Role of Indol-3-ylacetic Acid in the Induction of Enzymes during Seed Germination

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Biochemical morphogenesis has not attracted the same degree of attention in plants as in animals or in unicellular micro-organisms, notwithstanding the dramatic changes in the growth of plant tissues that occur under the influence of plant hormones. Differentiated single cells cultured in chemically defined media would be the ideal system for the study of the regulation of biosynthesis in plants. However, investigations with whole germinating seeds can also be considered as a practical approach for elucidating the

mechanism of homoeostasis in developing plant tissues. Some of the results obtained on the effect of indol-3-ylacetic acid on enzyme induction in germinating seeds of Bengal gram (*Cicer arietinum*) are presented.

Previous findings on the degradation of phosphatidylcholine during germination of this legume with the concomitant appearance of a membrane-bound phospholipase D (Talwalkar *et al.*, 1964, 1969) have been supplemented by the demonstration of degradation of protein and nucleic acid (Hadi, 1966) as well as the activation of ribosomal function during germination (Hadi & Krishna Murti, 1968).

To explore whether amino acids arising from protein degradation, apart from enriching the amino acid pool, were being channelled into alternative pathways and utilized for the synthesis of physiologically active non-protein nitrogenous compounds, the activities of enzymes known to mediate the metabolism of amino acids were investigated in gram seedlings. Tryptophan oxygenase, glutamate dehydrogenase, tyrosine transaminase and indol-3-ylacetate oxidase were not detectable in the dormant seeds but made their appearance after 48h germination. During this period there was 1000-fold stimulation of peroxidase activity compared with that of the dormant seed as well as significant increases in the pools of soluble carbohydrates and amino acids. Cycloheximide or azauridine, when present in the aqueous medium used for germination, not only inhibited elongation of the seedlings but also suppressed the induction of peroxidase and the accumulation of free carbohydrates and amino acids.

When induction was studied with seedlings *in vitro* incubated with substrate it was found that indol-3-ylacetic acid (1μM) or cyclic AMP (adenosine 3′:5′-cyclic monophosphate) (0.1mM) added exogenously to the medium stimulated tryptophan oxygenase activity two- to three-fold in 48–96h seedlings but did not affect the activity of tyrosine aminotransferase or glutamate dehydrogenase. The stimulation of tryptophan oxygenase activity by indol-3-ylacetic acid *in vitro* was inhibited by actinomycin D and cycloheximide. The stimulation of tryptophan oxygenase activity by indol-3-ylacetic acid or cyclic AMP was accompanied by increased incorporation of DL-[1-^{14}C]valine into proteins. Indol-3-ylacetic acid also stimulated the incorporation of [8-^{14}C]-adenine into cyclic AMP in 48–72h seedlings, from which it was inferred that indol-3-ylacetic acid presumably triggered a mechanism mediating the synthesis of cyclic AMP (Azhar & Krishna Murti, 1971*a*,*b*).

The relative rates of synthesis of cyclic AMP, indol-3-ylacetic acid, peroxidase and tryptophan oxygenase have been followed during germination to establish the correlation, if any, between enzyme synthesis and the appearance of indol-3-ylacetic acid or its mediator cyclic AMP. Purification of tryptophan oxygenase from induced and non-induced seedlings has been carried out to enable investigations on the modulation of its activity by indol-3-ylacetic acid.

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