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SHORT COMMUNICATION

THE INITIAL METABOLISM OF GERMINATING FERN SPORES

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Abstract—Label from tritiated water is distributed in a manner that suggests that germinating fern spores initially convert amino acids and hydroxy acids into oxo acids. Enzymes capable of catalysing these processes occur in resting spores.

TRITIATED water has been used by Wilson *et al.* to investigate metabolic processes during early germination of seeds[1,2] and pine pollen.[3] Fern spores are homologous to pollen grains, yet like seeds they develop into photoautotrophic plants. Some results of a comparative study are presented here.

Spores of *Cyathea medullaris* (Cyatheaceae), *Polystichum richardii* (Aspidiaceae), *Blechnum capense* (Blechnaceae) and other related species, gave almost identical labelling patterns. Glutamate was the most heavily labelled compound in all species studies, when the time interval was short. Label was also found in aspartate within half an hour of adding water. In all species except *Phymatodes diversifolium* (Polypodiaceae), glutamine, alanine and proline became labelled within 3 hr; malate and citrate also acquired significant label. In *Polystichum richardii* and *Blechnum* spp. asparagine also became labelled. The metabolite heavily labelled by *Phymatodes diversifolium* was not identified; a labelled compound occurs in a similar position on other chromatograms, overlapping the asparagine spot in some cases.

These results differ from those obtained with seeds[1] and pollen[3] in the labelling of proline and asparagine, and in the absence of label from γ-aminobutyrate. However, as in other systems, the initial reactions occurring in fern-spore germination may be ones that generate oxo acids from more stable amino acids and hydroxy acids.

As a result of treating cell-free acetone powders of *C. medullaris* with tritiated water. γ-Aminobutyrate became labelled, suggested that dry spores contain a glutamate decarboxylase; possibly it is not normally active because of subcellular organization. Intact spores of several species remain viable when washed with cold acetone, but after this treatment they incorporate label into γ-aminobutyrate and incorporate less into proline.

Dialysed extracts of an acetone powder of resting *Blechnum lanceolatum* spores were assayed for some enzyme activities that could produce the labelling patterns mentioned above. The results (Table 1) show that as well as high activities of aspartate and alanine

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TABLE 1. ENZYMES DETECTED IN EXTRACTS OF AN ACETONE POWDER OF *Blechnum lanceolatum* SPORES

Enzyme	Activity (nmole/min/mg protein)
Alanine aminotransferase[5]	30
Aspartate aminotransferase[6]	83
Glutamate dehydrogenase (NAD)[7]	6
Malate dehydrogenase (decarboxylating)[8]	7
Isocitrate dehydrogenase (decarboxylating)[9]	17
Isocitrate lyase[10]	0

aminotransferase, there are substantial levels of hydroxy acid dehydrogenases (NADP-linked 'malic' enzyme and isocitrate dehydrogenase), and of a NAD-specific glutamate dehydrogenase. All these give important oxo acid products. The initial incorporation of label into glutamate could be mediated by a transaminase or by glutamate dehydrogenase. The latter provides an alternative to isocitrate dehydrogenase as an initial source of α-oxoglutarate, and could account for the early predominance of label in glutamate.

Thus fern spores, like seeds,[1] may, in the initial phase of germination, convert chemically stable intermediates into metabolically important, but relatively labile, oxo acids.

EXPERIMENTAL

Fertile fern fronds were dried at 25° and the spores were passed through a 180-mesh sieve. Portions of spore material (5–10 mg: about 10^5 spores) were suspended in 50 μl (250 mc) tritiated water (Radiochemical Centre, Amersham, England) for various times, after which the procedures of Spedding and Wilson[1] were followed.

Acetone powders of resting fern spores were prepared by grinding dry spores in acetone at −16°. For enzyme assays, acetone powders were extracted by homogenizing with 0·2 M tris–HCl buffer (pH 7·4), and the extract dialysed. Protein was determined after Lowry *et al.*[4] Each enzyme was assayed at 25° by the method referred to in Table 1.

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