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ACTIVATION AND ALTERATION OF PLANT AND FUNGAL POLYPHENOLOXIDASE ISOENZYMES IN SODIUM DODECYLSULFATE ELECTROPHORESIS

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Key Word Index—Angiosperms; fungi; polyphenoloxidase; sodium dodecylsulfate; electrophoresis; activation.

Abstract—Electrophoretic analysis of polyphenoloxidase isoenzymes from a variety of angiosperms and from mushroom revealed that the enzymes remain active in the presence of 0.1 % sodium dodecylsulfate. Electrophoresis in the presence of sodium dodecylsulfate allows the detection of latent enzyme forms of polyphenoloxidase, and can also convert slower migrating enzyme forms to faster migrating forms. Electrophoresis in the absence of sodium dodecylsulfate followed by incubation in the presence of sodium dodecylsulfate can also be used to detect latent forms of polyphenoloxidase. Together, these approaches provide a method for screening latent enzymes and give some insight into the mechanism of activation by sodium dodecylsulfate.

INTRODUCTION

Polyphenoloxidase (*o*-diphenol: O_2 oxidoreductase, EC 1.14.18.1; tyrosinase, PPO) is a ubiquitous enzyme whose function in plants remains unknown. The enzyme has been shown to exist in multiple and interconvertible forms, many of which differ with regard to their development, subcellular location, and plant source [1]. The enzyme has been shown to exist in a latent state and can be activated by treatment with proteolytic enzymes [2], fatty acids [3], denaturants [4], and detergents [5]. Activation can also occur by removal of inhibitors [6], by conformational changes [7], and by acid treatment [7–9]. Various investigators have shown that PPO can be activated by treatment with the denaturant sodium dodecylsulfate (SDS) although the mode of its activation has not been explained [5, 10–13]. Since the enzyme can be activated by SDS, electrophoretic analysis of PPO isoenzymes in the presence of the detergent could be used as a tool for the detection of specific activated isoenzymes as well as for detecting any changes in the electrophoretic mobility of these forms. In this report we show that isoenzymes of PPO are active in the presence of SDS, that the electrophoretic pattern of isoenzymes is different in the presence of SDS from that in its absence, and that latent enzyme forms of PPO can be detected.

RESULTS AND DISCUSSION

Electrophoretic analysis in the presence and absence of SDS was performed on a variety of samples. The number and types of multiple isoenzyme forms were characteristic of each plant source. Representative samples from fresh extracts which showed different responses to SDS were selected here for comparison. From these samples the following generalizations were apparent.

Electrophoresis in the presence and absence of SDS indicated that the isoenzyme patterns of PPO from mushrooms and lettuce were not affected by SDS (Fig. 1).

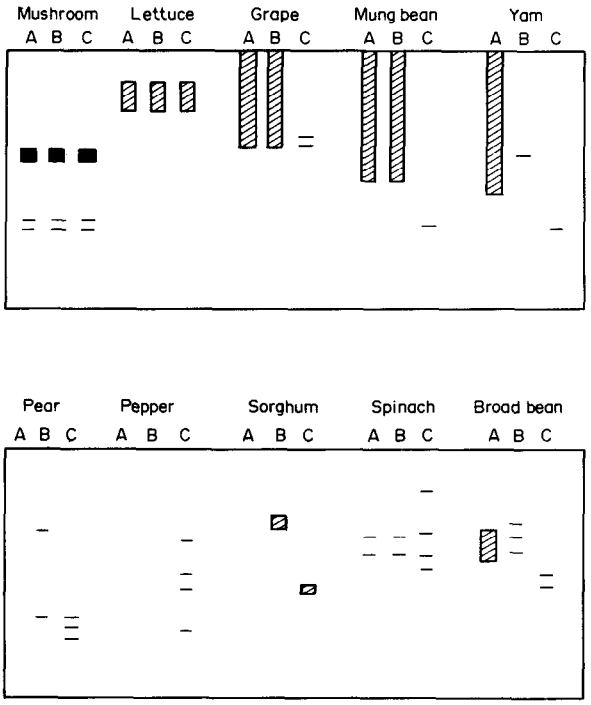

Fig. 1. Electrophoretic analysis of polyphenoloxidase isoenzymes in the presence or absence of SDS. Isoenzyme patterns of PPO were obtained using fresh extracts. Electrophoresis was performed as described in the Experimental. (A) Isoenzyme pattern from a gel run, incubated and stained for PPO activity in the absence of SDS. (B) Isoenzyme pattern from a gel run in the absence, but incubated and stained for PPO activity in the presence of 0.1 % SDS. (C) Isoenzyme pattern from a gel run, incubated and stained for PPO activity in the presence of 0.1 % SDS. (■) Most intense activity; (—) less intense activity; (▨) smear of activity (usually low levels), no definite enzyme bands. The direction of migration was from top (−) to bottom (+).

No change in the mobility of the individual isoenzyme forms was noted in fresh extracts nor were any new forms generated. This would suggest that for these cases SDS does not cause noticeable alteration in the charge to mass ratio from SDS binding. It also suggests that the enzyme from these sources is not dissociated into smaller subunits by SDS under partial denaturing conditions. The intensity of the individual isoenzyme forms appeared to be the same with or without the addition of SDS to the gels. Enzyme assays from these sources also showed no activation by SDS (data not shown).

Extracts from grapes, mung beans, yams and broad beans, on the other hand, showed an alteration in the PPO isoenzyme pattern when electrophoresed in the presence of SDS (Fig. 1). The pattern changed from a broad zone of activity in the absence of SDS into sharp, well-defined bands of enzyme activity in its presence. Electrophoresis in the absence of SDS followed by incubation with SDS did not change the overall pattern or mobility of the isoenzymes in grape and mung bean extracts (Fig. 1). Electrophoresis in the presence of SDS altered the mobility of these enzymes; yam and broad bean are, in this respect, different from grapes and mung beans.

In the absence of SDS, little or no activity was detected in extracts from pears, sorghum, peppers and broad beans, either with a spectrophotometric assay or by specific enzyme staining after electrophoresis (Fig. 1). In the presence of SDS, individual isoenzymes were readily detectable, suggesting that SDS converts many of these latent enzymes into fully active forms (Fig. 1). Electrophoresis in the absence of SDS, followed by staining in the presence of SDS, can detect latent enzymes in these extracts. This suggests that these enzyme forms are present but devoid of detectable activity. The mobility of these forms is substantially different from those present in electrophoretic conditions which included SDS (Fig. 1). It would appear that SDS causes an alteration in the enzyme from these sources which increases its electrophoretic mobility. Whether SDS causes an increase in the charge to mass ratio or dissociation into smaller fragments is not known.

In contrast to Meyer and Biehl who found that spinach phenolase was totally inhibited during SDS electrophoresis, we found that PPO from spinach leaves could be detected when electrophoresed in the presence of SDS [14]. They suggested that SDS electrophoresis cannot be used to study conversion of multiple forms of PPO by SDS because of SDS-mediated inactivation concomitant with activation. We found that in the presence of SDS, alteration of PPO isoenzyme forms could be observed in spinach and other plant extracts (Fig. 1). The discrepancies between their findings and ours remain to be resolved.

In recent years, several investigators have included SDS in the spectrophotometric assay for PPO activity and in their electrophoretic system for PPO analysis [14–19]. We are not aware of any reports analysing PPO isoenzymes electrophoretically in the presence or absence of SDS. We have shown that electrophoresis in the presence of SDS, or incubation of a gel with SDS following electrophoresis, allows detection of less active or latent enzymes. Electrophoretic analysis of SDS-activated PPO isoenzymes indicates that activation results in the conversion of slow moving forms to faster moving forms. This activation may be related to SDS binding or to partial denaturation into other forms with greater activity. From the results presented here, we cannot rule out the possibility that SDS removes some tightly bound inhibitor which affects the mobility and latency of the enzyme detected by electrophoresis, nor that SDS induces specific conformational changes in the enzyme. In any case, specific latent enzyme forms of PPO can be detected electrophoretically in the presence of SDS. However, caution must be used in the analysis of PPO isoenzymes since electrophoresis in the presence of SDS causes drastic changes in the mobility of PPO isoenzymes. Incubation with SDS after electrophoresis is probably the more reliable means of screening for these latent isoenzymes.

EXPERIMENTAL

Mung beans (*Vigna radiata* (L.) Wilczek cv Berken), broad beans (*Vicia faba* L. cv long Pod), sorghum (*Sorghum vulgare* (L.) Moench) and ornamental peppers (*Capsicum* cv Holiday Time) were grown in a greenhouse. All other fresh fruit and plant sources were obtained from local stores. Leaves from mung beans, sorghum, broad beans, ornamental peppers, lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) were used for PPO isolation. The whole fruit, tuber, or stalk was used from mushrooms (*Agaricus bisporus*), grapes (*Vitis vinifera* L.), yams (*Dioscorea sativa*) and pears (*Pyrus communis* L. cv d'Anjou). Me_2CO powders were prepared as described in ref. [20]. The powders were resuspended in 0.1 M NaPi buffer (pH 6.2) containing 0.05 M NaCl and stirred for 30 min, then centrifuged for 10 min at 9000 *g*. The supernatants were stored at −20° until use. Fresh extracts were prepared by homogenization in 0.1 M NaPi (pH 6.2) containing 0.05 M NaCl for 1 min, then centrifuged at 4° for 10 min at 9000 *g*. The supernatants were stored at −20° until use. Spinach extracts were freshly prepared each time before electrophoresis and/or enzyme assays. Enzyme assays (±0.05% SDS) were performed as described earlier [20, 21].

Vertical slab gel electrophoresis was performed according to the method of ref. [22] with or without the addition of SDS to the gel or electrophoresis buffer. Three gels were run simultaneously with equal amounts of each enzyme sample applied to replicate gels. Samples were not denatured before application. Electrophoresis was carried out for 3–4 hr at 100 V. After electrophoresis, one gel (A: without SDS in the separating and stacking gel) was incubated in 0.05 M NaPi (pH 6.5) for 30 min, then stained for PPO with 0.5 mM L-DOPA and 2 mM catechol in 0.05 M NaPi (pH 6.5). The second gel (B: without SDS in the separating and stacking gel) was incubated in 0.05 M NaPi (pH 6.5) buffer containing 0.1% SDS (w/v) for 30 min, then stained for PPO isoenzymes as above in buffer which contained 0.1% SDS. The third gel (C: containing 0.1% SDS (w/v) in the separating gel, stacking gel and electrophoresis buffer) was incubated in buffer containing 0.1% SDS, then stained for PPO isoenzymes as above in buffer which contained 0.1% SDS. With this combination of substrates, isoenzyme bands varied in intensity but were relatively permanent. All comparisons between gels were normalized to R_m between 0 and 1.

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