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PEROXISOMAL ENZYME ACTIVITY IN *TODEA BARBARA* GAMETOPHYTES AND SPOROPHYTES

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Abstract—Catalase and glycolate oxidase activity were observed in cultured gametophytes and sporophytes of the fern *Todea barbara* (L.) Moore. The biochemical characteristics of the glycolate-oxidizing enzyme in both plants indicates it is a glycolate oxidase. The results suggest that these plants are capable of photorespiration by a process similar to that occurring in leaves of higher plants.

INTRODUCTION

Two types of microbodies have been distinguished in higher plants on the basis of their biochemical constituents, glyoxysomes and peroxisomes. Glyoxysomes are primarily located in the endosperm or cotyledons of germinating fatty seedlings and contain enzymes for the degradation of fatty acids [1]. Peroxisomes are found predominantly in leaves and contain essential enzymes of the glycolate pathway which is involved in photorespiration [2]. Considerable information is available concerning the morphological and biochemical characteristics of both types of microbodies; their origin, function and transition from one type to another [3–5]. For the most part, this information has been obtained from studies utilizing flowering plants and conifers and little attention has been given to studying these organelles in ferns. Breidenbach [6] has called attention to the lack of biochemical evidence to support the cytological identification of microbodies in pteridophytes. The present investigation was undertaken to determine the nature of the microbodies in the thalloid, photosynthetic gametophytes and leafy sporophytes of a fern.

The sporophytes of several species of ferns are known to contain both catalase (EC 1.11.1.6) and glycolate oxidase (EC 1.1.3.1) activity and the suggestion has been made that these plants contain peroxisomes and are capable of participating in photorespiratory carbon metabolism [7, 8]. In fern gametophytes microbodies have been observed by electron microscopy [9–12] but there are no biochemical data available to identify them as glyoxysomes or peroxisomes. We recently examined the microbodies in germinating spores of *Dryopteris filix-mas* and, based on the close correlation between increased activity of glyoxysomal enzymes and lipid catabolism, we identified them as glyoxysomes [13]. This is the only report we are aware of that identified malate synthase (EC 4.1.3.2) and isocitrate lyase (EC 4.1.3.1) as components of microbodies in the haploid (ln) generation of ferns. We present here additional information indicating that catalase and glycolate oxidase, a central enzyme of the glycolate pathway and a characteristic peroxisomal enzyme, are present in both gametophytes and sporophytes of the fern *Todea barbara*.

RESULTS AND DISCUSSION

Low levels of catalase were consistently observed in preparations from gametophytes (Table 1). The concentration varied in the different preparations assayed and some of the factors responsible were the effectiveness of the grinding procedure, the length of time between transfer to fresh nutrient medium, and lengthy delays between tissue preparation and enzyme assay. Efforts were taken to minimize these effects. However, because of the variations obtained, the data from assays using plants cultured for different lengths of time were not combined. The data presented represent averages of duplicate assays of homogeneous material.

Catalase activity in the sporophytes was noted to be more than 50 times higher than that found in the gametophytes (Table 1). The sporophytes were generally 7–12 cm tall and when collected to be assayed bore several leaves characteristic of the sporeling stage. The leaves had large, vacuolate, irregularly shaped cells in contrast to the smaller, densely cytoplasmic cells that comprise most of the thalloid gametophyte. Protein content of the sporophytes was 2.4 mg/g fr. wt, while in the gametophyte, protein was only 0.6 mg/g fr. wt. Therefore, when catalase activity was expressed as activity/mg protein (Table 1) a 13-fold difference between activity in gametophytes and sporophytes was noted.

Table 1. Glycolate oxidase and catalase activity in gametophytes and sporophytes of *Todea*

	Glycolate oxidase activity*		Catalase activity†	
	g fr. wt	mg protein	g fr. wt	mg protein
Gametophyte	2.13	3.55	11.6	19.8
Sporophyte	7.56	3.16	619	258

* 1 Unit of activity = 7.97×10^{-4} nkat [14].
† 1 Unit of activity = K/sec [23].

Extremely low activity for glycolate oxidase was observed in gametophytes and sporophytes (Table 1). The activity, however, was localized in the particulate fraction indicating that it was membrane-bound, presumably in peroxisomes. In this study we were primarily interested in determining whether a glycolate-oxidizing enzyme was present in the plants, especially in the gametophytes, and if the enzyme was an oxidase or a dehydrogenase [7]. Quantitative recovery of the enzyme was not judged necessary. Nevertheless, considerable enzyme activity was located in the 11 000 rpm supernatant fraction although this is not included in the data of Table 1. As previously demonstrated [14], special precautions are necessary to prevent damage to the microbodies and liberation of the enzymes into the soluble fraction during tissue preparation.

Glycolate oxidase has been found in angiosperms, gymnosperms, and in sporophytes of ferns [6–8]. To our knowledge the enzyme has not been reported previously in fern gametophytes. However, the enzyme is known to be present in certain species of algae [15]. It is considered to be a flavoprotein with riboflavin phosphate (FMN) as its co-factor [16].

Another glycolate-oxidizing enzyme, glycolate dehydrogenase, has been identified in some groups of algae [17]. Although little is known about the structure of this enzyme, it has been localized in mitochondria [18] and is known to differ from glycolate oxidase in a number of biochemical properties [19].

Attention has been given to the distribution of the oxidase and the dehydrogenase among various groups of plants, and some effort has been made to determine whether or not there has been an evolutionary divergence of the glycolate-oxidizing enzymes [20]. Information is available which suggests that the distribution of the two enzymes is phylogenetically determined [7]. Therefore, it was of interest to examine *Todea* gametophytes as well as sporophytes to determine which of the glycolate-oxidizing mechanisms was active in these plants.

Several biochemical characteristics of the enzyme were examined to determine whether an oxidase or dehydrogenase was present (Table 2). The results of the assays for lactate stereospecificity indicate that, in sporophytes when L-lactate was the substrate, the enzyme activity was similar to that obtained when glycolate was provided as the substrate. In addition, there was no oxidation of D-lactate. According to Tolbert [19] and others [7], stereospecific preference for L-lactate is one of the principal criteria for the presence of an oxidase. In the gametophytes, the oxidation of L-lactate was 50% that of glycolate while the oxidation of D-lactate was, in different experiments, as low as 30% and as high as 43% that of glycolate. In primitive green algae the presence of glycolate dehydrogenase is indicated by the preferential oxidation of D-lactate while in higher plants the glycolate oxidase only oxidizes L-lactate. Our data imply that the gametophytes had enzymes which acted on both substrates. However, further experience with the assay indicates that the oxidation of D-lactate by gametophytes is probably an artifact caused by endogenous non-specific oxidation of the dye used in this assay. High endogenous activity has been reported for sporophytes [7].

Glycolate oxidase and glycolate dehydrogenase also could be distinguished by their different sensitivities to 2 mM KCN. Frederick *et al.* [7] found that the oxidase was insensitive to cyanide but the dehydrogenase was inhibited. In our studies the addition of KCN did not inhibit the oxidation of glycolate in either gametophytes or sporophytes. Furthermore, when the assays were conducted under aerobic rather than anaerobic conditions, glycolate-oxidizing activity was greatly reduced. Taken together, the results from substrate oxidation, cyanide inhibition, and oxygen sensitivity provide evidence for the activity of glycolate oxidase in both gametophytes and sporophytes.

Glycolate oxidase is a common marker enzyme for peroxisomes. The presence and activity of the enzyme in particulate fractions of gametophytes and sporophytes indicates that the catalase containing microbodies are of the peroxisomal type. Glyoxysomes have been identified in germinating fern spores [13]. However, the activity of the glyoxysomal enzymes isocitrate lyase and malate synthase diminishes rapidly after the filamentous prothallus develops. Measurable

Table 2. Characteristics of glycolate-oxidizing enzymes in *Todea*

Plant	Substrate	Samples averaged	Activity*
Gametophyte	Glycolate	4	2.13
	L-Lactate	2	1.29
	D-Lactate	2	0.90
Sporophyte	Glycolate	4	7.56
	L-Lactate	1	7.75
	D-Lactate	1	0

* 1 Unit of activity = 7.97×10^{-4} nkat [14].

levels of these glyoxysomal enzymes cannot be detected in mature gametophytes or sporophytes. Although there have been suggestions, based primarily on cytological observations, that peroxisomes exist in thalloid, green gametophytes, peroxisomal enzyme activity has only been reported in the adult sporophytes [7, 8]. This is not the case for bryophytes where glycolate oxidase activity has been measured in moss protonema [21]. In addition, CO_2 compensation points and rates of $^{14}CO_2$ release in light at different O_2 concentrations have been measured in a number of bryophyte gametophytes [22]. The evidence strongly suggests that these plants show the same pattern of photorespiration as higher plants with C_3 photosynthesis.

Results using similar techniques of gas analyses have not been reported for fern gametophytes but some data on the respiratory activity in a variety of fern sporophytes are available [8]. In all but one of the species examined the plants showed characteristics representative of C_3 plants, including high photorespiratory rates. The results presented here complement these earlier studies and demonstrate that peroxisomes can function during the growth of *Todea* gametophtes as well as during the growth of the leafy sporophyte. It is reasonable to assume that the glycolate produced during photosynthesis by these plants (DeMaggio, unpublished) can be metabolized by photorespiratory processes in the manner described for leaves of higher plants.

EXPERIMENTAL

Preparation of cell extract. Gametophyte and sporophyte of *T. barbara* were maintained on solid Knudon's medium in a controlled light and temp. chamber, with routine subculturing as previously described [10]. Plants used for enzyme extraction were collected at various times, weighed and carefully washed to remove all traces of medium. The plants were then finely chopped with a razor blade and ground in a chilled mortar in 0.5 M sucrose with 0.05 M K-Pi, pH 7.5 The ground material was periodically examined with a microscope and grinding continued until most of the cells were broken. The homogenate was passed through 8 layers of cheesecloth, the pellet discarded and the supernatant centrifuged at 1000 rpm for 20 min. The combined supernatants were centrifuged at 11 000 rpm for 30 min and the pellet suspended in a measured vol. of grinding medium and used for the assays.

Biochemical assays. Catalase assay was carried out as previously described [23]. Glycolate oxidase was assayed anaerobically using 2,6-dichlorophenolindophenol (DCPIP) as the electron acceptor [7]. The reaction was carried out in a Thunberg cuvette and the reagents added in the following order: 1.95 ml 200 μM K-PPi buffer, pH 8.5; 0.1 ml DCPIP (3×10^{-3} M); 0.1 ml FMN (2×10^{-3} M); 0.05 ml enzyme prepn; 0.1 ml Triton-X 100 (10%). Buffer was added to give a final vol. of 2.5 ml. Na glycolate (0.2 ml), pH 10, was added to the side-arm of the cuvette. The cuvette was evacuated and flushed ×3 with N_2 passed through Fieser's soln (16 g Na hydrosulfite, 6.6 g NaOH, 2 g anthraquinone B-sulfonate per 100 ml) to remove remaining O_2. Endogenous reduction of DCPIP by the enzyme was determined by measuring the decrease in A at 600 nm. The substrate in the side-arm was then added and further reduction in A_{660nm} was recorded. Enzyme activity was determined by subtracting the endogenous oxidation from the glycolate oxidation. A change of 1 A_{660nm} unit/min was equivalent to 4.78 nmol of reduced dye. In some cases 20 μM D- or L-lactate replaced glycolate as the substrate. When the prepn was tested for cyanide sensitivity, 2 mM KCN was added to the reagents in the cuvette.

Protein was determined according to established methods [24].

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