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Biochemistry of Fern Spore Germination

GLYOXYLATE AND GLYCOLATE CYCLE ACTIVITY IN *ONOCLEA SENSIBILIS* L.[1]

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ABSTRACT

In chlorophyll-containing spores of *Onoclea sensibilis*, depletion of lipid reserves during germination is correlated with increases in the activity of the glyoxylate cycle enzymes isocitrate lyase and malate synthase. In *Onoclea*, the heterotrophic activity associated with lipid catabolism occurs at the same time that autotrophic activity is taking place. Increases in chlorophyll content and in the activity of glycolate oxidase were recorded during the earliest stages of spore germination. In this species, there is no temporal separation of heterotrophic and autotrophic reactions. Concurrent increases in glyoxylate and glycolate cycle activities appear to occur naturally.

Considerable information is available concerning the biochemistry of seed germination (2, 9), but comparable information is lacking for fern spore germination. Recent evidence from this laboratory indicated that glyoxylate cycle enzymes were active during germination of spores of the fern *Dryopteris filix-mas* (7). In this species, there was a direct correlation between increases in activity of both isocitrate lysase and malate synthase and the hydrolysis of lipid reserves. As the spore coat ruptured and the photosynthetic prothallus developed, activity of glyoxylate enzymes ceased and an increase in activity of glycolate cycle enzymes was noted (A. E. DeMaggio, unpublished data). The shift from glyoxylate to glycolate metabolism is similar to that occurring during the germination of cucurbit seeds and is characterized by the successive participation of the two unique sets of enzymes (3, 24). The transition from a heterotrophic to an autotrophic pattern of development would appear to be similar in fatty seeds and spores.

There are, however, species of ferns that produce Chl-containing spores and, for these, a different nutritional pattern characterizes germination. Unlike non-green spores, these contain relatively large amounts of H_2O and are known to be metabolically active, short-lived, and capable of germinating readily in contact with the proper medium (17). Spores of *Onoclea sensibilis* L. are representative of this group and they have been used previously in physiological studies of spore germination (5, 19, 23). The lack of an obvious dormant period and the presence of functional chloroplasts indicate that the spores are metabolically distinct from seeds and non-green spores. In this report we present evidence that enzymes of both glyoxylate and glycolate pathways function during spore germination in the light and exhibit simultaneous increases in activity. From these findings, it is suggested that heterotrophic and autotrophic reactions are not temporally separated during germination of *Onoclea* spores but occur concurrently.

MATERIALS AND METHODS

O. sensibilis sporophylls were collected in Hanover, NH, during the spring, 1978. Spores were shaken from air-dried sporophylls and sporangia, sifted through lens paper, and refrigerated at 5 C until used. Spores (100 mg) were shaken in H_2O, to which a drop of Aerosol O.T. (Fisher Scientific Co., Fairlawn, NJ) had been added, and spread on wet filter paper in a Petri dish to germinate. Dishes were maintained in a room at 25 C and exposed daily to a 12-h light period of 250 ft-c provided by a combination of incandescent and fluorescent lamps. Ungerminated spores and spores germinated for specific lengths of time were collected, blotted between filter paper, and weighed and aliquots were counted in a hemocytometer. All subsequent steps were carried out at 4 C. Spores were ground by hand with a mortar and pestle, or in a glass tissue homogenizer, in buffer (6) and the crude homogenate was filtered through three layers of Miracloth before centrifuging at 270*g* for 10 min in a Sorvall RC5 centrifuge. The 270*g* supernatant ordinarily was used to determine total enzyme activity. For some determinations, a particulate fraction also was obtained by centrifuging the 270*g* supernatant at 10,800*g* for 30 min and suspending the pellet in 2 to 3 ml buffer. Changes in the activity of the enzymes examined during spore germination and postgerminative growth were similar in both fractions. Enzymes were assayed on a Gilford model 2000 recording spectrophotometer. ILA[3], MS, and GO were assayed as described (6, 11). Total lipids were estimated gravimetrically (10) and Chl was determined according to Arnon (1). Spores for electron microscopy were prepared and processed in the usual manner (22).

RESULTS

Characteristics of Spore Germination. Mature spores of *Onoclea* contain abundant chloroplasts and both lipid and protein storage products occupy the sparse cytoplasm (Fig. 1). During germination and postgerminative growth, the content of reserve lipid decreased (Fig. 2). As in other fern spores (7) and seeds (3, 24), there is a period during the early stages of germination when

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[3] Abbreviations: ILA, isocitrate lyase (EC 4.1.3.1); MS, malate synthase (EC 4.1.3.2); GO, glycolate oxidase (EC 1.1.3.1).

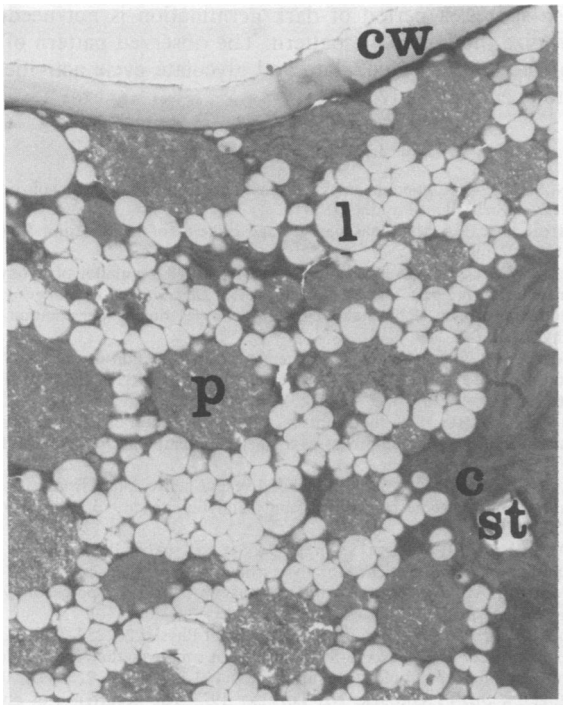

FIG. 1. Electron micrograph of *Onoclea sensibilis* spore germinated in the light for 2 days. cw, cell wall; l, lipid; p, protein; st, starch. × 8000.

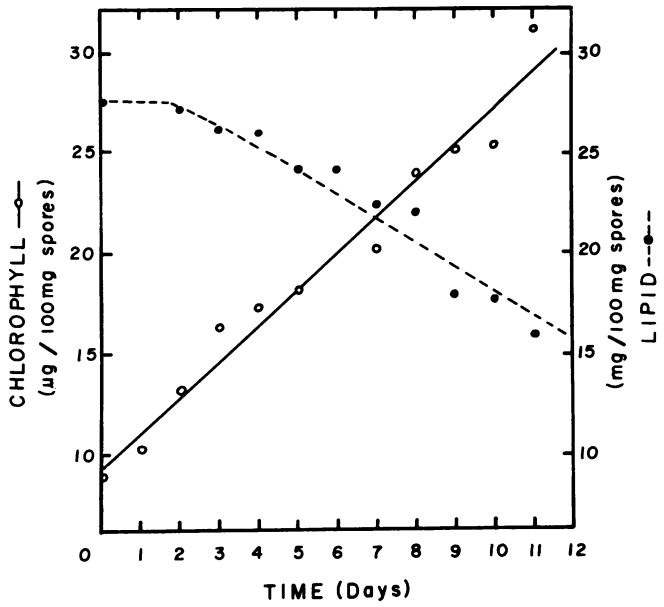

FIG. 2. Changes in the content of Chl and lipid during spore germination and postgerminative growth.

storage products are hydrolyzed and provide materials for the synthetic activities in early development. During the first few days of germination, the spores imbibed H_2O and enlarged as greening intensified. An ultrastructural examination of spores during hydration and early stages of germination indicates that considerable internal cytoplasmic activity took place during this time (8). The first obvious sign of germination, rupture of the spore coat, occurred after 5 days. The rhizoid and prothallial cells emerged from the spores at this time and continued their development. Under the growing conditions employed, multicellular filamentous prothalli were produced by 12 days.

Heterotrophic Reactions. As morphological changes in the spores occurred, the amount of reserve lipid gradually and consistently decreased (Fig. 2). Approximately 17% of the reserve lipid was hydrolyzed by the time the spore coat had ruptured at 5 days. At the end of 2 weeks, one-half of the stored lipid had been metabolized.

Glyoxylate cycle enzymes were present early during germination (Fig. 3). Appreciable levels of MS could be detected in ungerminated spores and small but measurable amounts of ILA also were observed. After 5 days germination, the activities of both enzymes had increased significantly. The activity of MS in the spores at that time had approximately tripled and ILA activity had increased 5-fold (Fig. 3). These increases took place while lipid reserves were being reduced and spores were undergoing internal differentiation prior to rupture of the spore coat. Maximum levels of activity for both enzymes appeared to be correlated with emergence of the rhizoid and prothallial cells from the spores after 5 days. ILA and MS levels then started to decline. At 7 days, the level of ILA was only 29% of that present at 5 days. On the other hand, MS activity decreased to 77% of that on day 5. By the 8th day, neither ILA nor MS could be detected. At that time, the spores had produced short photosynthetic filamentous prothalli and were capable of autotrophic growth.

Autotrophic Reactions. Chl was present in the ungerminated spores and its concentration increased during germination and postgerminative growth (Fig. 2). The amount of Chl in the spores had more than doubled before the spore coat opened and the rhizoid and prothallus emerged. The Chl content continued to increase linearly as the filamentous prothalli grew and became multicellular.

Measurements of photosynthetic activity were not performed but data are available indicating that the spores are photosynthetically competent (23). Starch grains were observed in many of the chloroplasts (Fig. 1) and increased starch deposition during germination was noted. These observations provide additional evidence that the chloroplasts are functional early during germination.

The glycolate pathway was detected by assaying spores for the peroxisomal enzyme GO. Low levels of enzyme activity were present in ungerminated spores and activity increased during germination (Fig. 3). By 5 days, enzyme activity had almost tripled and, on the 7th day, it exceeded that of both ILA and MS. Although data on enzyme activities beyond 7 days are not included here, they showed that GO activity continued to increase linearly to day 11. The spore coat by then had ruptured and a filamentous, photosynthetic prothallus had developed.

DISCUSSION

The depletion of lipid reserves in *Onoclea* spores appears to be closely correlated both with an increase in activity of glyoxylate

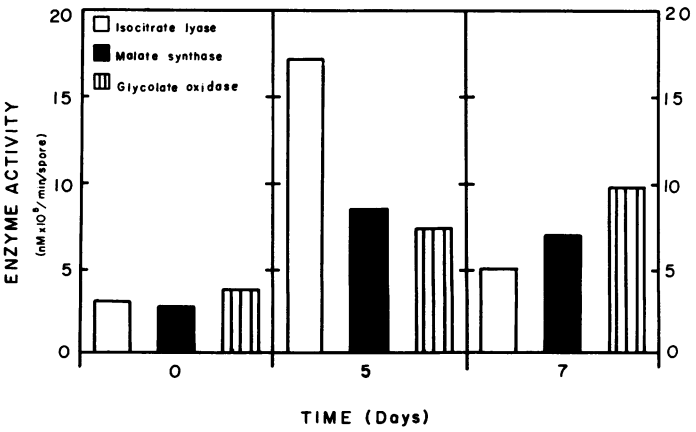

FIG. 3. Changes in activity of glyoxylate cycle enzymes (ILA and MS) and a glycolate cycle enzyme (GO) during spore germination and postgerminative growth.

cycle enzymes and early stages in spore germination. A parallel series of events also has been detected in non-green spores of *D. filix-mas* (7). Other investigators (12, 20) have called attention to the association of diminished lipid level and fern spore germination. In the observations presented here, both MS and ILA are active during spore germination and postgerminative growth. Slight activity of both enzymes could be demonstrated beginning on day 0. Both enzymes have also been detected in resting spores of *Anemia phyllitidis* (13), *Pteris vittata* (14), and *D. filix-mas* (7). However, Miernyk *et al.* (18) found measurable MS activity in resting *Onoclea* spores but were unable to detect ILA. Our experience with *Onoclea* suggests that activity of these enzymes diminishes as the spores age. The inability to measure ILA in resting spores (18) may be attributed to the length of time the spores were stored. Nevertheless, the presence of the glyoxylate cycle in the spores suggests that catabolism of lipids provides a source of materials and energy for early germination as it does in certain seeds (3, 9, 24).

In greening cotyledons, the appearance of GO activity coincides with the development of photosynthetic activity (3, 15, 24). In *Onoclea*, the enzyme is present in green, ungerminated spores and, during the initial stages of germination, its activity increases with the increase in Chl content. In non-green fern spores, *e.g. D. filix-mas* and *Thelypteris dentata* (21), Chl could not be detected until the spores had germinated and produced a prothallus. Not until this stage in development were levels of GO activity high enough to be measured (14). The presence of the enzyme in *Onoclea* spores and the observed change in activity as germination proceeded indicates that in this species photorespiration may be active from the start of germination.

Unlike non-green spores and most seeds, *Onoclea* spores exhibit simultaneous increases in both heterotrophic and autotrophic reactions. During initial stages of germination of these spores, the activity of enzymes in both glyoxylate and glycolate pathways increased. While lipid reserves were being depleted, the activity of MS and ILA increased. Simultaneously, there was an increase in GO activity. Ordinarily, the change from a heterotrophic to an autotrophic growth pattern in spores and fatty seeds is signaled by the gradual disappearance of glyoxylate cycle enzymes. Only then do the enzymes characteristic of the glycolate pathway appear.

Enzymes of the glyoxylate cycle are not commonly found in leaves of higher plants. Their functioning in chlorophyllous tissue is unusual when glycolate enzymes are active. However, the simultaneous appearance of high levels of glyoxylate and glycolate cycle enzyme activities has been experimentally induced in fatty cotyledons (3, 4, 15). More recently, Köller and Kindl (16) demonstrated that cucumber seeds kept for 3 days in the dark and then illuminated produced a co-ordinated increase in both MS and GO activities. It is possible to manipulate the environmental conditions during germination to regulate effectively the time of appearance and duration of glyoxylate and glycolate activities. In *Onoclea* spores, a period of dark germination is not needed to synchronize the metabolic pattern. The observed pattern of concurrent increases in glyoxylate and glycolate cycle activities appears to occur naturally.

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