

STUDIES ON ARGININE METABOLISM
IN GERMINATING SEEDS

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SUMMARY

The investigations reported in this thesis deal with the metabolism of arginine in germinating groundnut (Arachis hypogea L.) seeds with special reference to enzyme arginase, ornithine carbamyl transferase (OCT) and ornithine ketoacid aminotransferase (KAT). The enzyme carbamylating the diamine putrescine, putrescine carbamyl transferase (PCT) was also studied in order to check whether the carbamylation of ornithine and putrescine is carried out by the same enzyme or by two different enzyme proteins.

The protein content of the cotyledon and the embryo increased rapidly during development of groundnut seeds but decreased during germination. The enzyme arginase and OKAT of the cotyledon decrease during development. These enzymes showed very little decrease in the embryo. Storage for one year resulted in a considerable decrease in the activity of these enzymes in the cotyledon as well as embryo. During germination, in the cotyledon, both the enzyme increased upto 4th day of germination and then remained constant thereafter. In the embryo, however, these enzymes decrease after an initial increase around 3rd day of germination.

The enzyme OCT increased during early period of development and remained constant during the later period. In the embryo, the enzyme activity remained almost constant during development. Storage for one year resulted in about 40-50 % decrease in OCT activity in the cotyledon as well as embryo. During germination, the OCT activity of the cotyledon as well as embryo remained unchanged.

PCT activity decreased in the cotyledon and remained unchanged in the embryo during development. During germination, the enzyme decreased in the cotyledon but in the embryo it remained constant after an initial decrease.

The enzymes arginase and OKAT of groundnut cotyledon and embryo were inhibited by plant hormones at early stages of germination. The activity returned to control level with increase in the period of germination in the cotyledon but in the embryo it increased to morethan the control level. OCT and PCT activity of the cotyledon as well as embryo were not affected by plant hormones.

Plant hormones enhance the synthesis of ethylene in groundnut seeds. Experiments carried out with ethrel and chloroethanol, known to produce ethylene, showed effects similar to that of plant hormones. This suggested that the effect of plant hormones may be mediated through ethylene.

Previous studies from this laboratory have shown that plant hormones and ethylene producing compounds inhibit diamine oxidase of groundnut embryo, which would result in an increase in polyamine concentration in the tissue. The increased polyamines inhibit the activity of the enzyme agmatine iminohydrolase, involved in the biosynthesis of polyamines and arginine would then be channelled more for glutamate and proline formation by increase in arginase and OKAT activity. Feeding experiments with polyamines showed increase in arginase and OKAT of embryo.

Experiments carried out with cycloheximide suggested that the increase in the activity of arginase and OKAT in embryo was due to increase in synthesis of these enzymes in presence of plant hormones and ethylene producing compounds.

The regulatory control on the embryonal arginase and OKAT was not influenced by the accompanying cotyledon was demonstrated by cultivating the embryo in a synthetic medium. The pattern of changes in the enzyme activity was same as that for the embryo from whole seeds.

The studies on the differential characteristics of the cotyledon and embryo arginases indicated that these two may be different proteins. The two enzymes were purified separately from cotyledon and embryo to 150 and 40 fold

respectively. Presence of mercaptoethanol was essential for embryo enzyme, but it had no effect on the cotyledon enzyme. The two enzymes also differ in their energy of activation and heat stability.

The enzymes OCT and PCT were purified to about 278 and 133 fold respectively from cotyledons of dry groundnut seeds. It was not possible to separate the two activities during purification, but the ratio OCT/PCT ~~increased~~ considerably during purification. Also these two enzymes showed differences in some of their kinetic properties, like pH optima, affinity towards their respective substrates, heat stability and energy of activation.