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The use of continuous assays to characterize the oxidative cyclase that synthesizes the chlorophyll isocyclic ring

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1. A continuous spectroscopic assay has been developed for magnesium protoporphyrin monomethyl ester oxidative cyclase, which records either the dark formation of both free and protein-bound magnesium phaeoporphyrin or, following flash illumination, its corresponding chlorin. 2. The properties of the enzyme were studied in wheat etioplasts. 3. When plastids were pre-illuminated in the presence of NADPH all endogenous protochlorophyllide was converted into chlorophyllide and the product of dark incubation with magnesium protoporphyrin monomethyl ester was protein-bound magnesium 2-vinyl phaeoporphyrin a_5 monomethyl ester with either a vinyl or an ethyl group at position 4 of the macrocycle alone. 4. Rates of chlorin production from magnesium protoporphyrin monomethyl ester (up to 1240 pmol/h per mg of protein) were adequate to support known rates of plant chlorophyll synthesis. 5. The enzyme required NADPH and O_2 and had an approximate K_m of 0.5 μM for magnesium protoporphyrin IX monomethyl ester. Lipid-soluble metal-complexing agents inhibited enzyme activity: hydrophilic agents were ineffective. The strong inhibition of mycobactin suggested the involvement of iron ions. 6. Zinc protoporphyrin monomethyl ester, but not copper or nickel or metal-free protoporphyrin monomethyl esters, was a substrate; magnesium protoporphyrin dimethyl ester was inhibitory. 7. The activity of the enzyme was unchanged by prior greening of the plants. 8. The activity in isolated etioplasts was very dependent upon intactness of the plastid structure.

INTRODUCTION

Studies on chlorophyll synthesis with mutants of higher plants and photosynthetic bacteria (Jones, 1979) have suggested that the isocyclic ring of the chlorophyll nucleus is formed from the biosynthetic intermediate magnesium protoporphyrin IX monomethyl ester (MPE; see Scheme 1). The enzyme(s) involved in this reaction has (have) been detected in etioplasts of cucumber by Castelfranco and co-workers and called magnesium protoporphyrin IX monomethyl ester oxidative cyclase (Chereskin *et al.*, 1982).

They found that the production of the magnesium phaeoporphyrin, measured fluorimetrically following organic-solvent extraction of the assay mixture, was stimulated by the addition of NADPH and *S*-adenosylmethionine and required the presence of O_2. Fractionation and reconstitution studies demonstrated both membrane and soluble components involved in the process.

We have developed continuous assay methods for monitoring this enzyme activity that have the advantage of permitting observation of any transient intermediates in the reaction and avoiding laborious extraction of incubation samples. In the present paper we describe the development of such assay methods and describe the properties of the enzyme system found in wheat etioplasts, showing that they differ in some respects from those reported by the Castelfranco group.

MATERIALS AND METHODS

Preparation of wheat etioplasts

Seeds of wheat (*Triticum sativum* var. Avalon) supplied by British Seed Houses (Avonmouth, U.K.) were washed free of fungicide in running water, planted in Levington's compost (Fisons, Harston, Cambridge, U.K.) and grown in complete darkness for 7 days. Shoots were harvested and etioplasts prepared with illumination from a green safety light as previously described (Griffiths, 1975).

Porphyrins

Magnesium protoporphyrin monomethyl ester was prepared by extraction of the growth medium of the bacteriochlorophyll-less mutant N6 of *Rhodopseudomonas sphaeroides*, isolated by Dr. C. N. Hunter (Imperial College, London, U.K.). Magnesium 2,4-divinyl phaeoporphyrin a_5 monomethyl ester (DVPChlide) was similarly prepared from mutant V3 of *Rps. sphaeroides* (Jones & Saunders, 1972). Zinc, copper and nickel protoporphyrin monomethyl esters were prepared from protoporphyrin monomethyl ester by refluxing with the appropriate metal acetate in acetic acid (Smith, 1975, pp. 795–811) and purified by t.l.c. (see below). Magnesium protoporphyrin dimethyl ester was prepared from protoporphyrin dimethyl ester (Smith, 1975, p. 796).

Abbreviations used: MPE, magnesium protoporphyrin IX monomethyl ester; PChlide, magnesium 2-vinyl phaeoporphyrin a_5 monomethyl ester with either a vinyl or an ethyl group at position 4 of the macrocycle; DVPChlide, magnesium 2,4-divinyl phaeoporphyrin a_5 monomethyl ester.

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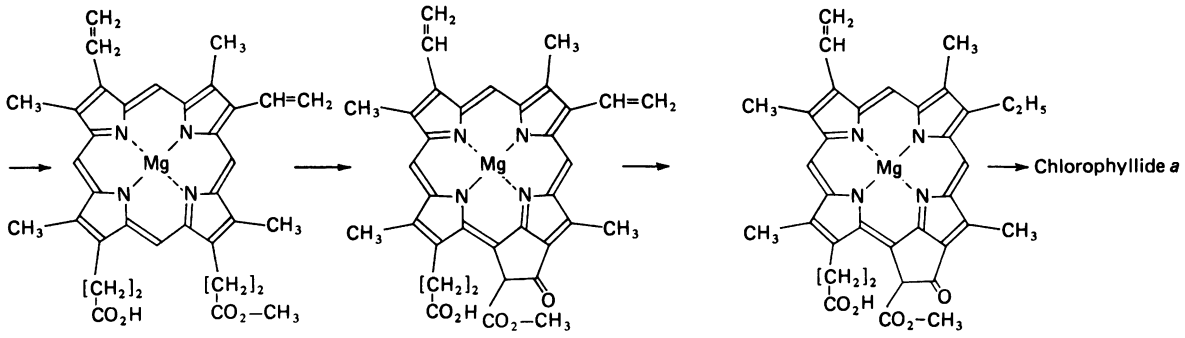

Magnesium protoporphyrin monomethyl ester | Magnesium 2,4-divinyl phaeoporphyrin a_5 monomethyl ester | Magnesium 2-vinyl phaeoporphyrin a_5 monomethyl ester

Scheme 1. Scheme for chlorophyll biosynthesis: biosynthetic sequence from magnesium protoporphyrin monomethyl ester (MPE) to chlorophyllide *a* (Chlide), via magnesium divinyl phaeoporphyrin a_5 monomethyl ester (DVPChlide) and magnesium 2-vinyl phaeoporphyrin a_5 monomethyl ester (PChlide)

Spectroscopic properties in methanol were as follows:

	MPE	DVPChlide	PChlide	Chlide
$\lambda_{max.}$ (nm)	589	623	623	663
ϵ_{mM} ($mM^{-1} \cdot cm^{-1}$)	18	36	36	91

T.l.c.

Metalloporphyrins were purified on 0.5 mm preparative thin layers of Silica gel 60H (Merck, Darmstadt, Germany) developed with toluene/ethyl acetate/aq. 95% (v/v) ethanol (4:1:1, by vol.). Elution of the material from the absorbent was achieved with aq. 10% (v/v) methanol after which the pigment was transferred into diethyl ether, washed several times with water, dried over NaCl and finally concentrated by evaporation under N_2.

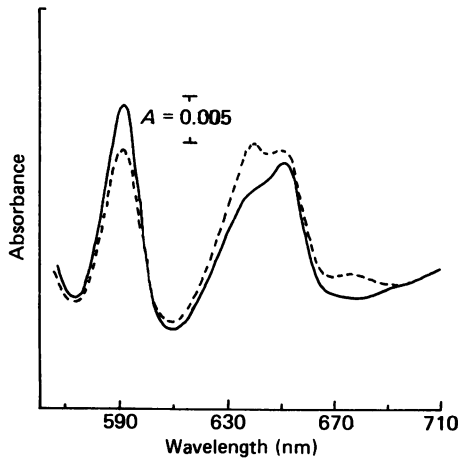

Fig. 1. Conversion of MPE by non-illuminated etioplasts

Freshly prepared wheat etioplasts (approx. 1.2 mg of protein) were added to 1.2 ml of isolation buffer supplemented with 2 mM-ATP and 0.5 mM-NADPH (as regenerating system) and the mixture was divided between two cuvettes. MPE (2.5 μmol in 5 μl of methanol) was added to the sample cuvette and the difference spectrum was recorded immediately (——) and after 45 min incubation in darkness (----).

Stock (approx. 500 μM) solutions of porphyrins for enzyme studies were prepared by dissolving them in methanol.

Etioplast incubation conditions

Incubation of etioplasts resuspended in isolation buffer (0.5 M-sucrose/2 mM-$MgCl_2$/20 mM-Tes/20 mM-Hepes buffer, pH 7.8) was carried out in thermostatically controlled glass cuvettes maintained at 21 °C with additions made as indicated.

Spectroscopy

The dual-wavelength spectrophotometer used in this work was fitted with a xenon flash actinic light, masked with a filter of saturated $CuSO_4$ solution, and the photomultiplier was protected with a Kodak Wratten filter no. 70. Conditions for the light-flashing regime were as described by Griffiths (1978) for the assay of protochlorophyllide reductase, i.e. the light-dependent formation of chlorophyllide absorbing at around 678 nm is measured.

The scanning spectrophotometer used here was described by Cross *et al.* (1984, 1985). Spectra were averaged and stored in an Apple IIE computer, and difference spectra were displayed as required.

RESULTS

Development of assay

Magnesium protoporphyrin monomethyl ester in organic solvents has characteristic strong absorption bands in the visible region at around 550 and 590 nm (Granick, 1951), whereas DVPChlide absorbs maximally in the red region of the spectrum at around 624 nm (Jones, 1969). The spectroscopic changes observed during the course of an incubation of isolated etioplasts are shown in Fig. 1. The etioplasts, supplemented with MPE, display prominent absorption bands (Fig. 1) at

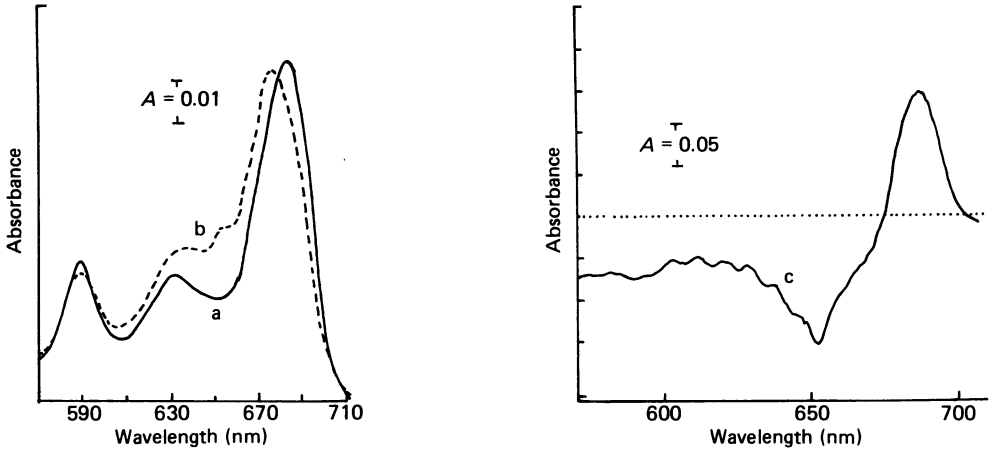

Fig. 2. Conversion of MPE by illuminated etioplasts and photoactivity of the product

Wheat etioplasts suspended and supplemented as indicated in Fig. 1 legend were first illuminated for 30 s, and the spectrum was recorded immediately after the addition of MPE (spectrum a) and 60 min later (spectrum b). The incubation sample was finally illuminated for 30 s and the light-minus-dark difference spectrum was recorded (spectrum c).

590 nm due to the added substrate and at approx. 634 and 650 nm due to the endogenous free and protochlorophyllide reductase-bound PChlide respectively. After incubation in darkness for 45 min a build-up of the free form of PChlide is seen associated with utilization of the added MPE by the cyclase enzyme system in the etioplast preparations (Fig. 1).

This experiment was repeated but after pre-illumination of the etioplasts supplemented with 1.5 μM-NADPH to convert all the endogenous pigment into the corresponding chlorophyllide absorbing maximally at approx. 676 nm (Fig. 2, spectrum a). On addition of MPE and incubation for 60 min in darkness the absorption of the latter at 590 nm diminishes as before, but now the product absorbs maximally at approx. 650 nm (Fig. 2, spectrum b) rather than 634 nm, indicative of protochlorophyllide reductase-bound PChlide. Subsequent illumination of this sample monitored by plotting the light-minus-dark difference spectrum (Fig. 2, spectrum c) confirms the photoactivity of the product, as shown by the decrease in absorption at 650 nm and increase at approx. 676 nm accompanying the illumination. This experiment demonstrates that the products of the cyclase reaction in the etioplasts are in a suitable form and location to act as substrates for protochlorophyllide reductase.

The information thus gained was used in designing the continuous dual-wavelength assay of cyclase activity shown in Fig. 3. The etioplast suspension supplemented with NADPH was first flash-illuminated to convert all endogenous PChlide into chlorophyllide, MPE ester was then added and flash illumination was continued. As the DVPChlide was formed it was converted by the flash into the corresponding chlorophyllide, absorbing at 676 nm. Assuming that the molar absorption coefficient of the product was 91 $mM^{-1} \cdot cm^{-1}$ (Griffiths, 1975) and knowing that the activity of the protochlorophyllide reductase activity was not limiting under these conditions, the rate of cyclase activity could be calculated. Rates for different etioplast preparations varied from 300 to 1240 pmol of magnesium phaeoporphyrin formed/h per mg of protein.

Another quantitative measurement of the cyclase activity was obtained when non-illuminated etioplasts were incubated with substrates (MPE and NADPH) in darkness in a scanning spectrophotometer. The disappearance of substrate and product accumulation can be clearly seen in scans of the difference in absorption between the incubation mixture at zero time and the substrate-supplemented mixture after various periods in darkness (Fig. 4). Utilization of the substrate is accompanied by accumulation of a product absorbing at approx. 634 nm. Assuming a molar absorption coefficient of 35 $mM^{-1} \cdot cm^{-1}$ for the formed magnesium phaeopor-

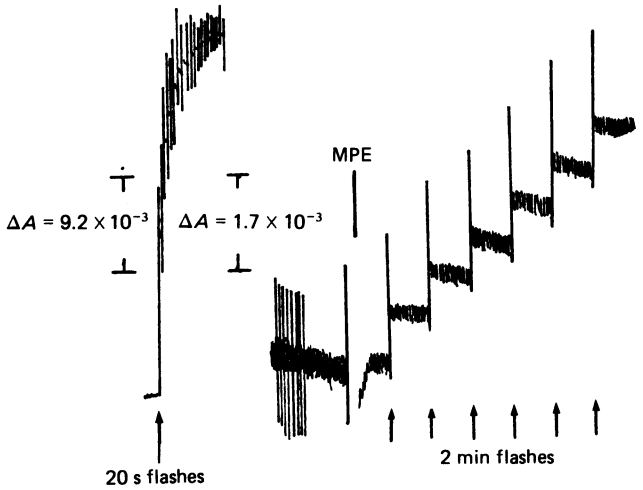

Fig. 3. Continuous coupled assay of MPE cyclase activity in wheat etioplasts

Freshly isolated wheat etioplasts (0.9 mg of protein) were added to 2.5 ml of isolation buffer containing 2 mM-ATP and 0.5 mM-NADPH and flash-illuminated while absorbance changes at 678 minus 710 nm (ΔA) were measured in a dual-wavelength spectrophotometer. The initial flashing at 1 flash/20 s converts endogenous PChlide into the corresponding chlorophyllide, after which 5 μmol of the substrate MPE was added and the flash rate changed to 1 flash/2 min and the sensitivity increased during the subsequent incubation.

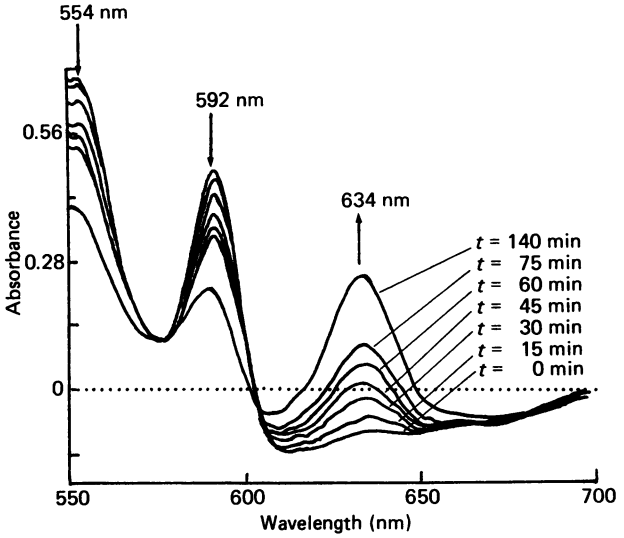

Fig. 4. Spectrophotometric assay of MPE cyclase in non-illuminated wheat etioplasts

The absorbance of a freshly isolated wheat etioplast preparation was recorded exactly as indicated in Fig. 1 legend and the spectrum stored, MPE (5 μM) was then added and the absorbance re-recorded after various periods of incubation in darkness. Finally the absorbance differences between these and the initial spectrum were plotted. The changes in direction of absorbance occurring at various wavelengths during the incubation are indicated by the arrows.

phyrin (see Jones, 1969), rates of cyclase activity could be estimated from these spectra. Such values agreed within 10% with those obtained simultaneously by the continuous flash assay procedure. Again, shoulders could also be clearly seen on the long- and short-wavelength sides of the 634 nm maximum in dark incubations (Fig. 4), suggesting that some spectroscopically distinct intermediates were also formed. These shoulders were less pronounced at the end of the incubation (Fig. 4).

Properties of the cyclase

The requirements of the cyclase for added nucleotide is shown in Table 1. NADPH was strongly stimulatory, whereas NADH, NAD^+ and $NADP^+$ increased the rates only slightly over the endogenous rate. ATP caused some slight increase in activity, but *S*-adenosylmethionine had no effect.

The response of the cyclase to changes in MPE concentration are shown in Fig. 5. Although accurate determination of K_m was not possible, the estimated value of 0.5 μM is rather lower than that found by Chereskin *et al.* (1982).

The cyclase reaction involves oxygenation of the type catalysed by a cytochrome *P*-450 system. However, CO, an inhibitor of cytochrome *P*-450 systems, had no inhibitory effect (Table 2). O_2 was necessary for activity. Very low concentrations of Triton X-100 completely abolished cyclase activity (Table 2), suggesting that the integrity of the etioplast was very important. This was confirmed with a variety of disruption methods, including detergents, sonication or hypo-osmotic lysis: all abolished activity. This apparent requirement for intactness makes the utilization of added NADPH

Table 1. Effects of cofactors on cyclase activity in wheat etioplasts

Etioplasts containing approx. 3 mg of protein were resuspended in 1.0 ml of etioplast isolation medium and supplemented with MPE to 5 μM and the cofactors as listed. Rates of cyclase activity were assessed from the absorption spectra recorded after various time intervals.

Cofactor	Cyclase activity (pmol of phaeoporphyrin formed/h per mg of protein)
None	258
NADPH (1 mM)	699
NADH (1 mM)	325
$NADP^+$ (1 mM)	335
NAD^+ (1 mM)	381
ATP (1 mM)	320
S-Adenosylmethionine (0.5 mM)	265

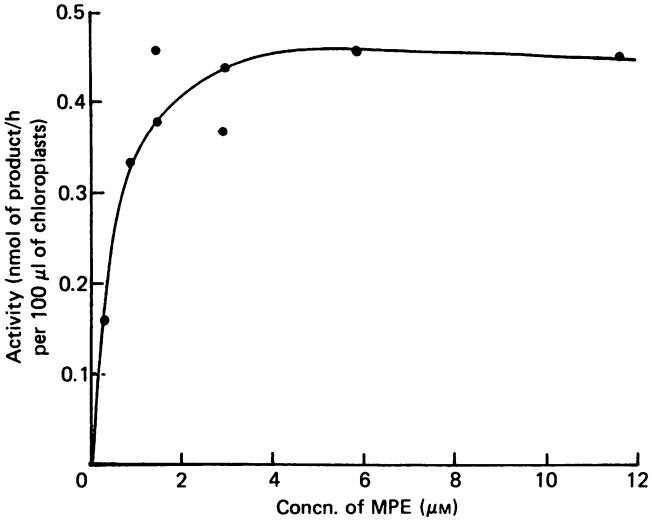

Fig. 5. Effect of substrate (MPE) concentration on cyclase activity in wheat etioplasts

Freshly isolated wheat etioplasts were incubated as described in Fig. 4 legend but with various concentrations of added MPE. Cyclase activity was calculated from the recorded spectra as described in the text.

surprising and suggests that the site of reduction is the outer face of the plastid.

The addition of chelating agents to the assay gave interesting results. 8-Hydroxyquinoline was an effective inhibitor, but its water-soluble non-permeant analogue 8-hydroxyquinolinesulphonate was completely ineffective over the same concentration range (Table 2). This suggests that the site of metal involvement is within the etioplast matrix or within the membrane. EDTA, which has a range of metal-chelating specificity similar to that of 8-hydroxyquinoline, was a relatively poor inhibitor (20% at 10 mM), but the iron-specific and lipid-soluble complexing agent mycobactin (Snow, 1970) gave complete inhibition at 500 μM. Desferrioxamine (1 mM), which is iron-specific but water-soluble, gave only 15–20% inhibition.

Table 2. Inhibitors of the cyclase in wheat etioplasts

Wheat etioplasts (approx. 2 mg of protein) were incubated in the presence of MPE and NADPH as described in Fig. 3 legend. Cyclase activity was determined in the presence of the inhibitors listed as described in the Materials and methods section. The results are expressed as percentage inhibitions of the rate obtained in the absence of any inhibitor.

Treatment	Inhibition (%)
Triton X-100 (5 μM)	100
CO	0
Omission of O_2	100
EDTA (10 mM)	18
8-Hydroxyquinoline (2 mM)	95
8-Hydroxyquinolinesulphonate (10 mM)	0
Mycobactin (0.5 mM)	100
Desferrioxamine (1 mM)	17

Table 3. Porphyrin-specificity of the cyclase

Etioplasts were incubated in the isolation buffer in the presence of 1 mM-NADPH with the pigments listed added at approx. 5 μM. Cyclase activity was monitored spectroscopically as outlined in the Materials and methods section.

Pigment	Cyclase activity (pmol/h per mg of protein)
Mg protoporphyrin IX ester	450
Zn protoporphyrin IX monomethyl ester	420
Ni protoporphyrin IX monomethyl ester	0
Cu protoporphyrin IX monomethyl ester	0
Protoporphyrin IX monomethyl ester	0

Porphyrin specificity of cyclase activity

A number of porphyrins and metalloporphyrins were assayed as alternative substrates or inhibitors of the cyclase (Table 3). Only zinc protoporphyrin monomethyl ester was effective as a substrate. The metal-free porphyrin showed no activity. Both zinc and magnesium phaeoporphyrins are known to act as substrates for protochlorophyllide reductase (Griffiths, 1980), indicating that the similarity of the co-ordination chemistry of magnesium and zinc porphyrins results in their similar binding to enzyme active sites. The mono- and di-methyl esters of the metal-free protoporphyrin IX at concentrations up to 5 μM had no effect on the MPE-dependent cyclase activity. Similarly protohaem at up to 10 μM showed no inhibition. Magnesium protoporphyrin dimethyl esters, however, proved inhibitory, with 5 μM resulting in complete inhibition.

Effect of light-induced chloroplast development *in vivo* on cyclase activity

Wheat shoots were illuminated for 10 h to permit the development of fully pigmented chloroplasts. Plastids were prepared from such tissue in the usual way and assayed for cyclase activity. The measured rate was 222 pmol of protochlorophyllide formed/h per mg of protein. Greening obviously does not increase the activity of the cyclase, nor does it lead to substantial destruction of the enzyme, as has been found for protochlorophyllide reductase (Mapleston & Griffiths, 1977).

DISCUSSION

The pathway of chlorophyll formation proposed by Granick (1951) has, since its early description, been subjected to only minor modifications (Ellsworth & Aronoff, 1969; Rebeiz & Lascelles, 1982). The original scheme was based largely on the various intermediates found to accumulate either in chlorophyll-less mutants of algae and photosynthetic bacteria or in higher-plant tissues after they had been fed with inhibitors of chlorophyll synthesis (Granick, 1951; Ellsworth & Aronoff, 1969). Confirmation of any proposed pathway usually relies upon demonstration of the reactions *in vitro* followed eventually by isolation and characterization of the individual enzymes. The aim of the work described in the present paper has been to demonstrate, and assay, the conversion of MPE into PChlide *in vitro*.

Absorbance changes following incubation of wheat etioplasts in darkness with added substrate and attributed to the conversion of MPE into PChlide are described here for the first time (Fig. 4). Castelfranco and co-workers (Chereskin *et al.*, 1983) have identified the compound produced by cucumber plastids under similar conditions as DVPChlide, and we assume a similar identity for the product formed here. After pre-illumination, incubation leads to the appearance of the characteristic absorption at 650 nm (Fig. 2) due to the ternary complex of substrate-charged reductase (Oliver & Griffiths, 1982). Illumination (of the incubation product) results in the photoconversion of the pigment into the corresponding chlorin (divinyl chlorophyllide?) observed spectroscopically as a loss in absorbance at approx. 650 nm accompanied by an increase of greater magnitude at approx. 678 nm (Fig. 2, spectrum c).

Since reductase activity is considerably in excess of that of the cyclase in these plastids, the overall rate of chlorophyll synthesis observed in this experiment must reflect the rate of the cyclization process. Measurement of chlorophyll therefore provides a convenient assay for the cyclase activity.

Assay of the cyclase by discontinuous monitoring of absorbance changes (Fig. 4) is facilitated by the fact that formation of the phaeoporphyrin is accompanied by an approximate doubling in absorbance value compared with the substrate (see Scheme 1). By coupling the cyclase reaction to the reductase enzyme (Fig. 3), considerable further advantage in assay sensitivity is achieved owing to the even greater absorbance of the chlorophyll so formed (see Scheme 1).

The rates of cyclase activity that we find in etiolated wheat etioplasts are consistently higher than those

obtained by Wong & Castelfranco (1984, 1985) with a discontinuous fluorimetric assay on cucumber plastids. Furthermore, whereas the latter workers can reconstitute the activity in fractionated plastid membranes by supplementation with a soluble protein fraction, in our experience with wheat plastids integrity of the etioplast structure is essential for observing activity. Thus the mildest of disruptive treatments, e.g. 20 μM-Triton X-100 or even the turbulence induced by bubbling the incubation solution with a gentle gas stream, resulted, in our hands, in irreversible loss of cyclase activity. The reason for this difference between the greening cucumber and etiolated wheat system could lie in the greater general fragility of the wheat plastids.

In view of the requirement for undamaged, presumably intact, plastids, for demonstrating cyclase activity with wheat extracts (see above), the observed specific stimulation of the process by the non-permeant NADPH (Table 1) is somewhat surprising. NADPH has also been reported to be a specific requirement for cyclase activity in the reconstituted cucumber system, whereas in the undisrupted system both NADPH and NADH proved to be stimulatory. It is obvious that further work is required for a rational explanation of the real role of NADPH in the cyclization process.

Chemically the cyclization reaction involves replacement of 2 H atoms in MPE by an O atom, and so it is not unexpected that the process has an absolute requirement for molecular O_2 (Table 1), as previously reported (Chereskin *et al.*, 1982). The failure of CO to inhibit the reaction, however (Table 2), eliminates the participation of a cytochrome *P*-450 type of mixed-function oxidase system in the process.

The involvement of iron in chlorophyll synthesis is a well-established phenomenon (Price, 1968). Very clear evidence for the role of iron in the cyclase reaction was obtained from specific inhibition of the process by added iron chelators such as mycobactin (complete inhibition at 50 μM) and 8-hydroxyquinoline (50% inhibition at 60 μM). Furthermore, the failure of a non-permeant analogue of the latter, namely 8-hydroxyquinoline-sulphonate, to inhibit (Table 2) implies that the iron-dependent reaction is localized on the inside of the impermeable plastid membrane.

The response of the plastid system to added NADPH, however, implies that another part of the system is obviously accessible to the outside of the inner membrane. This suggests that the cyclase system is made up of many components organized at different locations within the plastid. In fact, the involvement of both soluble and membrane-bound proteins in the cyclase process has been proposed by Castelfranco and his co-workers from their fractionation and reconstitution studies (Wong & Castelfranco, 1984).

It is of interest to compare this localization with that of other chlorophyll-biosynthetic enzymes, such as magnesium chelatase (outside of the inner membrane), magnesium protoporphyrin IX methylase (stroma) and protochlorophyllide reductase and chlorophyll synthase (inner membranes). This complicated arrangement of the different enzymes must demand a high degree of organization in the plastid for the co-ordinated operation of the pathway, so much so that any disruption of the etioplast might result in its inhibition. Furthermore it appears that the multicomponent cyclase system also needs precise organization (see above), which might help to explain the extreme sensitivity of the process to the detergent Triton X-100.

The porphyrin-specificity of the cyclase system indicates that the central metal plays an important and specific part in the process, reaction being confined to the magnesium and zinc chelates only, with neither the free porphyrin nor the nickel and copper chelates acting as substrates (Table 3). It has further been shown that the system has a low specificity for the 4-position, with both vinyl or ethyl groups here leading to activity (Wong & Castelfranco, 1985). In contrast, a vinyl group at position 2 appears to be a specific requirement for substrate activity. It may be noted that a very similar pattern of porphyrin specificity is shared by the light-requiring protochlorophyllide reductase (Griffiths, 1980). The reductase also undergoes a dramatic decrease in amount in response to illumination (Mapleston & Griffiths, 1977); however, in marked contrast, the cyclase shows no such change.

Mechanistically the cyclization process might be expected to involve the 6-hydroxy and 6-oxo compounds as cyclization intermediates (Fischer & Stern, 1940). In fact, some evidence for the involvement of these in experiments performed *in vitro* has been presented (Wong *et al.*, 1985). Whether any of the absorbance transients observed in the current work (Fig. 4) correspond to any of these compounds remains to be established.

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REFERENCES

Chereskin, B. M., Wong, Y.-S. & Castelfranco, P. A. (1982) Plant Physiol. **70**, 987–993
Chereskin, B. M., Castelfranco, P. A., Dallas, J. L. & Staus, K. M. (1983) Arch. Biochem. Biophys. **226**, 10–18
Cross, A. R., Parkinson, J. F. & Jones, O. T. G. (1984) Biochem. J. **223**, 337–344
Cross, A. R., Parkinson, J. F. & Jones, O. T. G. (1985) Biochem. J. **226**, 881–884
Ellsworth, R. K. & Aronoff, S. (1969) Arch. Biochem. Biophys, **130**, 374–383
Fischer, H. & Stern, A. (1940) Die Chemie des Pyrols, vol. 2, pp. 154–189, Akademische Verlag, Leipzig
Granick, S. (1951) Harvey Lect. **44**, 220–245
Griffiths, W. T. (1975) Biochem. J. **152**, 623–635
Griffiths, W. T. (1978) Biochem. J. **174**, 681–692
Griffiths, W. T. (1980) Biochem. J. **186**, 267–278
Griffiths, W. T. & Jones, O. T. G. (1975) FEBS Lett. **50**, 355–358
Jones, O. T. G. (1969) in Data for Biochemical Research (Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M., eds.), pp. 318–325, Clarendon Press, Oxford
Jones, O. T. G. (1979) in The Photosynthetic Bacteria (Clayton, R. D. & Sistrom, W. R., eds.), pp. 751–777, Plenum Press, New York
Jones, O. T. G. & Saunders, V. A. (1972) Biochim. Biophys. Acta **275**, 427–436
Mapleston, R. E. & Griffiths, W. T. (1977) Biochem Soc. Trans. **5**, 319–321
Oliver, R. P. & Griffiths, W. T. (1982) Plant Physiol. **70**, 1019–1025
Price, C. A. (1968) Annu. Rev. Plant Physiol. **18**, 239–248

Rebeiz, C. A. & Lascelles, J. (1982) in Photosynthesis: Energy Conversion in Plants and Bacteria (Govindjee, ed.), vol. 1, pp. 699–780, Academic Press, New York
Smith, K. M. (1975) Porphyrins and Metalloporphyrins, Elsevier, Amsterdam
Snow, G. A. (1970) Bacteriol. Rev. **34**, 99–125
Wong, Y.-S. & Castelfranco, P. A. (1984) Plant Physiol. **75**, 658–661
Wong, Y.-S. & Castelfranco, P. A. (1985) Plant Physiol. **79**, 730–733
Wong, Y.-S., Castelfranco, P. A., Goff, D. A. & Smith, K. M. (1985) Plant Physiol. **79**, 725–729

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