S U M M A R Y

SUMMARY

Polyamines which occur ubiquitously in all living organisms are believed to be involved in growth processes through their interaction with nucleic acids. In plants, however, they are also involved in the control of several stress related processes such as senescence and wounding as well as osmotic, temperature and salt stress. The protective effect of polyamines in these processes may be associated with the stabilization of the membrane structure. This has been demonstrated for instance, in the stabilization of protoplasts from oat leaf mesophyll cells, thylakoid membranes of barley chloroplasts and reduction of betacyanin efflux from beet root discs.

Guanidino compounds, which are structurally related to polyamines and share the same precursor arginine, are known to destabilize the membranes. They also interfere with the mitochondrial metabolism and transport of ions. The destabilization of membranes produced by guanidines could be reversed by polyamines.

There are well characterized membrane bound ATPases in mitochondria, chloroplasts and plasmalemma of plant cells. Membrane bound ATPase are controlled by cations, phospholipids, polyamines and guanidines. Peroxidases which are widely distributed in plants are associated with numerous catalytic functions, and are implicated in growth and development, hormonal balance and fruit ripening. Peroxidase has also been used as a parameter for various physiological conditions such as stress by salt, water and temperature, senescence, wounding, air pollution, infection by pathogens and γ-irradiation.

Since polyamines appear to protect plants against some of the above mentioned conditions and as polyamines and guanidines have been shown to have opposite effects on membrane stabilization, it was of interest to study the effect of polyamines and guanidines on membrane bound peroxidase and ATPase activities. Maize scutellum which is associated with the transport of nutrients and undergoes senescence, and is a rich source of membrane bound ATPase and peroxidase was selected for the studies. In most of the studies reported, slices prepared from the scutella were used for enzyme assays since it has been reported earlier that the two enzyme activities when measured in slices measure the plasmalemma bound enzyme. In some studies

homogenate was also used to measure the total activity of these enzymes. ATPase and peroxidase activities of scutellum slices increased till about 10th day of germination and reached to 72 and 152 units/g tissue respectively. Further studies using a series of diamines, polyamines and guanidines were carried out on the scutella excised from 4 day or 8 day germinated seedlings in order to demonstrate their effect on peroxidase and ATPase activities. It was found that with increasing chain length the diamines inhibited peroxidase activity and activated ATPase activity upto 1,4-diaminobutane. With further increase in the chain length there was a decrease in the inhibition of peroxidase and activation of ATPase by these compounds. Polyamines spermidine and spermine inhibited the peroxidase by 80% and activated ATPase by 45%. Among the guanidines tested guanidino acetic acid, guanidino-butyric acid, dodine and guazatine activated peroxidase by 40% and ATPase by 40-50%. None of the compounds tested, however, showed any effect on the peroxidase and ATPase activities of scutellum excised from 8 day germinated seedlings. The effect of these compounds was found to be concentration dependent on peroxidase and ATPase. The absence of any effect of these compounds on the scutella excised from the 8 day germinated seedlings may be because of the membrane disintegration and loss of membrane integrity of the scutellum with increased period of germination which was evident from the increased lipid peroxidation when measured as malondialdehyde content.

Studies carried out to demonstrate the interaction between polyamines and guanidines showed that the inhibitory effect of polyamines on peroxidase activity was reversed by guanidines but the polyamines could not reverse the activating effect of guanidines. These compounds though individually activated ATPase activity but did not show any additive effect when tested one after the other. Creatinine which had no free guanidino group had no effect by itself on peroxidase and ATPase and was unable to prevent the polyamine effect indicating that these compounds must be binding to the membranes by amino groups.

Further studies were carried out to see the effect of sulfhydryl compounds on these enzymes and their interaction with spermine and dodine. Sulfhydryl compounds activated both peroxidase and ATPase, They could reverse the inhibitory effect of spermine on peroxidase activity and spermine reversed the activating effect of sulfhydryl compounds. Guanidines when tested alongwith sulfhydryl reagents gave additive effect on peroxidase and ATPase activities. These results indicated that there must be some common binding sites on the membrane between polyamines and guanidines and between polyamines and sulfhydryl reagents. However, nonreversibility of guanidine binding by polyamines or sulfhydryl reagents and the additive response between guanidines and sulfhydryl reagents would indicate the presence of additional sites for guanidines.

Peroxidase and ATPase have been reported to be present in soluble as well as in various membrane fractions. Studies were carried out to see the effect of polyamines and guanidines on peroxidase and ATPase activities from different subcellular fractions. Polyamines inhibited peroxidase activity and activated ATPase activity in all the particulate fractions to varying degrees, the maximum effect being on the plasma membrane fraction. Guanidines activated both peroxidase and ATPase activities in all particulate fractions, the maximum being in the mitochondrial and plasma membrane fractions but none of the compounds tested had any effect on the enzymes present in cytosolic fraction.

Further studies were carried out to see whether the effect of these compounds is specific for maize scutellum or is a general phenomenon. It was found that these compounds have a similar effect on peroxidase and ATPase activities in the subcellular fractions prepared from a number of other tissues such as maize shoot and root, pea embryo, bengalgram embryo and barley root, indicating that the effect of these compounds is a general phenomenon occuring in all plant tissues.

In the later studies when membrane bound peroxidase and ATPase from mitochondrial and plasma membrane fractions were solubilized and tested for the effect of spermine and dodine it was found that these compounds have no effect on the solubilized enzyme. These results indicated that the

compounds can modulate only the membrane bound enzyme and do not have any effect on the enzyme present in the soluble fraction or the solubilized enzyme. This was further confirmed by the peroxidase reconstitution studies where it was found that though spermine and dodine could not prevent the reconstitution when they were bound to membrane, they affected the enzyme activity once the enzyme is reconstituted. The effect of spermine and dodine was same irrespective of the enzyme used for reconstitution i.e. whether it is the solubilized enzyme or the soluble enzyme present in cytosolic fraction. The effect was similar once it is reconstituted indicating that binding of enzyme to the membrane is a must for the compounds to show their effect.

It is well known that phytochrome mediation is a membrane related phenomenon and since polyamines and guanidines also bring their effect by membrane mediation, further studies were carried out to investigate the interaction between polyamines, guanidines and phytochrome. It was found that polyamines could reverse the increase in peroxidase activity brought about by the red light indicating that polyamines act opposite to that of red light. Guanidines, however, activated the enzyme in the control group to the level of red light treated group and the activation brought about by guanidines and red light

could be reversed by far-red light indicating that guanidines and red light must be acting in a similar way. It was also found that the polyamines and guanidines could modulate the phytochrome effect only in the particulate fraction and they have no effect on the enzymes present in the soluble fraction though red light affects enzymes from both particulate and cytosolic fractions.

Studies were also carried out to see the effect of hormones on membrane bound peroxidase and ATPase activities and it was found that except for etherel which inhibited peroxidase activity none of the other hormones had any effect on peroxidase and ATPase activities. The inhibitory effect of ethrel on peroxidase activity could be partially reversed by guanidino acetic acid and dodine but ethrel completely reversed the activating effect of guanidines.