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Effect of Ionic Detergents, Nonionic Detergents, and Chaotropic Agents on Polyphenol Oxidase Activity from Dormant Saffron (*Crocus sativus* L.) Corms

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Polyphenol oxidase (PPO; EC 1.14.18.1) catalyzes the hydroxylation of monophenols to *o*-diphenols (cresolase activity) and the oxidation of *o*-diphenols to *o*-quinones (catecholase activity), leading to browning in plants and produce. Further interest in the enzyme has been triggered by the active role that it plays in plant defense systems. PPO can be found in latent forms and is activated in vitro by various agents including urea, detergents, and proteases. The activation of PPO from several sources by sodium dodecyl sulfate (SDS) has been extensively investigated, but reports on the effect of other detergents or on the differential effect of detergents on each of PPO's activities are scarce. In addition, investigations on the enzyme in other plant parts besides fruits and vegetables are also scarce. Here, the effect of various detergents and chaotropic agents on PPO from dormant saffron (*Crocus sativus* L.) corm extract was investigated. SDS and sarkosyl activated the cresolase activity, while only SDS activated the catecholase activity. All other detergents tested, in milli- or micromolar concentrations, inhibited the cresolase activity but barely affected the catecholase activity. In contrast, urea and guanidine-HCl drastically inhibited the catecholase activity but moderately inhibited the cresolase activity. The same effects were obtained on the partially purified enzyme. Results identified a PPO, present in dormant corms, which was activated only by anionic detergents and was inhibited by other reputed activating agents such as urea. Results also emphasized the differences in structure and accessibility of the active sites for cresolase and catecholase activities.

KEYWORDS: Catecholase activity; chaotropic agents; corm; cresolase activity; ionic detergents; nonionic detergents; polyphenol oxidase; saffron

INTRODUCTION

Browning of damaged tissues in fresh fruits and vegetables results mainly from the oxidation of phenolic compounds and contributes significantly to quality loss (*1*). The enzyme primarily responsible for the browning reaction, polyphenol oxidase (PPO; EC 1.14.18.1), is an enzyme that includes two copper atoms in its active site (*2*). In the presence of oxygen, PPO catalyzes the ortho-hydroxylation of monophenols to *o*-diphenols (cresolase activity) and the subsequent oxidation of *o*-diphenols to their corresponding *o*-quinones (catecholase activity) (*3*). These, in turn, are polymerized to undesirable brown, red, or black pigments (*4*). One unusual characteristic of PPO is its ability to exist in an inactive or latent state (*5*). The enzyme can be released from latency or activated by a variety of treatments or agents including acid and base shock (*6*, *7*), urea (*8*), polyamines (*9*), anionic detergents such as sodium dodecyl sulfate (SDS) (*7*, *10*, *11*), proteases (*12*), and fatty acids (*13*, *14*).

In plants, PPO is predominantly located in the chloroplast thylakoid membranes, although the enzyme has also been detected in soluble fractions in homogenates from different vegetables (*15*, *16*), and its phenolic substrates are mainly located in the vacuoles; browning occurs when, upon any cell-damaging treatment, the enzyme and substrates come into contact, leading to rapid oxidation of phenols and production of dark pigments (*17*). The prevention of these undesirable reactions has always been a challenge for food scientists. For this reason, PPO has been studied in many fruits and vegetables including apples (*18*), grapes (*19*), peaches and pears (*20*), and eggplants (*21*). However, besides fruits and vegetables, plants also produce substances valued for their medicinal and/or flavoring properties, for which color and taste are of primary importance. Saffron, for example, has been renowned since ancient times as a spice as well as a healing agent for various ailments and even as a dye. It consists of the dried stigmas from the flower of *Crocus sativus* L. (hereafter, "saffron"), a triploid perennial plant adapted to overcome a dry dormant period in

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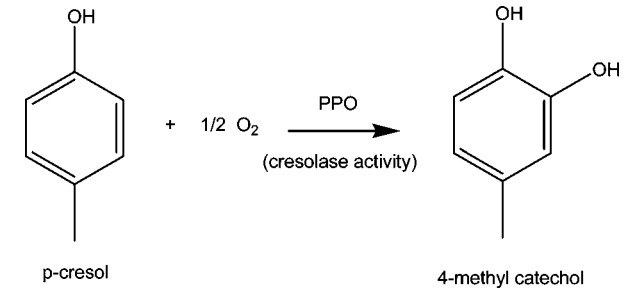
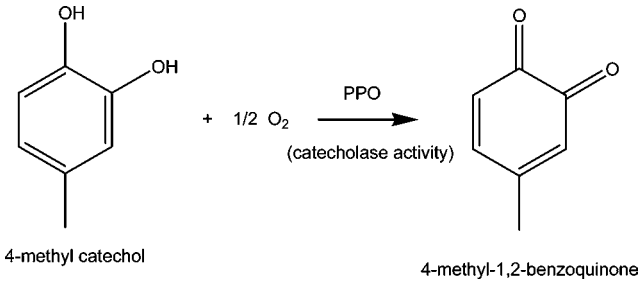
Scheme 1



Scheme 2



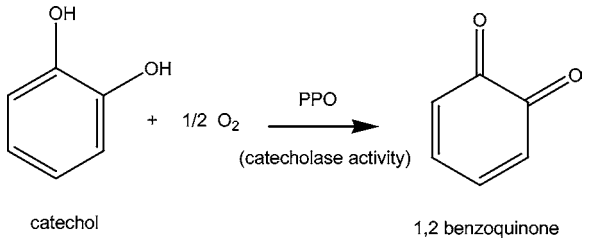
the form of an underground corm that is its only way of propagation. Although saffron has been cultivated for thousands of years, little is known on the basic aspects of its physiology and biochemistry and there is no reported investigation on the PPO activity in the plant. Given the importance of PPO in food quality, characterization of the enzyme from a wider variety of sources could help develop more effective methods in controlling browning of plants and produce. In addition, PPO has been found to play an active role in plant defense systems (*22*, *23*), triggering a further interest in investigating the properties of the enzyme. Furthermore, although reports on activation of PPO from various sources by specific agents have been made, there are few investigations on the effect of different detergents on PPO from a single source or on the differential effect of detergents on the cresolase and catecholase activities.

Thus, our objective was to investigate and characterize the PPO activity in saffron with an emphasis on the effect of various detergents and chaotropic agents on the cresolase and on the catecholase activities, thereby characterizing the saffron PPO activity and investigating the effect of different detergents on PPO from a single source. One of the most important saffron organs is its corm, and some of our previous studies suggested the ability of the corm to fine-tune its metabolism according to environmental conditions such as oxygen deprivation by flooding or exposure to high salinity (*24*, *25*). Even during the dormancy period, the corm has to survive diverse kinds of stress and the enzymes involved in its defense mechanisms need to be active. Given the importance of the corm in the survival and propagation of the plant and also given the role attributed to PPO in plant defense systems (*22*, *23*), the studies presented here were conducted in saffron corm extract; the corms studied were in the dormant state. A number of assays were also conducted on PPO partially purified from saffron dormant corm extract.

MATERIALS AND METHODS

Chemicals. 1,2-Benzenediol (catechol), SDS, nonaethylene glycol octylphenol ether (Triton X-100), nonaethylene glycol octylphenyl ether (NP-40), urea, guanidine chloride (GnHCl), and DEAE cellulose were purchased from the Sigma Chemical Co.; CM-Sephadex C-25 was from Pharmacia; sodium N-lauroylsarcosinate (Sarkosyl) was purchased from

Scheme 3



Fluka; polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene sorbitan monoleate (Tween 80), sodium cholate, sodium deoxycholate, *p*-cresol, and all of the other chemicals used in this study were purchased from the Merck Chemical Co. All chemicals were of reagent grade.

Corms and Extract Preparation. Saffron corms were obtained from the University of Tehran farm located in Karaj, near Tehran. Dormant corms were unearthed, depleted from their sheathing leaves, and cleaned from any dirt particles. Extracts were prepared from corms each weighing between 3 and 6 g, according to Keyhani and Sattarahmady (*26*). Briefly, corms were homogenized in 0.01 M phosphate buffer, pH 7.00, containing 0.02% phenylmethanesulfonyl fluoride as the protease inhibitor. After centrifugation at 3000*g* for 10 min, then at 35000*g* for 30 min, a clear, transparent supernatant termed "crude extract" was obtained and used for our studies. Protein determination was done by the Lowry method (*27*).

Enzymatic Activity Assays. *Cresolase Activity Assay.* The cresolase activity in saffron corm extract was determined spectrophotometrically by following the increase in absorbance at 420 nm due to the oxidation of *p*-cresol to 4-methyl-1,2-benzoquinone that would take place according to **Schemes 1** and **2**.

Catecholase Activity Assay. The catecholase activity was determined spectrophotometrically by following the increase in absorbance at 400 nm due to the oxidation of catechol to 1,2-benzoquinone that would take place according to **Scheme 3**.

The assays were conducted at room temperature (~22−25 °C) in 3 mL reaction mixtures containing 0.1 M phosphate buffer, pH 6.7, 0.1 mg/mL corm extract, and either 17 mM *p*-cresol or 22 mM catechol. The steady-state rate was defined as the slope of the linear zone of the product accumulation curve. A preliminary pH activity profile, performed with each substrate, identified pH 6.7 as an optimum pH in both cases. The substrate concentrations picked were equivalent to 1.5 K_m values.

Effect of Detergents and Chaotropic Agents on the Enzymatic Activities. The enzymatic activities were also measured in the presence of increasing concentrations of ionic detergents (SDS, sarkosyl, sodium cholate, and sodium dodecyl cholate), nonionic detergents (Triton X-100, Tween 20, Tween 80, and NP-40), and chaotropic agents (urea and GnHCl). The concentrations used in each case were kept within the range where no aggregation was observed and were determined as a function of the sensitivity of the enzymatic activities.

Activity Assays in the Presence of Ionic Detergents. The cresolase activity was assayed in 0.1 M phosphate buffer at pH 6.7 with 17 mM *p*-cresol as the substrate, in the presence of either 0−0.7 mM SDS, 0−0.5 mM sarkosyl, 0−14 mM sodium cholate, or 0−1.0 mM sodium deoxycholate. The concentration ranges remained below the critical micelle concentration (CMC) for each detergent. The catecholase activity was assayed in 0.1 M phosphate buffer at pH 6.7 with 22 mM catechol as the substrate, in the presence of either up to 0.7 mM SDS, up to 40 mM sarkosyl, up to 150 mM sodium cholate, or up to 9 mM sodium deoxycholate.

Activity Assays in the Presence of Nonionic Detergents. The cresolase activity was assayed in 0.1 M phosphate buffer at pH 6.7 with 17 mM *p*-cresol as the substrate, in the presence of either 0−30 μM Triton X-100, 0−65 μM Tween 20, 0−60 μM Tween 80, or 0−83 μM NP-40, where all concentrations remained below each detergent CMC. The catecholase activity was assayed in 0.1 M phosphate buffer at pH 6.7 with 22 mM catechol as the substrate, in the presence of either 0−150 mM Triton X-100, 0−80 mM Tween 20, 0−75 mM Tween 80, or 0−160 mM NP-40, where all concentrations went above each detergent CMC.

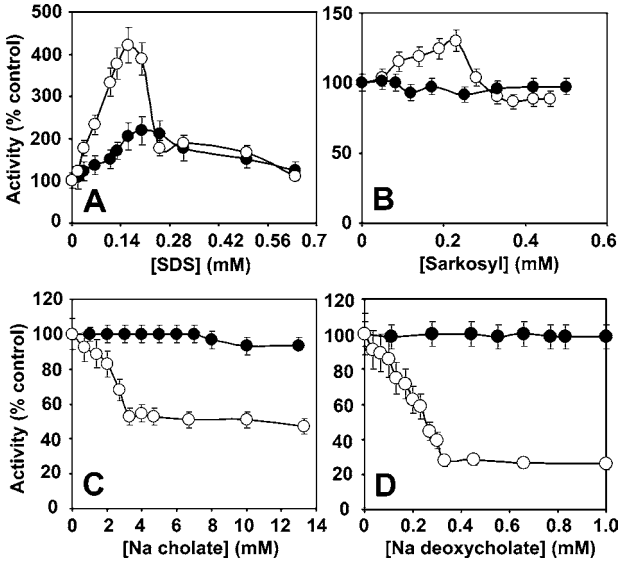


Figure 1. Effect of ionic detergents on saffron corm PPO activity. The cresolase (○) and catecholase (●) activities were assayed in the presence of various concentrations of SDS (**A**), sarkosyl (**B**), sodium cholate (**C**), and sodium deoxycholate (**D**). Assays were conducted at room temperature (~22–25 °C) in 0.1 M phosphate buffer, pH 6.7, containing 0.1 mg/mL corm extract and either 17 mM *p*-cresol as the substrate for the cresolase activity or 22 mM catechol as the substrate for the catecholase activity.

Activity Assays in the Presence of Chaotropic Agents. The cresolase activity was assayed in 0.1 M phosphate buffer at pH 6.7 with 17 mM *p*-cresol as the substrate, in the presence of either 0−100 mM urea or 0−16 mM GnHCl. The catecholase activity was assayed in 0.1 M phosphate buffer at pH 6.7 with 22 mM catechol as the substrate, in the presence of either 0−3.5 mM urea or 0−90 mM GnHCl.

PPO Partial Purification. Eighty milliliters of dormant saffron corm extract prepared as described above was brought up to 30−80% $(NH_4)_2SO_4$ and, after extensive dialysis against 0.1 M phosphate buffer, pH 7.00, and 0.02% PMFS, was fractionated by passage through a CM-Sephadex C-25 column equilibrated with the same buffer. A peak exhibiting PPO activity was eluted and further fractionated by passage through a DEAE-cellulose column equilibrated with the same phosphate buffer and elution with a 0−500 mM NaCl gradient. PPO-containing fractions were eluted at low NaCl concentrations. Cresolase and catecholase activities were assayed on the partially purified enzyme as described above, except that 0.035 mg/mL partially purified PPO was used instead of 0.1 mg/mL corm extract.

RESULTS

Assays Conducted with Dormant Saffron Corm Crude Extract. Both cresolase and catecholase activities, assayed as described in the Materials and Methods, were detectable in dormant saffron corm extract. Under our experimental conditions, no lag period was observed in the expression of either activity. Although such lag periods have often been reported for other systems, it remains dependent on the source of the enzyme and on the nature of the substrate used (*28*); thus, the absence of the lag period recorded in this work represents one of the features of the PPO under study.

Effect of Ionic Detergents. *Effect of SDS.* Upon addition of up to 0.7 mM SDS to the reaction mixture, both the cresolase and the catecholase activities were stimulated (**Figure 1A**). The extent of stimulation depended on the concentration of detergent and reached a maximum at 0.16 mM SDS. With *p*-cresol as the substrate, more than a four-fold increase in PPO activity was observed in the presence of 0.16 mM SDS, while with catechol as the substrate, a two-fold increase in activity was observed in the presence of the same (0.16 mM) SDS concentration (**Figure 1A** and **Table 1**). Further increases in SDS concentration progressively reduced the activation, and the effect observed with either substrate became similar. In the presence of 0.25 mM SDS, the activity observed with *p*-cresol as substrate was 1.7 times the control while that observed with catechol as the substrate was two times the control; in the presence of 0.3−0.65 mM SDS, the activities observed with either *p*-cresol or catechol as the substrate were the same, and they were 1.8, 1.5, and 1.2 times the control for, respectively, 0.32, 0.5, and 0.64 mM SDS (**Figure 1A**).

Effect of Sarkosyl. The addition of up to 0.25 mM sarkosyl to the reaction mixture resulted in up to a 30% increase in the activity when *p*-cresol was used as the substrate (**Figure 1B**). No effect was observed in the presence of 0.3 mM sarkosyl. Higher detergent concentrations (up to 0.5 mM) caused a 10% decrease in PPO activity with *p*-cresol as the substrate (**Figure 1B**). Over the same concentration range (0−0.5 mM), sarkosyl had no effect on the enzymatic activity with catechol as the substrate (**Figure 1B**).

In the presence of higher sarkosyl concentrations, cloudiness prevented further investigations with *p*-cresol as the substrate; with catechol as the substrate, up to 40% inhibition was observed in 40 mM sarkosyl (**Table 1**).

Effect of Sodium Cholate and Sodium Deoxycholate. In the presence of either sodium cholate or sodium deoxycholate, a decrease in PPO activity was observed when *p*-cresol was used as the substrate. The inhibition increased with increasing detergent concentration, until a plateau was reached. In the presence of sodium cholate, the PPO activity decreased progressively to 50% of the control as the detergent concentration increased progressively to 3 mM; in the presence of higher sodium cholate concentrations, the PPO activity was still reduced to 50% of the control (**Figure 1C**). When sodium deoxycholate was used, the PPO activity decreased progressively to 30% of the control in the presence of 0.3 mM detergent and remained at that level even in the presence of 1 mM sodium deoxycholate (**Figure 1D**). Higher detergent concentrations could not be used when *p*-cresol was the substrate because of the formation of aggregates, leading to inconsistency and no reliability in the results.

Over the same ranges of concentrations (1−15 mM for sodium cholate and 0.02−1 mM for deoxycholate), no effect was observed on PPO activity when catechol was used as substrate (**Figure 1D**). However, because higher detergent concentrations could be used in the presence of catechol, it was observed that PPO activity was reduced by 20% in 50−150 mM sodium cholate but that it remained unaffected in up to 9 mM sodium deoxycholate (**Table 1**).

Effect of Nonionic Detergents. The addition of Triton X-100, Tween 20, Tween 80, or NP-40 to the reaction mixture led to a decrease in the PPO activity, whether *p*-cresol or catechol was used as the substrate. The extent of inhibition, however, varied with the detergent used.

With *p*-cresol as the substrate, up to 80% inhibition was observed in the presence of 30 μM Triton X-100; 50% inhibition was observed in the presence of 40−65 μM Tween 20, as well as in the presence of 68 μM NP-40, and 60% inhibition was observed in the presence of 32−60 μM Tween 80 (**Figure 2A**). Over the same ranges of concentrations (0−30 μM for Triton X-100, 0−65 μM for Tween 20 and for Tween 80, and 0−83 μM for NP-40), no effect was observed on PPO activity when catechol was used as the substrate (**Figure 2B**). However, because higher detergent concentrations could be used in the

Table 1. Differential Effects of Ionic Detergents, Nonionic Detergents, and Chaotropic Agents on the Cresolase and on the Catecholase Activity of Saffron Corm PPO[a]

	concentration range (mM)	effect on cresolase activity (% control)[b]	effect on catecholase activity (% control)[b]
		ionic detergents	
SDS	0–0.7	activation (400%)	activation (200%)
sarkosyl	0–0.25	activation (130%)	no effect
	0.25–0.5	inhibition (90%)	no effect
	0.5–40		inhibition (60%)
sodium cholate	0–14	inhibition (50%)	no effect
	14–150		inhibition (80%)
sodium deoxycholate	0–0.7	inhibition (30%)	no effect
	0.7–9		no effect
		nonionic detergents	
Triton X-100	0–0.030	inhibition (20%)	no effect
	0.030–180		inhibition (40%)
Tween 20	0–0.064	inhibition (50%)	no effect
	0.064–85		inhibition (80%)
Tween 80	0–0.060	inhibition (40%)	no effect
	0.060–80		inhibition (85%)
NP-40	0–0.065	inhibition (50%)	No Effect
	0.065–150		inhibition (75%)
		chaotropic agents	
urea	0–3.5	inhibition (92%)	inhibition (15%)
	3.5–100	inhibition (70%)	
GnHCl	0–3	inhibition (80%)	inhibition (65%)
	3–10	inhibition (70%)	inhibition (50%)
	10–16	inhibition (70%)	inhibition (45%)
	16–90		inhibition (25%)

[a] The maximum effect obtained for each concentration range is reported. [b] The values in parentheses represent the activity expressed as % control.

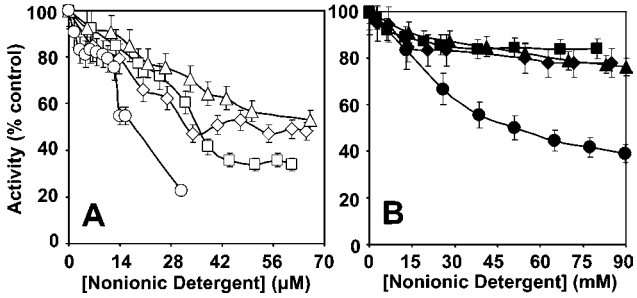


Figure 2. Effect of nonionic detergents on saffron corm PPO activity. The cresolase (**A**) and catecholase (**B**) activities were assayed in the presence of various concentrations of Triton X-100 (○), Tween 20 (◇), Tween 80 (□), and NP-40 (△) (open symbols were used in part **A**, and closed symbols were used in part **B**). Assays were conducted at room temperature (~22–25 °C) in 0.1 M phosphate buffer, pH 6.7, supplemented with 0.1 mg/mL corm extract and either 17 mM *p*-cresol as the substrate for the cresolase activity or 22 mM catechol as the substrate for the catecholase activity. Note the difference between the detergent concentration ranges used in part **A** (0–30 μM for Triton X-100, 0–64 μM for Tween 20, 0–60 μM for Tween 80, and 0–65 μM for NP-40) and the detergent concentration ranges used in part **B** (0–90 mM for Triton X-100, 0–85 mM for Tween 20, 0–80 mM for Tween 80, and 0–90 mM for NP-40).

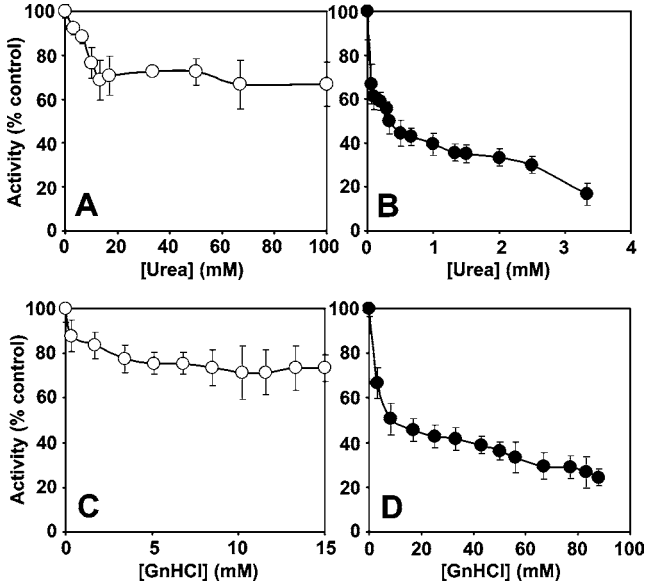


Figure 3. Effect of chaotropic agents on saffron corm PPO activity. Increasing concentrations of urea were added to the assay mixtures for cresolase (**A**) and catecholase (**B**) activities; similarly, increasing concentrations of GnHCl were added to the assay mixture for cresolase (**C**) and catecholase (**D**) activities. Assays were conducted at room temperature (~22–25 °C) in 0.1 M phosphate buffer, pH 6.7, in the presence of 0.1 mg/mL corm extract and either 17 mM *p*-cresol as the substrate for the cresolase activity or 22 mM catechol as the substrate for the catecholase activity.

presence of catechol, it was observed that the PPO residual activity was 40% of the control value in 90 mM Triton X-100, 80% of the control value in 40 mM Tween 20, 85% of the control value in 38 mM Tween 80, and 75% of the control value in 83 mM NP-40. Doubling the concentration of either detergent did not cause any further inhibition of the catecholase activity (**Table 1**).

Effect of Chaotropic Agents. *Effect of Urea.* The addition of increasing concentrations of urea to the assay mixture resulted in increasing inhibition of the cresolase activity, so that 30% inhibition was reached with 17 mM urea; no further inhibition was observed for up to 100 mM urea (**Figure 3A**). When the catecholase activity was assayed in the presence of urea, up to 60% inhibition was observed in 0.5 mM urea; a further increase in urea concentration resulted in more inhibition so that 85%

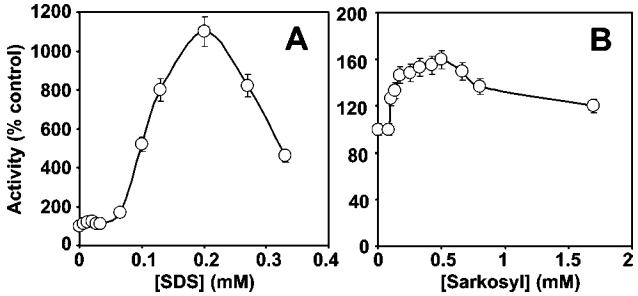


Figure 4. Effect of SDS and sarkosyl on partially purified saffron corm PPO. The cresolase activity was assayed in the presence of various concentrations of SDS (**A**) or sarkosyl (**B**). Assays were conducted at room temperature (~22–25 °C) in 0.1 M phosphate buffer, pH 6.7, containing 0.035 mg/mL corm extract and 33 mM *p*-cresol as the substrate.

inhibition of the catecholase activity was observed in 3.5 mM urea (**Figure 3B**).

Effect of GnHCl. Up to 30% inhibition of the cresolase activity was observed upon addition of up to 10 mM GnHCl; the inhibition remained at the same level in up to 16 mM GnHCl (**Figure 3C**). On the other hand, up to 55% inhibition of the catecholase activity was observed in 17 mM GnHCl; a further increase in GnHCl to 90 mM led to as much as 75% inhibition of the catecholase activity (**Figure 3D**).

Assays Conducted with Partially Purified PPO from Dormant Saffron Corm. The partially purified PPO obtained as described in the Materials and Methods exhibited both cresolase and catecholase activities. However, the latter was detectable only on freshly collected fractions and was rapidly lost upon storage, whether at 4 or −20 °C. Indeed, after 24 h, the residual activity detectable with catechol as the substrate was only 60% of the original activity, whether samples were stored at 4 or −20 °C. After 72 h, the residual activity dropped to 10% of the original value and no activity was detectable after 1 week. A similar loss of catecholase activity upon storage was reported for partially purified PPO from various sources such as Chinese cabbage (*Brassica rapa* L.) (*29*) and wild potato (*30*). The cresolase activity also diminished during storage but was recovered by increasing the substrate (*p*-cresol) concentration to 33 mM. Thus, because of the irrecoverable loss of catecholase activity upon storage, further investigations were conducted on the cresolase activity of partially purified PPO.

The effect of ionic detergents on the enzymatic (cresolase) activity of partially purified PPO was similar to that found with the extract, except that the extent of stimulation by SDS and sarkosyl was more important and that maximum stimulation by sarkosyl occurred at a higher detergent concentration (0.5 mM for the partially purified enzyme instead of 0.25 mM for the extract) (**Figure 4A**,**B**). The partially purified enzyme also showed a higher sensitivity to cholate, being inhibited at a lower detergent concentration and to a larger extent: 0.7 mM cholate caused 86% inhibition in the partially purified enzyme (**Table 2**) while 4 mM cholate caused only 50% inhibition in the extract (**Figure 1C**). Sensitivity to deoxycholate was also, to some extent, higher in the purified enzyme: 91% inhibition observed with 0.5 mM deoxycholate (**Table 2**) in the partially purified enzyme vs 70% inhibition in the extract with up to 1 mM deoxycholate (**Figure 1D**).

The effect of nonionic detergents on the cresolase activity of the partially purified enzyme was also similar to that observed with the extract with some differences in sensitivities. Triton X-100 inhibited the enzymatic activity to the same extent in the partially purified fraction and in the extract (**Table 2** and **Figure 2A**). However, both Tween 20 and Tween 80 inhibited the cresolase activity to a greater extent in the partially purified enzyme than in the extract. As the Tween 20 concentration increased from 35 to 60 μM, inhibition of the cresolase activity was raised from 45 to 85% in the partially purified enzyme (**Table 2**) but remained at 50% in the extract (**Figure 2A**). Tween 80 in concentrations of 15, 20, and 25 μM caused, respectively, 20, 45, and 60% inhibition of the cresolase activity in the partially purified enzyme (**Table 2**) and 15, 25, and 30% inhibition of the cresolase activity in the extract (**Figure 2A**). In contrast, with NP-40, the cresolase activity was inhibited to a greater extent in the extract than in the partially purified enzyme. NP-40 concentrations of 55 and 120 μM brought about, respectively, 20 and 30% inhibition of the cresolase activity in the purified enzyme (**Table 2**) but as much as 45 and 50% inhibition of the cresolase activity in the extract (**Figure 2A**).

Table 2. Effect of Ionic Detergents and Nonionic Detergents on the Cresolase Activity of Partially Purified Saffron Corm PPO[a]

	concentration range (mM)	effect on cresolase activity (% control)[b]
	ionic detergents	
SDS	0–0.35	activation (1,100%)
sarkosyl	0–0.8	activation (160%)
	0.8–1.8	activation (120%)
sodium cholate	0–0.1	inhibition (80%)
	0.1–0.3	inhibition (70%)
	0.3–0.7	inhibition (14%)
sodium deoxycholate	0–0.1	inhibition (90%)
	0.1–0.3	inhibition (75%)
	0.3–0.7	inhibition (9%)
	nonionic detergents	
Triton X-100	0–0.020	inhibition (45%)
Tween 20	0–0.035	inhibition (65%)
	0.035–0.060	inhibition (15%)
Tween 80	0–0.015	inhibition (80%)
	0.015–0.020	inhibition (55%)
	0.020–0.025	inhibition (40%)
NP-40	0–0.055	inhibition (80%)
	0.055–0.120	inhibition (70%)

[a] The maximum effect obtained for each concentration range is reported. [b] The values in parentheses represent the activity expressed as % control.

DISCUSSION

Although in plants PPO has been predominantly located in the chloroplast thykaloid membrane, the enzyme has also been detected in soluble fraction in homogenates from different vegetables (*16*). The present investigation was conducted on extracts obtained from saffron corms that are subterranean organs devoid of chloroplasts; the extract prepared as described in the Materials and Methods consisted of the soluble fraction of the corms homogenate, and the PPO activity reported here refers to the soluble enzyme. Each assay was performed at least in triplicate, and each experiment was repeated three times. The results were expressed as means of the values obtained, and the standard deviations of the means were calculated and shown on the figures.

Whether membrane-bound or soluble, PPO retains its intriguing property of being able to exist in a latent state from which it can be released by a variety of treatments, such as exposure to SDS, that are reputed to inactivate enzymes (*15*). While activation by SDS has been reported for PPO from a wide range of sources (*10*, *11*, *14*, *31*−*34*), the effect of other detergents has been investigated on a limited basis only. Reported data on SDS indicated that the degree of activation as well as the

optimum SDS concentration varied depending on the source of the enzyme or isoenzyme studied as well as on the experimental conditions, such as pH or the nature of the substrate used, and whether the monophenolase (cresolase) or diphenolase (catecholase) activity was assayed (*11*, *15*, *34*). In this investigation, both the cresolase and the catecholase activities detectable in dormant saffron corm extract were tested in the presence of various concentrations of a number of ionic and nonionic detergents as well as chaotropic agents. As summarized in **Tables 1** and **2**, results showed that only a couple of ionic detergents, namely, SDS and sarkosyl, would activate saffron corm PPO activity and that the cresolase and the catecholase activities exhibited different sensitivities toward the various detergents and agents used. Except in the cases of SDS and urea, the cresolase activity was sensitive to much lower detergent/agent concentrations than the catecholase activity.

A number of mechanisms have been proposed for the activation of PPO by SDS, and they all point to the involvement of conformational changes in the enzyme during activation (*11*, *15*, *34*); similarly, activation by other factors, for example, acid shock or protease treatment, is also attributed to conformational changes in the enzyme (*34*). In the model proposed by Gandía-Herrero et al. (*15*), the enzyme would undergo conformational changes toward a more active form upon the binding of discrete molecules of SDS, the number of SDS molecules needed to express maximum activity depending on the nature of the substrate used. Our results showed that, among the ionic detergents tested in this study, only SDS and sarkosyl would activate saffron corm PPO, while cholate and deoxycholate inhibited the enzymatic activity. Assuming that activation results from the binding of discrete detergent molecules to the enzyme in order to trigger some local conformational changes, the inhibition observed with cholate and deoxycholate may be due to steric hindrance upon binding of these bulkier detergents to the enzyme. Along the same line, all of the nonionic detergents used in this study were even larger than cholate and deoxycholate and may thus cause inhibition upon binding to the enzyme by preventing access of the substrate to the active site because of steric hindrance.

For nonionic detergents, as long as the detergent concentrations remained below their CMC, the cresolase activity was inhibited, while the catecholase activity remained unaffected. The inhibition of catecholase activity at higher detergent concentrations may be due to the entrapment of either the enzyme or the substrate in detergent micelles. An interesting observation was the differential sensitivity of the cresolase and catecholase activities toward detergents, suggesting a difference in the structure and accessibility of PPO's active site for cresolase activity and that for catecholase activity; incidently, differences in the mode of attachment of monophenols and diphenols to PPO have been proposed by others (*35*, *36*). Initial access to at least one Cu^{2+} in the active site by monophenols or to either one or both Cu^{2+} by diphenols requires subtle alterations around the enzyme's active site that may vary for each isoform of the enzyme within one species as well as across different species. Whether either bidentate binding to both copper ions or monodentate binding to one copper ion takes place with diphenolic substrates is still under debate (*35*).

The results obtained with the partially purified enzyme confirmed those obtained with the extract. The enhanced activation observed with SDS and sarkosyl may be due to the loss, during purification, of some natural activators present in the extract; alternatively, it may be due to conformational changes to the enzyme that may be particularly sensitive in this species of plant. Structural changes during purification may also explain the loss of catecholase activity.

Reports have been made on the activation of PPO by urea or other agents such as proteases (*8*, *12*, *34*). In this work, no activation of the enzymatic activity by urea or by another chaotropic agent such as GnHCl was found. Espín and Wichers have shown, in their work on latent mushroom tyrosinase, that two isoforms of the enzyme exhibited different kinetic features and that one isoform was activated by protease, while the other was activated by SDS (*34*). Thus, different isoenzymes may be activated only by some agents and not by others. This was also observed in a study of the effect of various agents, including detergents, on the activation of PPO in extracts of bran from several wheat (*Triticum aestivum*) cultivars (*8*). The present study shows that the isoforms of PPO present in saffron corm soluble extract are activated by SDS but not by urea. Interestingly, while no more than 30% inhibition was observed in the presence of up to 100 mM urea for the cresolase activity, up to 80% inhibition of the catecholase activity was observed in the presence of only 3.5 mM urea. Similarly, GnHCl caused no more than 30% inhibition of the cresolase activity but up to 75% inhibition of the catecholase activity.

Thus, the catecholase activity of soluble PPO from saffron corm was much more sensitive to chaotropic agents than the cresolase activity, while the cresolase activity was much more sensitive to detergents than the catecholase activity. These findings indicated a difference in the conformation and in the microenvironment of the respective active sites in the enzyme. Even in the presence of SDS, cresolase was activated to 400% of the control without SDS, while catecholase was activated to only 200% of the control without SDS. The marked difference in behavior toward chaotropic agents between the cresolase activity and the catecholase activity, along with the different sensitivities of the two activities toward detergents, indicates that, at least for the soluble PPO in saffron corm extract, substrate accessibily and perhaps even the structure and localization of the active center for external *o*-diphenols and endogenous *o*-diphenols produced in situ after the cresolase reaction, were different.

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