination (Table IV). The only enzyme stimulated by light was IPP isomerase. The enhancement of light-enhanced carotenoid formations and IPP isomerase activity were very similar (Tables I and IV). Further comparison of its activity and that of prenyl transferase activity showed that the reaction catalyzed by IPP isomerase is the rate-limiting step in this region of the terpenoid biosynthetic pathway (data not shown). One other example of a light-stimulated IPP isomerase is known in *Aspergillus giganteus* (El-Jack et al., 1987). In this fungus IPP isomerase is one of several enzymes of the carotenoid pathway that is involved in light-regulated carotenogenesis.

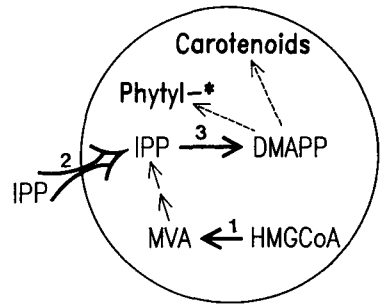

Figure 3. Changes in the plastidic terpenoid metabolism related to increased carotenoid biosynthesis in the course of development to a mature chloroplast: light-dependent transient changes of hydroxymethylglutaryl CoA reductase (1), enhanced supply by exogenous IPP (2), and increased IPP isomerase activity (3).

There are reports in the literature that refer to light-stimulated carotenoid biosynthesis during greening of plants. Wong et al. (1982) showed that the plastidic hydroxymethylglutaryl-CoA reductase activity of pea doubled after etiolated plants were illuminated for 2 h; however, this effect was later reversed. The changes in the activity of this enzyme may be related to the results of Heintze et al. (1990), who demonstrated a shift from autonomy in synthesis of isoprenoids to increased import of IPP into plastids during their development. Increased supply of exogenous IPP to the developing chloroplast requires higher activities of the subsequent rate-limiting enzyme, IPP isomerase. From this point of view the light-enhanced activity of IPP isomerase associated with the development of a functioning chloroplast fits well into the overall model of light regulation of carotenoid biosynthesis presented in Figure 3.

Table IV. *In vitro conversion of radioactively labeled substrates into reaction products of different enzymes involved in the carotenogenic pathway (dpm mg^{-1} protein h^{-1})*

Enzyme preparations were from chloroplasts isolated from 11-d-old etiolated maize seedlings preilluminated as indicated. Incubations were carried out for 2 h with 0.3 to 0.4 mg of protein. The amounts of substrates were equivalent to 0.125 μCi except for phytoene synthase assays, where 0.05 μCi was employed. Values are means of three to eight determinations.

Enzyme (substrate)	Preillumination			Stimulation Factor over 5 h
	0 h	3 h	5 h	
IPP isomerase	11,780 ± 2,986	21,068 ± 4,653	32,706 ± 6,284	2.8
FPP synthase	117,596 ± 33,690	112,892 ± 40,951	135,236 ± 34,606	1.2
GGPP synthase	60,212 ± 26,918	81,467 ± 37,475	66,835 ± 12,030	1.1
Phytoene synthase	10,266 ± 2,388	12,114 ± 3,498	12,320 ± 2,105	1.2

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