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REGULATION OF BOUND PEROXIDASE BY POLYAMINES AND GUANIDINES IN MAIZE SCUTELLUM

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Abstract—Peroxidase bound to the membrane either ionically or covalently, but not the free enzyme, is inhibited by polyamines and activated by guanidines. The ionically bound peroxidase detached from the membrane by Ca^{2+}, or the peroxidase present in the cytosolic fraction, can be associated with the membrane fraction from which the ionically bound enzyme is removed by Ca^{2+}. The reconstituted membrane fraction, either with the enzyme solubilized by Ca^{2+} or with the cytosolic enzyme, can again be modulated by these compounds by changing the affinity of the enzyme for its substrate.

INTRODUCTION

Peroxidases are widely distributed in plants [1] and have been implicated in growth and development [2, 3], hormonal balance [4] and various stress conditions induced by salinity [5, 6], dessication [7], temperature [8], wounding [9], air pollution [10], infection by pathogens [11, 12], γ-irradiation [13] as well as membrane stability [14]. Peroxidases are known to be present in soluble and membrane fractions [9, 15]. The membrane-bound enzyme may be bound ionically or covalently; the ionically bound enzyme can be solubilized by Ca^{2+} [15]. Release of wall-bound peroxidase by Ca^{2+} has been reported in other studies [16, 17]. Penel and Greppin [18] however found that the pelletability of peroxidase was increased by Ca^{2+} in the hypocotyl hooks of *Cucurbita pepo* and it is suggested that this cation may play a role in the intracellular localization of peroxidases.

In a previous study [19] we reported that the membrane-bound peroxidase activity of excised maize scutellum was inhibited by polyamines and activated by guanidines as a result of their binding to the membranes. The inhibition by polyamines was reversed by guanidino compounds but the activation by guanidines was not reversed by polyamines. Peroxidase present in the soluble fraction or the ionically bound peroxidase from the particulate fraction after solubilization by Ca^{2+} was not affected by these compounds. The present paper reports the results of a study of the reconstitution of the ionically bound peroxidase after its solubilization from the membrane and the kinetics of modulation of bound peroxidase by polyamines and guanidines.

RESULTS AND DISCUSSION

The membrane fraction (40 000 *g*) isolated from the scutella of 4-day-old germinated seedlings was treated with 0.8 M $CaCl_2$ to remove the ionically bound enzyme from the one bound covalently. The results reported in Table 1 (expt I) show that *ca* 85% of the enzyme present on the membrane could be solubilized by Ca^{2+} and 15% remained associated with the membrane, indicating that a major fraction of the membrane-bound enzyme is associated ionically. The membrane fraction (fraction 2b), from which the ionically bound enzyme was removed, was mixed with the solubilized enzyme after dialysis (fraction 3) and allowed to dialyse for a further period of 48 hr (fraction 4). After centrifugation, fraction 4 showed that *ca* 50% of the solubilized enzyme was reassociated to the membrane fraction (fraction 5b) and the reconstituted enzyme was again modulated by polyamines and guanidines (fraction 6). The data for spermine, a polyamine, and dodine, a guanidino fungicide, are given, though a similar effect was obtained by other polyamines and guanidines which affect this enzyme [19]. Spermine inhibited the reconstituted enzyme by 60% while dodine activated it by 50%. These results suggest that the ionically bound peroxidase was modulated by the compounds only when it was associated with the membrane. Several studies have suggested that polyamines, being cationic, mimic the effect of cations such as Ca^{2+} or Mg^{2+} [20–25]. In the present study, however, the effect of polyamines and guanidines does not appear to be due to their cationic nature since the treatment of the membrane fraction with polyamines and guanidines, unlike Ca^{2+}, could not solubilize the bound enzyme and all the bound enzyme remained associated with the membrane (data not given). These results, as well as those from our previous study [19] that the covalently bound enzyme remaining attached to the membrane was still modulated by these compounds, suggests that the modulation of the enzyme occurs only when the enzyme is associated to the membrane ionically or covalently.

In the above mentioned experiment the reconstitution was carried out with the enzyme solubilized from the membrane itself. To investigate if the enzyme present in the cytosolic fraction could also be attached to the membrane from which the ionically bound enzyme has been removed, the reconstitution in fraction 4 was carried out with cytosolic enzyme (expt II). The results showed

Table 1. Reconstitution of peroxidase from plasma membrane of maize scutellum and the effect of spermine and dodine on the reconstituted enzyme

Fraction	Peroxidase activity (units) Expt I*	Expt II†
1. Plasma membrane	86	86
2. After solubilization with 0.8 M $CaCl_2$		
(a) Supernatant	73	73
(b) Precipitate	12	12
3. Fraction 2a after dialysis	67	67
4. Reconstitutiom of fraction 2b with fraction 3/or cytosolic enzyme	74	72
5. Fraction 4 after centrifugation at 105 000 *g*		
(a) Supernatant	30	32
(b) Precipitate	44	40
6. Fraction 5b + spermine	18	20
Fraction 5b + dodine	66	64

*Expt I: plasma membrane fraction was treated with 0.8 M $CaCl_2$ for 1 hr and then centrifuged at 105 000 *g* to separate the solubilized enzyme in the supernatant (2a) and the bound enzyme in the precipitate (2b). The solubilized enzyme (2a) was dialysed to remove Ca^{2+}. The dialysed fraction 3 was then mixed with the precipitate (2a) in the dialysis bag and was further dialysed for 48 hr. After this period, fraction 4 was centrifuged at 105 000 *g* to separate the supernatant (5a) and the precipitate (5b) fractions. A portion of fraction (5a) was then pretreated with spermine or dodine (1 mM) for 1 hr before assaying the enzyme.

†Expt II: was the same as expt I except that the reconstitution at stage 4 was carried out with cytosolic enzyme obtained after centrifugation of the homogenate at 105 000 *g*.

that even the cytosolic enzyme could be associated to the membrane fraction and the reconstituted enzyme is modulated by polyamines and guanidines in the same way as the ionically bound enzyme, suggesting that the cytosolic enzyme may not be different from the ionically bound enzyme since both are regulated by the compounds after their association with the membrane. These results suggest that polyamines, guanidines and Ca^{2+} may play an important role in regulating peroxidase activity in tissues. The mechanism of Ca^{2+} regulation may be simply to control the level of enzyme bound to the membrane while the polyamines and guanidines have a more direct role in modulating the activity of the enzyme associated with the membrane.

In the above study the effect of the compounds was tested after the enzyme solubilized by Ca^{2+} or the

Table 2. Effect of spermine and dodine on the reconstitution of peroxidase from plasma membrane of maize scutellum

Fraction	Peroxidase (units) reconstituted in presence of —	Spermine	Dodine
1. Plasma membrane	77		
2. After solubilization with 0.8 M $CaCl_2$			
(a) Supernatant	66		
(b) Precipitate	10	3	14
3. Fraction 2a after dialysis	67		
4. Reconstitution of fraction 3 with fraction 2b	67	37	89
5. Fraction 4 after centrifutation at 105 000 *g*			
(a) Supernatant	20	22	19
(b) Precipitate	46	15	70

Plasma membrane fraction was solubilized by $CaCl_2$ as described in Table 1. The precipitate (2b) was then treated with spermine or dodine (1 mM) for 1 hr centrifuged at 105 000 *g* to remove the free spermine and dodine. The precipitate was then reconstituted with the solubilized enzyme after dialysis as described in Table 1. After reconstitution the fraction 4 was centrifuged at 105 000 *g* to separate the supernatant and precipitate.

cytosolic enzyme was reassociated with the membrane fraction. However, to test whether the compounds will affect the reconstitution process itself, the membrane fraction from which the ionically bound peroxidase was removed was first treated with the compounds before carrying out the reconstitution. The results reported in Table 2 show that spermine and dodine had their normal inhibitory and activating effect on the reconstituted enzyme and the reconstitution process was not affected by the binding of the compounds to the membrane before reconstitution. Also the binding of the compounds to the membrane fraction from which peroxidase was previously detached did not alter its subsequent binding, suggesting that the groups involved in attaching the compounds to the membrane were not involved in attaching the enzyme to the membrane.

The nature of the inhibition of bound peroxidase by spermine and activation by dodine was studied at varying H_2O_2 concentrations and the results when plotted in reciprocal form showed that K_m for H_2O_2 was increased by spermine and decreased by dodine (Fig. 1) suggesting that the modulation by these compounds is brought about by changing the affinity of the enzyme for its substrate.

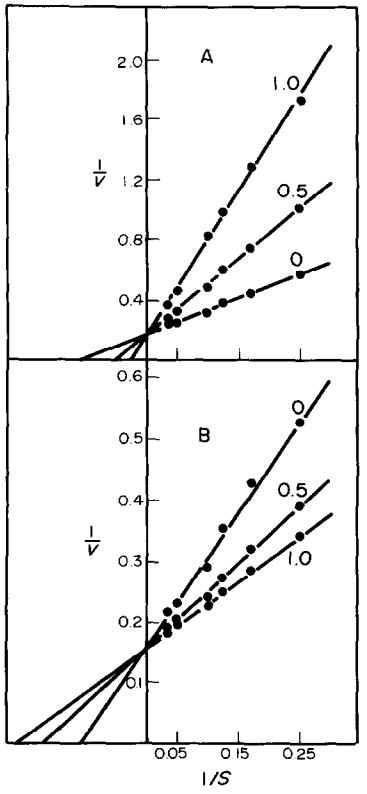

Fig. 1. Double reciprocal plot of peroxidase activity at various concentrations of H_2O_2 in the absence and presence of spermine (A) or dodine (B). The concentrations (mM) of spermine and dodine are indicated on the lines.

EXPERIMENTAL

Plant material. Maize seeds (*Zea mays* L. Var. Ganga-2) were obtained from National Seed Corporation, Baroda. The germination, preparation of membrane fraction and assay of the enzyme was as described earlier [19].

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