Evidence for the Allosteric Nature of IAA Oxidase System in *Phaseolus mungo* Hypocotyls

The synergistic effect of sodium metabisulphite with IAA in the production of adventitious roots in hypocotyl cuttings of *Phaseolus mungo* reported earlier from this laboratory lends support to the view that IAA effects are caused through IAA oxidation products. This is contrary to the view of other workers, who consider that IAA oxidation causes detoxification of IAA in the plant system.

In in-vitro experiments that are carried out to determine the activity of IAA oxidase in tissue homogenates, the concentrations of IAA that are used as substrate are fairly high (10^{-4} M). If the destruction of IAA in vivo also occurs at this rate, the plant tissue will be depleted of its endogenous IAA within 1–10 min, as its biosynthesis is considered to proceed at a very slow rate. As the maintenance of a proper balance between the synthesis and
degradation of IAA is necessary for normal physiological function in plants, it seems rather improbable that IAA degradation in vivo can occur at the high rates at which it occurs in vitro experiments with tissue homogenates.

The present paper deals with the results of some experiments that were carried out to shed light on this point, using IAA oxidase system in the tissue homogenates of hypocotyls of *Phaseolus mungo*.

Seedlings of *Phaseolus mungo* were raised as described earlier. 3 cm portions below the cotyledonary nodes were used for IAA oxidase activity determined by the method described elsewhere. It was found that the acetone-precipitated proteins that were dissolved in citrate-phosphate buffer at pH 6.0 were able to oxidize only 4.0 μg of IAA in 60 min (Figure 1). The IAA oxidase activity of the acetone-precipitated proteins was then determined at 7 different pH. The activity curve is hyperbolic with a peak at pH 4.0 and decreasing values both with the increasing as well as decreasing pH (Figure 1).

Another experiment was carried out to study the effect of enzyme concentration on its activity in relation to substrate level. 2 concentrations of enzyme proteins, i.e. 437.6 μg and 4,000 μg, were dissolved in citrate-phosphate buffer at pH 4.0 and the concentrations of IAA used as substrate varied from 30 to 150 μg.

The activity curve shows sigmoid tendency with the higher concentration of enzyme proteins (Figure 2). Thus, the oxidation rate increased linearly with the increase in the substrate concentration from 30 to 100 μg IAA but decreased with further increase in substrate level. In marked contrast to this, a considerably higher oxidation rate was recorded with lower (437.6 μg) concentration of the enzyme at lower substrate (30 and 45 μg IAA) levels. However, the activity in this case decreased with the increasing concentration of substrate till 60 μg IAA, beyond which it did not change much.

The results thus show that, at low substrate levels, the rate of IAA oxidation was much higher with lower than with higher enzyme concentrations, although the activity with higher enzyme concentrations exhibited a sigmoid relationship with the change in the concentration of the substrate.

This behaviour of IAA oxidase is characteristic of allosteric enzymes with 2 sites, a primary binding site and a secondary site. This behaviour may be explained on the basis of a model assuming 2 binding sites, or it may be conceived that IAA oxidase system consists of closely associated enzymes, one of which may be an oxidase and the other a peroxidase. These may represent 2 binding sites, as postulated earlier. The primary site has a higher affinity for IAA but low catalytic activity, whereas the secondary site has a low affinity for IAA but high catalytic activity. The secondary site (site II) opens as a result of the allosteric transformation, when the primary site (site I) is saturated. Such a model can explain why IAA oxidation is high at low enzyme concentration with low substrate levels but low at high enzyme concentration with the same substrate level.

This model also explains the two views regarding the role of IAA oxidase in plant systems. Most workers have studied the activity of the enzyme in diluted tissue homogenates in vitro experiments, using high concentrations of IAA as substrate. The high activity that is reported in these experiments is due to low enzyme concentration and represents the activity of site II. This may not be the case in intact cells, as the concentration of IAA available endogenously may not be adequate even to saturate the primary site (site I). Due to allosteric transformations, therefore, IAA oxidase system increases or decreases the rate of IAA degradation, depending upon the amount of IAA available in its vicinity.

The presence of such an enzyme system is very significant as site I, the oxidase site, may be considered to be concerned in the production of oxidation products which cause physiological responses, while site II, the peroxidase, may perform a regulatory role and may cause detoxification of the excessive IAA when present in a plant tissue.