

Note

Activation of tobacco leaf polyphenol oxidase by sodium dodecyl sulfate

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The effect of sodium dodecyl sulfate (SDS) on purified tobacco leaf PPO (PPO II) was investigated at various pHs and temperatures. SDS increased the activity of PPO II due to the formation of SDS-PPO II complex, leading to conformational changes, thus making access to active center easier. The relationship between the activity and the molar ratio of SDS-PPO II to PPO II showed that the critical point reached a plateau of activity at the molar ratio of about 1.2. The pH had a significant effect on interaction between SDS and PPO II, as compared to PPO II. The optimum catalytic temperature of the complex rose by 10°C, suggesting that stabilization of the structure had been improved by the formation of complex.

Keywords: Sodium dodecyl sulfate (SDS), polyphenol oxidase, tobacco leaf, pH effect, temperature effect

Polyphenol oxidase (PPO), a copper-containing enzyme is mainly involved in synthesis of pigments, such as melanin in plants¹. Usually, it is formed during the tissue development and bound in the chloroplast thylakoid membranes. The intriguing character of the enzyme is its ability to keep itself in inactive or latent state², until it is activated by various kinds of treatment or agents, such as acid and base shock³, urea⁴, polyamines⁵, SDS⁶⁻⁹, proteases¹⁰⁻¹¹, and fatty acids^{10,12}. The activation by SDS is particularly interesting, since most of the enzymes are inhibited by SDS; the activation is due to a limited conformational change in the latent enzyme^{6,13}, demonstrated by intrinsic fluorescence experiments⁷. Although reports on PPO from fruits are available, the studies on leaf PPO are lacking¹⁴⁻¹⁷. From tobacco, a PPO from transgenic tobacco leaves¹⁷ and a laccase-type PPO (100 kDa) from lignifying xylem¹⁸ have been reported. Earlier, we purified PPO I and -II, having

molecular weights of 35,800 and 35,600 Da, respectively from tobacco¹⁹. PPO II has shown only the activity of catalytic oxidation of *o*-diphenol, but not *p*- or *m*-diphenols, and monophenols (such as L-tyrosine). It can be activated by adding about an equal mole of N_3^- . Their complex is initially assigned as a terminal mode²⁰, but, the exact mechanism is still not understood. In the present paper, we report the activation of tobacco leaf PPO II by SDS, at different pH and temperatures. Our results are significantly different to those reported for PPO from transgenic tobacco leaves¹⁷, which have been ascribed to the effect of activated state of PPO.

DEAE-Sephadex A-50 and Sephadex G-75 were purchased from Pharmacia Corporation, Sweden and SDS from Calbiochem, Germany. All other reagents were of analytical grade and used without further purification.

Fresh tobacco leaves (*Nicotiana tabacum*) harvested directly from the field were washed and kept in refrigerator below 4°C for about 24 hr. PPO II was essentially purified according to the method described earlier¹⁹, with some modification in the order of column chromatography: DEAE-Sephadex A-50, followed by Sephadex G-75 and a second DEAE-Sephadex A-50. Protein concentration was determined using the method of Bradford²¹. The enzyme activity was monitored spectrophotometrically at 420 nm, as described¹⁹.

The enzyme assay solution contained 25 mM catechol (*o*-diphenol) and 50 mM sodium phosphate buffer (pH 6.5). The final SDS concentrations were 0, 0.05, 0.10, 0.50, 1.00 mM, respectively. The pH studies were carried out using 50 mM sodium citrate buffer (pH 3.0-5.0) or sodium phosphate buffer (pH 6.0-7.0) or Tris-HCl buffer (pH 8.0-9.0) in the presence and absence of SDS at each pH. The effect of temperature was investigated between 20-80°C. All samples above were measured after incubating for 10 min.

The simplified plot (Fig. 1) of the activity ratio of SDS-PPO II to PPO II vs SDS concentration showed that activity was increased, particularly at SDS concentration ranging from 0 to 0.10 mM (by 50%); the most effective concentration was 0.50 mM, where the activity was improved by 86%. When SDS effect,

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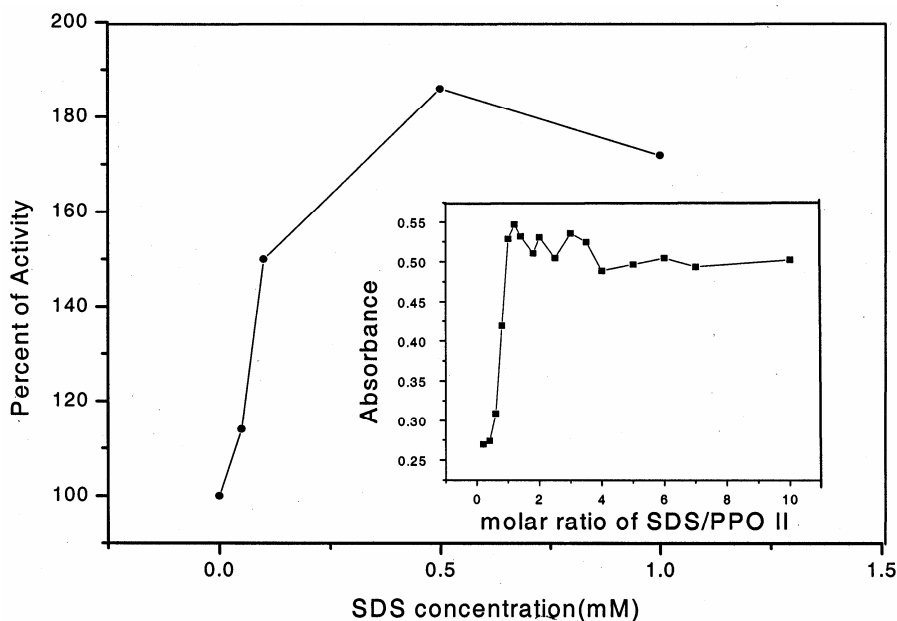


Fig. 1—Effect of SDS concentration on the relative activity of PPO II at pH 6.5 at room temperature [The activity of 100% was defined as that of PPO II in the absence of SDS. Inset: the effect of SDS on PPO II activity (absorbance at 420 nm) vs molar ratio of SDS-PPO II to PPO II]

i.e., the enzyme activity vs molecular ratio of SDS to PPO II was further analyzed (Fig. 1 inset), it reached a plateau with slight fluctuation (owing to the error of measurement). The critical molar ratio of SDS-PPO II between steep increase and plateau lay at the point of about 1.2. The K_m values determined by Lineweaver-Burk plot (at 10°C and 25 mM catechol as substrate) were 5.8 mM and 38.4 mM, for SDS-PPO II complex (1.2/1, molar/molar) and PPO II, respectively, suggesting that SDS-PPO II complex is superior to PPO II in the binding affinity for the substrates.

It is obvious that SDS shows effects on PPO II activity, although the effect is less than that observed for PPOs from broad bean⁷, mushroom²², banana²³ and apple leaf¹⁷. The optimum SDS concentration (0.5 mM) for tobacco PPO II is also much lower than PPOs mentioned above, except in the case of mushroom PPO²². The difference in their activation may be possibly due to the fact that tobacco PPO II was in an active state after its purification, similar to the crude PPO from transgenic tobacco leaf¹⁷. At higher SDS concentration, the activity of PPOs may: (i) still increases with the increasing SDS concentration, but tardily, such as in broad bean PPO⁷; (ii) the activity declines slightly and then reach a plateau, example, in apple leaf PPO¹⁷ and PPO II in the present study; (iii) the activity is decreased

steeply, with least effect on activation, such as in banana PPO²³. However, in either case SDS affects PPO's activity significantly.

Critical micelle concentration (CMC) of SDS was taken into consideration to explain the abnormal phenomena in previous reports^{7,24}. SDS concentration required for maximal activation was related to the amount of SDS monomers in the solution²⁵. SDS could successfully activate tobacco leaf PPO II below its CMC. SDS monomers would congregate into micelles at CMC and then amount of its monomers enters into a steady state, corresponding to the critical molar ratio of SDS-PPO II of about 1.2. The activity would thus not increase any further, irrespective of amount of SDS added (Fig.1 inset), while the slight decrease in activity could be observed, possibly due to the influence of SDS micelles.

pH is a vital factor that affects the activity of SDS-PPO II. The activity curves of both SDS-PPO II complex (molar ratio 1.2/1) and PPO II vs pH are shown in Fig. 2. The optimum pH for SDS-PPO II was 6.5, which is consistent with that for PPO II. The activity of SDS-PPO II at various pHs shows similar tendency with that of PPO II, differing only in the magnitude of enhancement. The graph shows strong dissimilarity with PPOs obtained from broad bean⁷, table beet¹⁶, mushroom²² and apple leaf¹⁷, where the

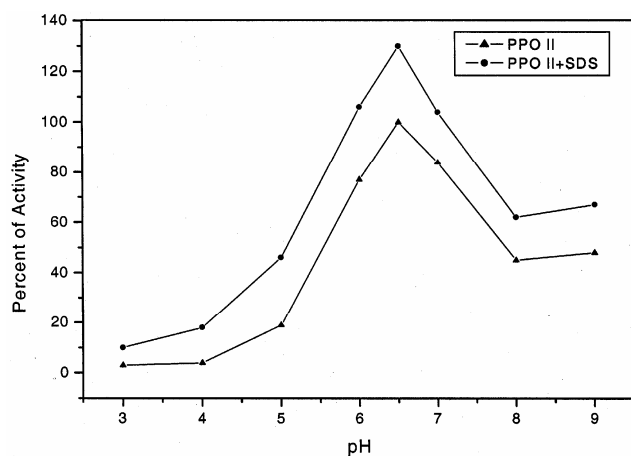


Fig. 2—Effect of pH on the relative activity of SDS-PPO II (molar ratio 1.2/1) and PPO II at 20°C [The activity of 100% was defined as that of PPO II at its optimum pH of 6.5]

optimum pH is greatly shifted and in acidic condition, SDS completely inhibited the PPO activity. However, the partly purified transgenic tobacco leaf PPO¹⁷ showed the unusual inhibition by SDS, even at its optimum pH , indicating that PPO is in an active state, which is responsible for the inhibition.

The activities slowly re-increased in both cases at $pH > 8$ (Fig. 2), possibly due to the changes of copper environment in the basic solution, as we indicated earlier²⁶. As SDS contains a long hydrophobic alkyl chain, it possibly induces changes in the secondary configuration of PPO II, instead of modifying the catalytic type-3 copper center. Thus, the rigidity of the “pocket” around the active site may be alleviated and more suitable for aromatic substrates to access. Considerable activity is observed for SDS-PPO II complex at acidic pH , where PPO II showed almost no activity, indicating that SDS may play a role that slightly retards the acidic invasion on the active center. Thus, although pH affects the composition of the active sites, it shows little influence on the interaction between SDS and PPO II.

The temperature effect on both SDS-PPO II (molar ratio 1.2/1) and PPO II is shown in Fig. 3. SDS stimulated the activity at all temperatures tested. The optimum temperature for SDS-PPO II was 50°C, which was about 10°C higher than that of PPO II¹⁹. The maximal activity of SDS-PPO II is almost 4 times of that at 20°C and the value for PPO II is only about 1.7. These can be ascribed to the increase in CMC of SDS with the increasing temperature and the ascent in activity with the multiplication of SDS monomers binding to PPO II. The factor is predominant before it

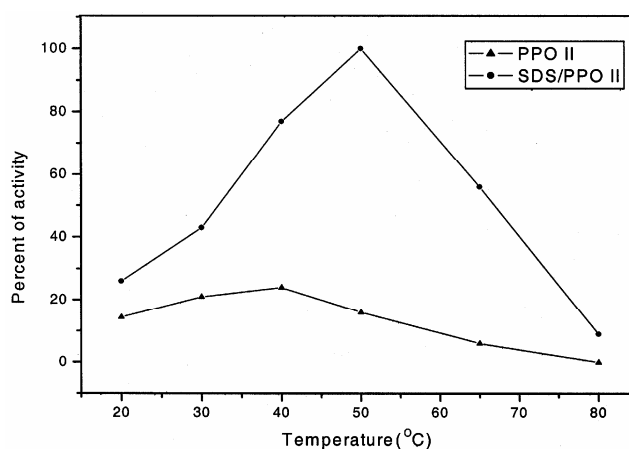


Fig. 3—Effect of temperature on the relative activity of SDS-PPO (molar ratio 1.2/1) [The activity of 100% was defined as that of SDS-PPO II at its optimum temperature of 50°C]

reaches the optimum temperature. The disadvantage factor comes from thermal destruction and the decreased levels of O₂, which act as the co-substrate of PPO II, thus causing a quick decline in its activity at higher temperature. At 65°C, SDS-PPO II retains 56% of its optimum activity, while PPO II retains only 25%, indicating that SDS might stabilize the structure, so as to endure a higher temperature.

It can be concluded that SDS can increase the activity of PPO II due to the formation of SDS-PPO II complex, possibly through a conformational change. SDS concentration needed for maximal activation is related to the amount of SDS monomers in solution. When CMC is reached, the activity does not increase any further. The pH mainly affects the composition of the active site of PPO II, but show little effect on SDS-PPO II complex. Compared with PPO II, the optimum catalytic temperature of the complex rises by 10°C, possibly induced by the increasing SDS monomers that bind to PPO. Thus, SDS might be helpful in the stabilization of the copper center.

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