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P700 OXIDATION AND ENERGY TRANSFER IN NORMAL MAIZE AND IN CAROTENOID-DEFICIENT MUTANTS

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SUMMARY

1. Particles enriched in System 1 and 2, respectively, were prepared by digitonin fragmentation of chloroplasts from normal maize and from mutants which accumulate lycopene or ζ-carotene instead of the normal carotenoids and which have a reduced chlorophyll content. Although both mutants had a relatively high carotenoid content in relation to chlorophyll, the System-1 particles showed about the same carotenoid to chlorophyll ratio as particles from normal maize Particles from the lycopene mutant showed approximately a normal ratio of light harvesting to reaction center chlorophyll (P700). In the ζ-carotene mutants the amount of light harvesting chlorophyll was somewhat reduced, both in relation to the number of System-1 reaction centers and to the amount of protein.

2. Action spectra for P700 oxidation indicate a 30–50 % efficiency of energy transfer from carotenoids to chlorophyll in System-1 particles of normal maize, increasing with increasing chloroplast development during greening. With the lycopene mutant the transfer efficiency was 30–50 %. In the ζ-carotene mutant the transfer efficiency from ζ-carotene was estimated to be somewhat higher The quantum efficiencies for P700 oxidation for light absorbed by chlorophyll *a* by System-1 particles from mutant maize were about twice as low as for normal maize

3. With normal maize, after prior etiolation, the formation of functional System-1 reaction centers lags behind the synthesis of chlorophyll in the early phases of greening. Approximately at the time of beginning of granum formation, when about 20 % of the chlorophyll had been synthesized, the ratio of chlorophyll to P700 and the distribution of chlorophyll between the two pigment systems was found to reach a constant value, suggesting a close relation between the regulatory mechanisms for these phenomena and the development of the photosynthetic membrane system.

INTRODUCTION

The role of carotenoids in photosynthesis is only partly understood. Numerous studies (see ref. 1) suggest that, at least in algae and higher plants, carotenoids are

Abbreviation: P700, reaction center pigment absorbing at about 700 nm

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essential for the development of the normal photosynthetic structure and function. Absence of carotenoids or a strongly reduced carotenoid content have been found to be associated with an abnormal development of chloroplast structure, increased photosensitivity, and impaired photosynthesis. Action spectra of chlorophyll fluorescence and of photosynthesis have shown that energy transfer from carotenoids to chlorophyll occurs with an average efficiency of 30–50 % (ref. 2). More recently it has been found that this relatively low number can be explained by the fact that only β-carotene transfers energy with high efficiency, and that xanthophylls are inactive[3,4]. In dark grown leaves of higher plants the main carotenoid is lutein[5], while an extensive accumulation of β-carotene only occurs during greening. Experiments on detergent fractionation indicate that most of the β-carotene is associated with Photosystem I (refs. 6 and 7).

This paper reports the results of a study on pigment content, photochemistry and energy transfer with particles obtained by digitonin fractionation of chloroplasts from normal and etiolated maize and from carotenoid-deficient mutants, which accumulate lycopene and ζ-carotene instead of the normal carotenoids Amongst other things, the experiments indicate that, although the chlorophyll content of the mutants was strongly reduced, the presence of β-carotene is not essential for the normal functioning of the primary photochemical reaction of System I. System-I particles prepared from the mutants were largely similar in properties to those from normal maize. Action spectra for photo-oxydation of P700, the reaction center pigment absorbing at about 700 nm, showed energy transfer from carotenoids to chlorophyll *a* (with less than 100 % efficiency) in System-I particles from normal and mutant plants. The efficiency was found to increase gradually during greening.

MATERIALS AND METHODS

Material

Chloroplasts were prepared from 7–9-day-old leaves of normal, lycopene and ζ-carotene seedlings of maize. The seed material was a segregation population of inbred heterozygotes for lycopene and ζ-carotene genes[8]. In some experiments with etiolated plants (when indicated), the commercial variety Caldera 561 was used. Leaves in various stages of greening after prior etiolation were produced by growing the seedlings in complete darkness during 6–8 days followed by an illumination of 25 lux (Phillips, Holland, No. 33 daylight fluorescent tubes) for various periods of time. For a comparison of chloroplasts from normal and from mutant plants the seedlings were grown continuously at 25 lux.

Preparation of chloroplasts particles

Chloroplasts were isolated by grinding the leaves in the medium given by AVRON[9]: 0.4 M sucrose, 0.05 M Tris, 0.01 M NaCl, 0.02 M sodium ascorbate supplemented by 0.005 M of cysteine·HCl, in order to avoid the effects of tannins in the homogenate[10]. The pH was 7.8. The chloroplasts were collected by centrifugation at 3000 × *g* for 20 min and resuspended and washed thoroughly in the medium of ANDERSON AND BOARDMAN[11]: 0.05 M phosphate buffer (pH 7.2) containing 0.3 M sucrose and 0.01 M KCl. Fragmentation of chloroplasts was carried out by a 30-min treatment with 0.5 % digitonin (Roger R. Brunschwig, Amsterdam) at 0°. Non-

fragmented chloroplasts were removed by centrifugation at 3000 × *g* for 20 min. In the case of mature chloroplasts this sediment contained 25–30 % of the total chlorophyll. With etiolated leaves the percentage of the chlorophyll recovered in non-fragmented chloroplasts varied between 10 to 30 %, increasing with progressing development Aliquots of the supernatant were taken as samples representing the bulk of fragmented chloroplasts. The remainder was subjected to serial centrifugations to obtain fractions sedimenting at different speeds. Sediments were resuspended in 0.05 M phosphate buffer (pH 7.2) containing 0.01 M KCl. Absorption spectra were recorded with a Cary Model 14R spectrophotometer equipped with a scattered transmission accessory

Pigment determination

Pigments were extracted with acetone and transferred to ethyl ether. Chlorophyll *a* and *b* contents were calculated from the absorbances measured at 662 and 644 nm, respectively[12]. Carotenoid content of normal chloroplasts was calculated on basis of the extinction coefficient (ε_{mM}) of β-carotene of 124 $mM^{-1} \cdot cm^{-1}$ at 480 nm. Lycopene content was estimated at 485 nm ($\varepsilon_{mM} = 187$) The amount of ζ-carotene was measured at 400 nm ($\varepsilon_{mM} = 124$), correcting for the absorbance of chlorophyll *a* at this wavelength.

P700 measurements

Spectra of light-induced absorbance changes were determined with a difference spectrophotometer described earlier[13]. The amounts of P700 in the various preparations were calculated from the decrease of absorbance around 700 nm upon illumination with blue light (410–490 nm) of saturating intensity. An extinction coefficient (ε_{mM}) for P700 of 73 $mM^{-1} \cdot cm^{-1}$ at the maximum of about 700 nm was applied, the same as that of chlorophyll *a* at 662 nm in 80 % acetone.

Action spectra of P700 oxidation were measured by comparing at each wavelength the amount of P700 oxidized at a certain light intensity with a response *versus* intensity curve made at 430 nm. The actinic light was provided by an Osram 900-W Xenon lamp in combination with a Bausch and Lomb 500-mm monochromator. Stray light was reduced by means of Schott BG12 or BG38 filters. Light intensities were measured with a vacuum photocell, calibrated by means of a thermopile. Quantum efficiencies for P700 oxidation were calculated from the initial rates of absorbance decrease at about 700 nm upon illumination

2,3,5,6-Tetramethyl-*p*-phenylenediamine was a kind gift from Dr A. Trebst, Gottingen All measurements were done at room temperature (approx 22°).

Protein determination

Aliquot samples of suspensions containing chloroplasts or chloroplast fragments were precipitated with trichloroacetic acid to a final concentration of 5 %. After the precipitate had been allowed to settle for 30 min at 0–5°, it was collected by centrifugation at 5000 × *g* for 10 min The precipitate was washed three times with water. After a wet combustion its nitrogen content was determined by the Nessler method[14].

RESULTS AND DISCUSSION

Comparison of particles obtained from normal and mutant maize

Table I shows the distribution of chlorophyll *a* and chlorophyll *b*, carotenoids, P700 and protein in fractions obtained from normal and mutant chloroplasts by digitonin fragmentation.

For normal and lycopene maize both the chlorophyll *a* and chlorophyll *b* and the chlorophyll *a* + *b*/P700 ratio (columns 2 and 4) indicate a partial separation of Systems 1 and 2 by digitonin treatment with a separation efficiency similar to that obtained with spinach chloroplasts[11,15]. With the ζ-carotene mutant the chlorophyll *a* + *b*/P700 ratio similarly indicates a separation However, here the chlorophyll *a*/chlorophyll *b* ratio was high in the non-fractionated chloroplasts and not much different in the various fractions, which suggests that System 2 in this mutant either contains little chlorophyll *b* or is little developed.

For normal maize, the distribution of chlorophyll in the various fractions was somewhat different from that reported for spinach chloroplasts[11] where more than half of the amount of chlorophyll originally present was found in heavy particles and only 10–15 % was recovered in the 144000 × *g* fraction. This is probably due to differences between species and also to the low intensity of illumination which is optimal for the chlorophyll formation of the mutants but low for the normal leaves. In lycopene and ζ-carotene chloroplasts there was an increased percentage of chlorophyll in the supernatant. Only a small fraction of chlorophyll was found in the large fragments. This might suggest a deficiency of System-2 formation, but may also reflect a reduced stability to withstand detergent solubilization The shape of the low-temperature emission spectra suggests the presence of System-2 pigment in both mutants[16].

Both mutants, but especially the ζ-carotene mutant, showed a relatively high carotenoid content in non-fractionated chloroplasts and in the large particle fraction. However, the chlorophyll/carotenoid ratio in the small particles was roughly the same for mutant and normal chloroplasts Preliminary analyses suggest that a few percent of the total carotenoid content of lycopene chloroplasts consists of β-carotene, which seems to be mainly associated with the 144000 × *g* sediment. No carotenoids other than ζ-carotene were detected in non-fractionated chloroplasts and small fragments prepared from the ζ-carotene mutant.

P700 contents related to the amount of chlorophyll were similar for normal and lycopene chloroplasts, while chloroplasts from the ζ-carotene mutant contained considerably more P700. In the small fragments from ζ-carotene chloroplasts the relative amount of P700 was also higher than in the corresponding normal particles, whereas the light fraction of lycopene chloroplasts contained somewhat less P700. Small particles of normal and lycopene chloroplasts had about the same protein/chlorophyll ratio. For the ζ-carotene mutant this value was roughly three times higher. Protein/chlorophyll ratios were high in the 144000 × *g* supernatants, which contained the soluble and solubilized proteins and presumably also the chloroplastic ribosomes

Light-induced difference spectra for the small particle fraction from normal and mutant maize are given in Fig. 1. All spectra showed the characteristic shape for the bleaching of P700. One of the main differences was in the location of the far-red band which was at 700 nm for the normal material, but at somewhat shorter wavelength

TABLE I

PIGMENT AND PROTEIN CONTENT OF CHLOROPLAST FRACTIONS FROM NORMAL, LYCOPENE AND ζ-CAROTENE MAIZE

The data are average values of 3–5 experiments with different samples The average chlorophyll *a* + *b* contents of the leaves used in these experiments were 550 nmoles per g fresh weight for the normal, 182 nmoles per g fresh weight for the lycopene mutant and 86 nmoles per g fresh weight for ζ-carotene mutant leaves P700 measurements were carried out in the presence of $1 \cdot 10^{-6}$–$4 \cdot 10^{-6}$ M *N*-methylphenazonium methosulphate or $1.2 \cdot 10^{-5}$ M 2,3,5,6-tetramethyl-*p*-phenylenediamine and $3.6 \cdot 10^{-4}$ M ascorbate

Material	*Chlorophyll a + b* (%)	*Chlorophyll a* / *Chlorophyll b*	*Chlorophyll a + b* / *Carotenoid(s)*	*Chlorophyll a + b* / *P700*	*Protein** / *Chlorophyll a*
Normal maize					
Fragmented, non-fractionated chloroplasts	100	3.4	4.9	279	2.1
10000 × *g* sediment	35	2.5	4.7	418	0.9
50000 × *g* sediment	18	3.1	5.1	263	1.2
144000 × *g* sediment	36	6.0	5.2	142	2.6
144000 × *g* supernatant	11	3.1	2.2	251	12.2
Lycopene mutant					
Fragmented, non-fractionated chloroplasts	100	3.2	2.9	280	3.8
10000 × *g* sediment	14	2.3	1.2	435	2.2
50000 × *g* sediment	11	3.1	4.6	243	3.0
144000 × *g* sediment	33	5.8	7.1	179	3.0
144000 × *g* supernatant	42	3.0	4.8	269	26.0
ζ-Carotene mutant					
Fragmented, non-fractionated chloroplasts	100	5.7	0.9	148	12.9
10000 × *g* sediment	6	4.4	0.2	265	16.0
50000 × *g* sediment	8	4.4	0.8	188	14.8
144000 × *g* sediment	39	4.8	6.2	92	9.1
144000 × *g* supernatant	47	6.6	1.8	164	13.0

* Expressed in matoms of protein nitrogen per mmole of chlorophyll *a*.

for both mutants, apparently reflecting differences in structural development of the chloroplast. With the lycopene mutant, the main red band was clearly broadened, compared to the other spectra, possibly by the occurrence of slightly different types of reaction centers with overlapping bands of P700.

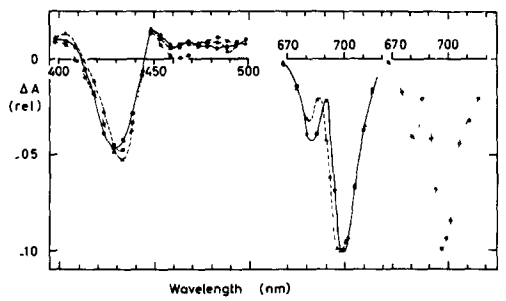

Fig 1 Difference spectra obtained by illumination of a suspension of 144000 × *g* particles of normal (●—●), lycopene (▲---▲), and of ζ-carotene mutant maize (○·····○) in the presence of $6 \cdot 10^{-4}$ M 2,3,5,6-tetramethyl-*p*-phenylenediamine and $1\,8 \cdot 10^{-3}$ M ascorbate Illumination by red or blue light of saturating intensity The spectra have been normalized in the height of the red band in order to facilitate comparison

Action spectra and quantum yield of P700 oxidation

Action spectra for P700 oxidation of 144000 × *g* particles of normal, lycopene and ζ-carotene chloroplasts are presented in Figs. 2–4. For all three preparations the action spectra indicate transfer of energy from carotenoids to chlorophyll *a* in effecting P700 oxidation. With particles prepared from normal and lycopene maize the efficiency of energy transfer can be estimated to be 30–50 % by comparison of the action and absorption spectra of these particles.

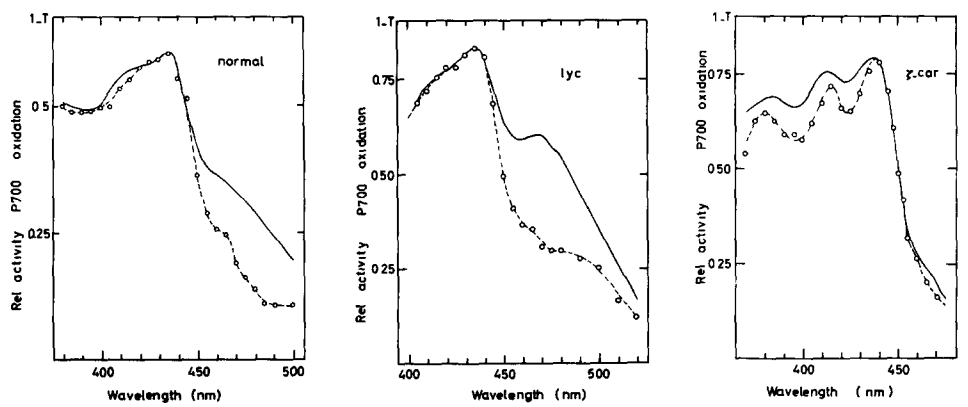

Fig 2. Action spectrum for P700 oxidation (○---○) and absorption (1 − *T*) spectrum (———) of a suspension of 144000 × *g* particles from normal maize in the presence of 2,3,5,6-tetramethyl-*p*-phenylenediamine and ascorbate

Fig 3. Action spectrum for P700 oxidation (○---○) and absorption spectrum (———) of 144000 × *g* particles from lycopene mutant maize.

Fig. 4. Action spectrum for P700 oxidation (○---○) and absorption spectrum (———) of 144000 × *g* particles from ζ-carotene mutant maize.

The action spectrum for the ζ-carotene particles, which clearly shows the characteristic bands of ζ-carotene at 375 and 415 nm, indicates efficient energy transfer from ζ-carotene to chlorophyll *a*. Apparently the ζ-carotene is located in close proximity to the chlorophyll, probably within the photosynthetic membrane. A quantitative estimate of the transfer efficiency (perhaps 60–80 %) is difficult because of the overlapping absorption of chlorophyll *a* and ζ-carotene in this region. The location of the absorption spectrum of ζ-carotene would favor energy transfer to the soret band of chlorophyll *a* which might enhance the probability of transfer as compared to that from carotenoids absorbing at longer wavelength.

Quantum efficiencies for P700 oxidation in the 144000 × *g* sediment fraction from chloroplasts of normal and mutant maize were measured in actinic light of 430 nm The light intensity varied between $5 \cdot 10^{-11}$ and $20 \cdot 10^{-11}$ Einstein·cm^{-2}·sec^{-1}, the quantum efficiencies obtained were independent of the intensity in this region. With small particles from normal material a quantum efficiency of 0.5 molecule/*hv* was measured Similar numbers (0 3–0.7) have been reported for the corresponding particles from spinach[17] and for intact blue-green and red algae[18,19]. Quantum efficiencies for P700 oxidation by small particles prepared from the mutants were about half as high: 0.20 and 0 26 for lycopene and ζ-carotene particles, respectively These lower quantum efficiencies, apparently due to a loss of energy in chlorophyll not associated with the reaction center of System 1, are possibly caused by a lower resistance of the structure to digitonin treatment. With the ζ-carotene mutant an alternative explanation, in line with the low chlorophyll/P700 and chlorophyll/protein ratio, would be loss of energy due to loose packing of the chlorophyll and a corresponding higher average distance between chlorophyll molecules.

P700 and energy transfer in chloroplasts from greening leaves

Digitonin fractionation was also applied to chloroplasts prepared from leaves of normal maize at various stages of greening after prior etiolation. In some respects the characteristics of chloroplasts from greening leaves were found to be similar to those prepared from the carotenoid mutants. In the early phases of greening most of the chloroplast was recovered in the 144000 × *g* sediments and supernatant, and

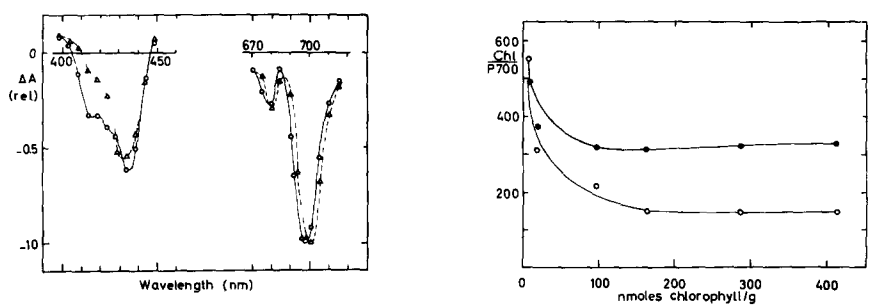

Fig. 5. Difference spectra (normalized) of 144000 × *g* particles from greening leaves of maize (variety Caldera 561), after 6 h (○—○) and 48 h (△---△) of illumination after prior etiolation Further experimental conditions were the same as for Fig 1

Fig 6 Relative amounts of chlorophyll *a* + *b*/P700 in fragmented, non-fractionated chloroplasts (●—●) and in particles sedimenting at 144000 × *g* (○—○) from leaves at different stages of greening The measurements were done after 3, 6, 9, 12, 24 and 48 h of illumination after prior etiolation The abscissa gives the chlorophyll *a* + *b* content per g of fresh weight leaf material

the light-induced absorption difference spectrum showed a similar shift of the red maximum of P700 (Fig. 5) as was observed with the mutants. The quantum efficiency for P700 oxidation by 144000 × *g* particles in light of 430 nm was low initially and increased from 0.15 molecule/*hv* after 9 h to 0.25 after 48 h of illumination

Fig 6 shows the relative amounts of P700 in non-fractionated chloroplast preparations and in particles sedimenting at 144000 × *g* At the beginning of the greening process the formation of P700 lagged behind the synthesis of chlorophyll, and the 144000 × *g* sediment was not enriched in P700. After about 10–12 h of light, when about 20% of the chlorophyll present in light-grown leaves had been synthesized, the chlorophyll/P700 ratio stopped decreasing and stayed at remarkably constant level during the further phases of greening, both in the unfractionated chloroplasts and in the small particle fraction. This stage approximately coincides with the onset of granum formation[20], which suggests that the mechanisms that regulate the formation of reaction centers in a fixed proportion to the synthesis of light harvesting chlorophyll, and the distribution of pigments between the two pigment systems are structurally coupled to the development of the normal photosynthetic membrane system.

The efficiency spectra for P700 oxidation in small particles (Fig 7) showed an increasing efficiency of carotenoid participation during greening. Similar effects have been observed for the action spectra of chlorophyll fluorescence of etiolated bean leaves[3, 21].

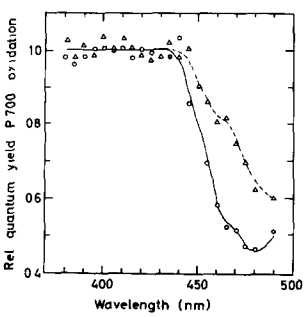

Fig 7. Relative efficiency per absorbed quantum as a function of wavelength for P700 oxidation by 144000 × *g* particles from greening maize leaves; O—O, after 9 h of illumination, Δ---Δ, after 48 h of illumination

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