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Polyphenol Oxidation by *Vicia faba* Chloroplast Membranes

STUDIES ON THE LATENT MEMBRANE-BOUND POLYPHENOL OXIDASE AND ON THE MECHANISM[1] OF PHOTOCHEMICAL POLYPHENOL OXIDATION

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ABSTRACT

The mechanism whereby light effects polyphenol oxidation was examined with *Vicia faba* chloroplast membranes known to contain a bound latent polyphenol oxidase. Results obtained with the inhibitors 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-idopropyl-*p*-benzoquinone (DBMIB) indicated an involvement of the noncyclic electron transport pathway in the light-dependent oxidation of polyphenols, such as dihydroxyphenylalanine (DOPA). Further evidence was provided by experiments in which (*a*) DOPA replaced H_2O as electron donor for the photoreduction of NADP, (*b*) NADP replaced O_2 as electron acceptor in the photochemical oxidation of DOPA, and (*c*) the variable fluorescence associated with photosystem II was increased by DOPA. The photochemical oxidation of DOPA by *V. faba* chloroplast membranes was insensitive to KCN and to antibodies against purified latent polyphenol oxidase. The results are consistent with the conclusion that the light-dependent oxidation of polyphenols by *V. faba* chloroplast membranes is achieved independently of the latent membrane-bound polyphenol oxidase. Electrons derived from polyphenols seem to enter the noncyclic electron transport chain on the oxidizing side of photosystem II and to react with O_2 at an unidentified site on the photosystem I side of the DCMU/DBMIB blocks.

The physiological mechanism for the activation of latent polyphenol oxidase remains an unanswered question. Present results suggest that activation could occur through either acidification or the release of free fatty acids.

It is well-established that chloroplasts isolated from certain plants contain a latent membrane-bound PPO[3] that can be solubilized and activated by detergents such as SDS (11, 12, 18, 22). Chloroplast membranes from such plants can also oxidize polyphenols photochemically in the absence of SDS and the function of light has been ascribed to an activation of the latent PPO enzyme (24). No mechanism has been proposed to account for this type of activation.

To satisfy our interest in the role of light in enzyme regulation, we investigated the mechanism whereby light effects polyphenol oxidation by chloroplast membranes of this type. We now report evidence that the light-dependent oxidation of polyphenols by such chloroplast membranes (isolated in this case from *Vicia faba*) is achieved independently of the latent membrane-bound PPO. The evidence indicates that the electrons released in the photochemical oxidation of polyphenols enter the oxidizing side of PSII in a manner analogous to known PSII donors and are transferred via noncyclic electron transport to O_2 at a site on the system I side of the DCMU and DBMIB blocks. Photochemical polyphenol oxidation by chloroplast membranes isolated from plants with an active PPO (for example, spinach) seems to occur via this same route. The mode of activation of the latent PPO enzyme *in vivo* in plants such as *V. faba* thus seemingly is independent of light.

MATERIALS AND METHODS

Plant Material. *V. faba* (Broad Windsor horsebean, Northrup King and Co., Fresno CA) was greenhouse-grown in University of California mix under natural environmental conditions (2).

Reagents. Biochemicals were purchased from Sigma Chemical Co. All other reagents were purchased from commercial sources and were of the highest quality available. Ferredoxin and ferredoxin-NADP reductase (a gift from R. Chain) were isolated as described by Buchanan and Arnon (4) and Shin *et al.* (21), respectively.

Preparation of Chloroplast Fragments and Chloroplast Extract. Chloroplasts were isolated from freshly harvested chilled leaves in a preparative solution containing 0.35 M sucrose and 25 mM Tris-HCl buffer (pH 7.9). Fifty g of leaflets were blended and the slurry was filtered and centrifuged as described by Kalberer *et al.* (10). The sedimented intact chloroplasts were osmotically broken by suspension in a solution containing 50 mM Tris-HCl buffer (pH 7.9) and 1 mM $MgCl_2$ (henceforth called buffer B) and the chloroplast membrane fragments were collected by centrifugation for 5 min at 27,000*g*. For obtaining the soluble enzyme fraction, the Chl concentration of the resuspended intact chloroplasts was adjusted to 1.0 mg/ml (1) before centrifugation and the resultant supernatant fraction (chloroplast extract) was saved. For obtaining chloroplast membrane fragments, the pellet from the centrifugation step was washed by suspension in 40 ml buffer B and collected by centrifugation as above. The pelleted membranes were resuspended in buffer B to a Chl concentration of 1 mg/ml. For aging, the washed chloroplast membranes were stored overnight at 6 C.

Assay Methods. PPO was assayed either spectrophotometrically or polarographically with DOPA as substrate. In the spectropho-

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[3] Abbreviations: PPO, polyphenol oxidase; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DOPA, L-dihydroxyphenylalanine; DPC, diphenyl carbamate.

tometric assay, the newly formed oxidized DOPA derivative (DOPAchrome) was determined by measuring the A at 500 nm in a Gilford model 220 spectrophotometer. In the polarographic assay, DOPA-dependent O_2 uptake was followed by using a Rank Bros. O_2 electrode calibrated as described by Hiyama *et al.* (8). For routine assay, PPO activity was measured spectrophotometrically in the presence of 4 mM SDS (see below). The photoreduction of NADP by washed chloroplast membranes was measured by the procedure of McSwain and Arnon (17).

Analytical Methods. Fluorescence emission by washed chloroplast membrane fragments was measured at room temperature in the narrow spectral region around 695 nm. This method maximizes the fluorescence of variable yield from PSII and minimizes the contribution from PSI (20). Fluorescence was measured at room temperature (15) by using the fluorometer described by Malkin *et al.* (15).

Polyacrylamide gel electrophoresis was carried out according to Davis (6). Protein was determined with a modified Lowry procedure (14).

Isolation of Bound PPO. Membrane-bound PPO was purified from washed chloroplast membrane fragments that were prepared as above except that the final Chl concentration was 2.5 mg/ml. One kg chilled leaflets was used as the starting material for isolation of the chloroplast membrane fragments. Bound PPO was released by sonicating 10-ml aliquots of the chloroplast membrane suspension kept on ice for a total of 105 s (30-s pulse, followed by a 10-s rest), using a Branson sonifier (model S125) set at power setting 3. Following sonication, the aliquots were combined and centrifuged at 105,000g for 90 min. The green precipitate containing the chloroplast membranes was discarded. The supernatant fraction was applied to a DEAE-cellulose column (2 × 30 cm) which had been equilibrated beforehand with 50 mM Tris-HCl buffer (pH 7.9) (subsequently designated buffer A) and the column was eluted with 600 ml of a 0 to 0.4 M NaCl linear gradient added to buffer A. Fractions (6.6 ml) were collected and assayed for latent PPO activity by using the above SDS assay. Column fractions showing the highest activity were pooled, concentrated by dialysis *versus* solid sucrose, and dialyzed against buffer A overnight. The dialysate was clarified by centrifugation (105,000g for 30 min) and 5 ml was applied to a Sephadex G-100 column (2 × 90 cm) equilibrated with the same buffer. Fractions (2.5 ml) were collected at a flow rate of 16 ml/h and assayed for PPO activity as before. The fractions containing the highest activity were pooled and concentrated as above. The concentrate of the pooled Sephadex G-100 fractions was applied to a 2- × 5-cm hydroxyapatite column (Bio-Gel HTP, Bio-Rad Labs., Richmond, CA) that had been equilibrated with buffer A. The column was subsequently eluted with 200 ml buffer A supplemented with a linear gradient of 0 to 0.35 M K-phosphate buffer (pH 7.7). Fractions (2.5 ml) were collected and assayed for PPO activity. The peak fractions were pooled, concentrated by sucrose dialysis, and stored at −10 C. The approximate yield of PPO was 2.5 mg. The specific activity in the SDS spectrophotometric assay was 328 $\Delta A_{500\ nm}$/mg protein·min.

Preparation and Purification of PPO Antibody. Anti-PPO γ-globulin and control γ-globulin were prepared from New Zealand albino rabbits as described by Breazeale *et al.* (3) except that the initial injection contained 1.4 mg enzyme in 0.4 ml buffer A and subsequent injections contained 0.8 mg enzyme in 0.23 buffer A. The γ-globulin fraction was purified by ammonium sulfate and DEAE-cellulose fractionation and stored at −15 C at a concentration of 70 mg/ml.

RESULTS AND DISCUSSION

LIGHT-DEPENDENT DOPA OXIDATION BY WASHED CHLOROPLAST MEMBRANES

The first question to be dealt with was whether chloroplast membranes isolated from *V. faba*, like those from other plants known to contain a membrane-bound latent PPO, catalyze a light-dependent oxidation of polyphenols (24). The results of these experiments provided an affirmative answer to this question: the washed membranes catalyzed an oxidation of DOPA that was strictly light-dependent when monitored either spectrophotometrically by measuring DOPAchrome formation (Fig. 1, left panel) or polarographically by measuring O_2 uptake (Fig. 1, right panel). A requirement for light was also observed when DOPA was replaced by catechol, another known substrate for PPO (data not shown).

With DOPA as substrate, the rate of oxidation measured in the light under the conditions of Figure 1 was 80 μmol O_2 uptake/mg Chl·h. The rate of DOPAchrome formation seen in parallel experiments was 125 A units/mg Chl·h, which corresponds to about 75% the rate of DOPA oxidation catalyzed in the dark by the enzyme activated by 4 mM SDS. When followed either spectrophotometrically or polarographically, the oxidation of DOPA by the *V. faba* membranes was proportional to the light intensity (up to at least 5×10^5 ergs/cm^2·s) and was negligible at light intensities less than 1×10^3 ergs/cm^2·s (data not shown; *cf.* ref. 24). Both red (680 nm) and blue (420 nm) light could drive the oxidation of DOPA.

DOPA AS AN ELECTRON DONOR IN NONCYCLIC ELECTRON TRANSPORT

The above results are consistent with the idea that photosynthetic electron transport is necessary for the light-dependent oxidation of DOPA by *V. faba* chloroplast membranes. If so, DOPA might be expected to serve as an electron donor for the noncyclic transport of electrons to an added acceptor other than O_2. Table I shows that this was the case: aged chloroplast membranes, which had lost the capability to oxidize H_2O, utilized DOPA as a donor for the photoreduction of NADP. The rate of NADP reduction, measured with DOPA in the presence of 2 mM ascorbate, was 2 to

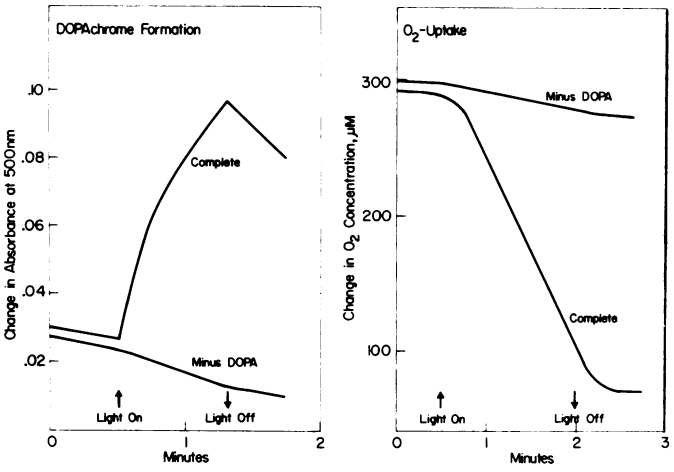

FIG. 1. Requirement for light for the oxidation of DOPA by *Vicia faba* chloroplast membranes. When DOPAchrome formation was measured, the reaction mixture contained once-washed chloroplast membranes equivalent to 100 μg Chl, 100 μmol Tricine buffer (pH 8.0), and 2 μmol L-DOPA. Volume, 1.0 ml. Temperature, 22 C. Light intensity, 5×10^4 ergs/cm^2·s with a Corning 2-64 filter. The reaction was carried out in 2-mm glass cuvettes. Following 1 min incubation in the dark, the light was turned on and the change in A at 500 nm was measured over the initial 1-min reaction period. When O_2 uptake was measured, the reaction mixture was as above except that 0.2 μg sodium azide was included and Tris buffer replaced Tricine. The reaction was carried out in an O_2 electrode at 18.8 C. Light intensity, 1×10^4 ergs/cm^2·s. Reaction volume and dark incubation was as above. After turning on the light, the rate of O_2 uptake was measured continuously over the initial 2-min reaction period.

Table I. *Demonstration of DOPA as an Electron Donor in Photoreduction of NADP by Aged Vicia faba Chloroplast Membranes*

The reaction mixture contained once-washed *Vicia faba* chloroplast membranes equivalent to 50 μg Chl, 120 μg spinach chloroplast ferredoxin, 70 μg spinach chloroplast ferredoxin-NADP reductase, 330 μg bovine liver catalase, 100 μg *Aspergillus niger* glucose oxidase, and the following (in μmol): ADP, 5; K_2HPO_4, 5; $MgCl_2$, 2; NADP, 2; and Tricine-HCl buffer (pH 8.0), 100. Volume, 1.0 ml. As indicated, 2 μmol L-DOPA and 2 μmol sodium ascorbate were added. The reaction mixture was vacuum-purged and equilibrated with argon three times. Glucose, 0.01 ml of a 1 M solution kept under N_2, was injected with a syringe. The indicated NADPH trap consisted of 10 μmol oxidized glutathione and 7 μg yeast glutathione reductase. The reaction was carried out under N_2 in rubber stopper-sealed, 2-mm cuvettes. Light intensity, 1×10^5 ergs/cm^2·s (Corning filter 2-64). Temperature, 22 C.

Electron Donor	NADP Reduced	
	Complete	+ NADPH Trap
	$\Delta A_{340\ nm}$/mg Chl·h	
None	9.6	4.8
Ascorbate	16.8	4.8
DOPA + ascorbate	36.0	2.4

Table II. *Replacement of Oxygen by NADP in Light-dependent Oxidation of DOPA by Vicia faba Chloroplast Membranes*

Conditions were as given in Table I.

Treatment	Light-dependent DOPAchrome Formed[a]
	$A_{500\ nm}$/mg Chl·h
No added acceptor	9
+ Reaction mixture[b]	66

[a] Corrected for changes in A in the absence of DOPA. Parallel treatments in air gave DOPA oxidation rates of 138 and 102 in the absence of an added acceptor and + NADP, ferredoxin, ferredoxin-NADP reductase, respectively.

[b] Two μmol NADP, 120 μg ferredoxin, and 70 μg ferredoxin-NADP reductase were included in the reaction mixture.

3 times the rate observed with ascorbate alone. Ascorbate was added in these experiments to reduce and decolorize the newly formed DOPAchrome that interfered with the spectrophotometric measurement of NADPH at 340 nm.

That DOPA can supply electrons to the noncyclic electron transport chain was also evidenced by experiments in which DOPAchrome formation was followed when DOPA oxidation was coupled to reduction of NADP rather than of O_2 (Table II). The rate of DOPA oxidation under conditions in which NADP served as electron acceptor was about one-third that observed with O_2. NADP served as an electron acceptor only in the presence of the soluble proteins required for the reduction of NADP from H_2O (*i.e.* ferredoxin and ferredoxin-NADP reductase). These requirements support the conclusion that electrons derived from DOPA proceed to NADP via the known noncyclic pathway. Further evidence for this conclusion was provided by the finding that the photooxidation of DOPA and the photoreduction of NADP (from H_2O) showed similar light saturation curves (data not shown).

PORT OF ENTRY OF DOPA-BASED ELECTRONS IN NONCYCLIC ELECTRON TRANSPORT

The results presented so far show that electrons derived from DOPA can enter the noncyclic electron transport chain, but there is no indication of their port of entry. To add information on this point, we tested the effect of specific inhibitors of noncyclic electron transport, DCMU and DBMIB, on light-dependent DOPA oxidation. The results (Table III) demonstrated that the light-dependent oxidation of DOPA catalyzed by *V. faba* chloroplast membranes was sensitive to both of these inhibitors, whether measured with O_2 or with NADP as electron acceptor. The extent of inhibition of DOPA oxidation by both DCMU and DBMIB was similar to that observed for the photoreduction of NADP with H_2O as electron donor. These results are in accord with the conclusion that electrons derived from DOPA enter the noncyclic electron transport chain on the PSII side of the DCMU and DBMIB blocks. Because of the very oxidizing oxidation-reduction potential of DOPA (E_o = +800 mv; ref. 5) it was considered likely that DOPA electrons enter the chain on the oxidizing (H_2O) side of PSII.

DOPA was therefore tested for an effect on variable fluorescence, a light-induced change of chloroplasts that is ascribed to PSII. In this system, an increase in the fluorescence emission near 695 nm (variable fluorescence) is considered to represent a reduction of the primary electron acceptor complex of PSII (20). Such an increase is readily observed in untreated freshly isolated chloroplasts in which H_2O serves as the electron donor. When chloroplast preparations are treated specifically to inhibit H_2O oxidation, the variable fluorescence changes disappear and reappear only on the addition of an artificial PSII electron donor such as DPC.

When screening treatments that could reduce variable fluorescence, it was observed that certain treatments applicable to spinach chloroplasts (*e.g.* washing with Tris or hydroxylamine-EDTA solutions) were ineffective with *V. faba* preparations (9, 25). Aging, by contrast, was found to be satisfactory; storing the washed *V. faba* chloroplast membranes for 18 to 24 h at 6 C diminished the light-dependent fluorescence that was observed with H_2O as electron donor. Variable fluorescence increased upon the addition of either DPC or DOPA to the reaction mixture (Table IV). These findings thus support the conclusion that, like DPC, DOPA supplies electrons to the oxidizing (H_2O) side of PSII.

IS MEMBRANE-BOUND PPO INVOLVED IN LIGHT-DEPENDENT DOPA OXIDATION?

Purification of Membrane-bound PPO. Although the above results demonstrate that *V. faba* chloroplast membranes oxidize

Table III. *Effect of Inhibitors on Light-dependent DOPA Oxidation and NADP Reduction by Vicia faba Chloroplast Membranes*

Oxidation or Reduction	Inhibition by 10 μM DCMU[a]	Inhibition by 1 μM DBMIB[a]
	%	
DOPA oxidation[b]	95	85
NADP reduction[c]		
H_2O as donor	95	95
DOPA as donor	85	85
DCIP + ascorbate as donor	18	23

[a] In parallel experiments, control rates of 180 $A_{500\ nm}$/mg Chl·h and 24 μmol NADP reduced/mg Chl·h were measured for DOPAchrome formation and DOPA-dependent NADP reduction, respectively.

[b] DOPAchrome formation was measured under the conditions given in Fig. 1 except that illumination was provided by a Baird atomic interference filter at 670 nm.

[c] NADP reduction was measured with freshly prepared chloroplast membranes in air as given in Table II. As indicated, 2 μmol DOPA and 0.5 μmol 2,6-dichlorophenolindophenol (DCIP) plus 2 μmol sodium ascorbate, were used as electron donors.

Table IV. *Identification of DOPA as a PSII Electron Donor by Its Enhancement of Variable Fluorescence of Vicia faba Chloroplast Membranes*

The reaction mixture (in a cuvette of 1-cm light path and 3-ml capacity) contained once-washed *Vicia faba* chloroplast membranes equivalent to 60 μg Chl, 300 μmol Tris-HCl buffer (pH 7.9), 3 nmol DCMU, and 3 μmol $MgCl_2$. Volume, 3.0 ml. As indicated, 6 μmol L-DOPA or 1.5 μmol DPC (delivered in 30 μl ethanol) were added. Following 15 s in the dark, the sample was exposed to the measuring beam and the fluorescence was measured for 30 s (F_o). The sample then was exposed to the actinic light for 1 min and the maximum fluorescence was recorded. Variable fluorescence (F_v) was reported as the difference between F_o and F_{max}.

Electron Donor	Fluorescence Emission		
	F_o	F_v	F_v/F_o
None	5.2	5.4	1.04
DPC	5.0	7.5	1.50
DOPA	5.2	7.4	1.42

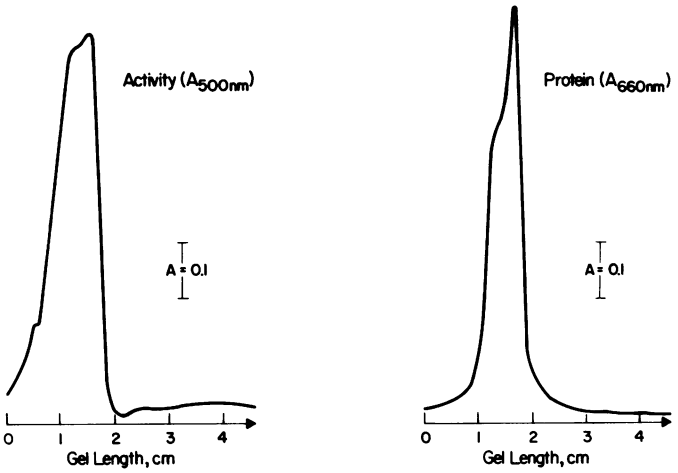

FIG. 2. Densitometric traces of protein and enzyme activity with PPO purified from *Vicia faba* chloroplast membranes. Purified PPO (90 μg) was subjected to electrophoresis in native polyacrylamide tube gels (7.5%). PPO activity was determined by incubating the gel for 60 min in a solution containing 50 mM Tris-HCl buffer (pH 7.9), 4 mM SDS, and 2 mM L-DOPA. Gels then were incubated in 100 mM Tris-HCl buffer (pH 7.9) for 15 min and subsequently scanned for *A* at 500 nm. Protein was determined by Coomassie blue staining (6).

polyphenols via the noncyclic pathway, they give no clue as to whether the latent membrane-bound PPO is necessary for the initial oxidation reaction. It was thought that an answer to this question might be provided by experiments designed to show whether a specific antibody against the latent membrane-bound PPO would inhibit the light-dependent oxidation of DOPA. In earlier experiments, it was observed that essentially all of the latent PPO of *V. faba* chloroplasts was membrane-bound and only traces of activity were recovered in the chloroplast-soluble protein fraction (chloroplast extract). To obtain PPO for the antibody studies, the membrane-bound enzyme was solubilized by sonication and purified by chromatography on DEAE-cellulose, Sephadex G-100, and hydroxyapatite columns. The enzyme preparation so obtained was free of other proteins, as determined by polyacrylamide gel electrophoresis (Fig. 2). The purified enzyme consisted of two active forms which behaved as a single component throughout the purification procedure and were resolved only by electrophoresis. No effort was made to separate the two enzyme forms prior to injection into rabbits for the production of PPO antibody. Multiple forms of PPO from other plants have been described (18).

Effect of PPO Antibody on DOPA Oxidation. Purified PPO elicited the formation of an active antibody which showed a single precipitin line when tested against the PPO antigen in Ouchterlony double diffusion (precipitation occurred up to a 1:16 dilution; *cf.* ref. 3). Reaction of the antibody with PPO was also evidenced by polyacrylamide gel electrophoresis experiments which revealed that the PPO antibody inhibited by more than 90% the mobility of the purified enzyme in gels prepared and developed as described in Figure 2 (data not shown). Under the same conditions, the control γ-globulin fraction and BSA were without effect. In addition to its capability to react with the purified PPO enzyme, the PPO antibody reacted with the membrane-bound enzyme, as evidenced by the "clumping" and ensuing increase in the rate of sedimentation of the membranes. It was not possible to test the effect of the antibody on the activity of the SDS-activated PPO due to the copious precipitate that formed on the addition of SDS to the antibody treated enzyme. However, when both the (purified) solubilized and the membrane-bound enzyme were activated by acid treatment (see below), activity was sensitive to the PPO antibody. There was, however, no effect of the antibody on the photochemical oxidation of DOPA under the same conditions, indicating that light-dependent polyphenol oxidation by *V. faba* chloroplasts is effected via the noncyclic electron transport chain independently of the latent membrane-bound PPO. Further evidence for this conclusion was provided by the finding that KCN (at 5 mM) totally inhibited the solubilized (SDS-activated) enzyme but had little, if any, effect on the photooxidation of DOPA (data not shown).

Activation of PPO by other means. The mechanism by which the latent PPO is activated *in vivo* thus remains an interesting question. During the course of this work, a number of agents were tested for their capacity to activate the enzyme with negative results (H_2O_2, DTT, the oxidized and reduced forms of glutathi-

Table V. *Acid-induced Activation of PPO Purified from Vicia faba Chloroplast Membranes*

PPO that was purified through the Sephadex G-100 step (72 μg in 0.05 ml) or once-washed *Vicia faba* chloroplast membranes equivalent to 100 μg Chl (in 0.05 ml) was preincubated in 0.25 ml of 100 mM solutions of each of the indicated buffers. After respective preincubation times of 3 and 10 min for the purified PPO preparation and the chloroplast membranes, 0.1 ml of each was injected into a reaction mixture that contained 200 μmol Tris-HCl buffer (pH 8.0) and 2 μmol L-DOPA. Volume, 1.0 ml. Temperature, 22 C. The reaction was carried out for 30 min in test tubes (12 × 200 mm) and *A* at 500 nm then was measured with a Gilford model 252 spectrophotometer.

Preincubation Buffer	PPO Activity[a]	
	Purified PPO	Chloroplast Membranes
	$A_{500\,nm}$/30 min	
Mops		
pH 7.7	0.00	0.05
pH 6.5	0.00	0.02
Na-citrate		
pH 6.5	0.00	0.05
pH 6.0	0.00	0.02
pH 5.5	0.01	0.05
pH 5.0	0.00	0.02
pH 4.5	0.02	0.01
pH 4.0	0.09	0.10
pH 3.5	0.16	0.27
pH 3.0	0.10	0.15

[a] In parallel experiments, activation by 4 mM SDS resulted in an increase in *A* of 0.94 and 0.24 for the PPO preparation and chloroplast membranes, respectively.

one, thioredoxins, and such lipid constituents as phosphatylcholine). The only treatments found to activate the latent enzyme, other than detergents, were incubation at an acid pH (pH 3 to 4) essentially as described earlier by Kenten (11) and treatment with fatty acids as recently reported by Golbeck and Cammarata (7). As shown in Table V, pH-induced activation was observed both with the purified PPO preparation and with the membrane-bound PPO. Activation was maximal at pH 3.5 and was not observed at pH values more alkaline than 4. A 5-min incubation under acid conditions gave maximal activation. As for the fatty acids, activation was observed after treatment of the purified enzyme with palmitic, oleic, or linoleic acid. However, the activation of the purified PPO by these fatty acids was less than 5% the maximum activation observed with SDS. It remains to be seen whether the activation effected by either acid pH or free fatty acids is of physiological significance (cf. refs. 13, 18, 19).

In summary, the present findings indicate that, contrary to previous notions (24), the latent membrane-bound PPO of *Vicia faba* chloroplasts is not involved in photochemical polyphenol (DOPA) oxidation. The evidence suggests that the oxidation of polyphenols by chloroplasts with a latent bound PPO is effected via the same mechanism operative in chloroplasts deficient in latent PPO, *i.e.*, via noncyclic electron transport (cf. 24). In the noncyclic oxidation system, electrons released in polyphenol oxidation enter the electron transport chain on the oxidizing (water) side of photosystem II and react with O_2 on the photosystem I side of the DCMU/DBMIB block (23). The exact site of transfer of the DOPA-derived electrons to O_2 is not known.

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