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The Occurrence of Glycolate Dehydrogenase and Glycolate Oxidase in Green Plants

AN EVOLUTIONARY SURVEY[1]

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ABSTRACT

Homogenates of various lower land plants, aquatic angiosperms, and green algae were assayed for glycolate oxidase, a peroxisomal enzyme present in green leaves of higher plants, and for glycolate dehydrogenase, a functionally analogous enzyme characteristic of certain green algae. Green tissues of all lower land plants examined (including mosses, liverworts, ferns, and fern allies), as well as three freshwater aquatic angiosperms, contained an enzyme resembling glycolate oxidase, in that it oxidized L- but not D-lactate in addition to glycolate, and was insensitive to 2 mM cyanide. Many of the green algae (including *Chlorella vulgaris*, previously claimed to have glycolate oxidase) contained an enzyme resembling glycolate dehydrogenase, in that it oxidized D- but not L-lactate, and was inhibited by 2 mM cyanide. Other green algae had activity characteristic of glycolate oxidase and, accordingly, showed a substantial glycolate-dependent O_2 uptake. It is pointed out that this distribution pattern of glycolate oxidase and glycolate dehydrogenase among the green plants may have phylogenetic significance.

Activities of catalase, a marker enzyme for peroxisomes, were also determined and were generally lower in the algae than in the land plants or aquatic angiosperms. Among the algae, however, there were no consistent correlations between levels of catalase and the type of enzyme which oxidized glycolate.

The production of glycolate in chloroplasts during photosynthesis and its metabolism in peroxisomes via the "glycolate pathway" constitute central aspects of photorespiratory carbon metabolism in higher plants (47). Glycolate is synthesized also by the green algae (49), and a complete glycolate pathway has been demonstrated in certain unicellular species of this group (3, 27, 32). There seem to be some dissimilarities, however, between the glycolate pathway of the algae and that of higher plants: one major difference lies in a key enzyme, that which oxidizes glycolate to glyoxylate. In higher plants this enzyme, termed "glycolate oxidase," is a flavoprotein which transfers electrons to molecular O_2 to form H_2O_2. In addition to glycolate, this oxidase can oxidize L-lactate but not D-lactate, and it is insensitive to cyanide. The enzyme reported in unicellular algae, on the other hand, does not utilize molecular O_2 as an immediate electron acceptor and has been termed "glycolate dehydrogenase" (5, 33). Moreover, it can oxidize D-lactate but not L-lactate, and it is inhibited by cyanide. Glycolate oxidase and glycolate dehydrogenase may also differ in their subcellular localization. Whereas glycolate oxidase characteristically occurs together with catalase in the peroxisomes (microbodies) of angiosperm leaves (50), the localization of glycolate dehydrogenase has not yet been established; nor is it even certain that organelles resembling leaf peroxisomes exist to any great extent in autotrophically growing green algae.

The plants which have been studied to date with respect to the glycolate oxidizing enzymes, *i.e.* unicellular green algae and various angiosperms, represent opposite extremes as regards evolutionary specialization toward structural complexity. Apart from a preliminary survey of several plants (33), little is known about which of these enzymes, if either, is present in the many green plant groups of intermediate evolutionary development. In this perspective, we have investigated the distribution of glycolate dehydrogenase and glycolate oxidase among various forms of green algae, bryophytes, ferns, and fern allies. To provide supplementary information which may be relevant to the status of peroxisomes in these plants, we have also examined levels of catalase, an important peroxisomal marker. The information gained from these studies should provide a useful framework for future considerations of glycolate metabolism in diverse plant groups and may be helpful eventually in reconstructing the phylogeny of the peroxisome as an organelle involved in glycolate metabolism.

MATERIALS AND METHODS

Plant Materials. Most algae were obtained from our laboratory cultures of stocks originating from the Indiana University Culture Collection (44, 45), and all were grown autotrophically on mineral media. Indiana stocks and media used for their culture included *Protosiphon botryoides* (Kütz.) Klebs (No. 99) and *Chlorella pyrenoidosa* Chick (No. 395), medium of Norris *et al.* (36); *Dunaliella tertiolecta* Butcher (No. LB 999), McLachlan medium (31); *Eremosphaera viridis* DeBary (No. LB 34), Waris solution (44) + 10% soil water; *Spirogyra varians* (Hass.) Kütz. (No. LB 479), Hughes medium (22); *Hormidium flaccidum* A. Br. (No. 322, hereafter referred to by the newer generic designation of *Klebsormidium*), "Bold's Basal Medium" (35); *Stigeoclonium helveticum* var. *maius* (No. 441) and *Ulothrix fimbriata* Bold (No. 638), "Tris Buffered Inorganic Medium" (43); *Coleochaete scutata* Bréb (No. LB

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610), Juller's liquid medium + soil extract (41); and *Microspora sp.* (No. LB 472), modified Hill's medium (38). A stock culture of *Chlorella vulgaris* (211-11h) was supplied by Dr. W. Kowallik (26) and grown in Norris medium (36). All of the algae listed above were grown as axenically as possible in 100-ml or 1000-ml quantities of medium contained in 250-ml Erlenmeyer flasks or 2800-ml Fernbach flasks. Cultures were maintained in a growth chamber at 18 to 20 C, and approximately 600 ft-c of light was supplied by a combination of incandescent and fluorescent bulbs. Some cultures were shaken and some were continuously aerated. *Codium sp., Enteromorpha sp.,* and *Ulva sp.* were collected by John Burris at La Jolla, California. *Nitella sp.* was shipped from Northwest Biological Laboratories, Ltd. (Victoria, British Columbia), while *Oocystis polymorpha* Groover et Bold was grown and supplied by John Gerrish at Michigan State University. *Netrium digitus* was supplied by Harvey Marchant, University of Colorado.

Sources of nonalgal species were as follows: *Athyrium sp., Equisetum sp., Leptodictyum riparium, Mnium sp., Onoclea sensibilis* L., *Osmunda regalis* L., and *Polytrichum sp.* were collected during the summer months near East Lansing, Michigan; *Psilotum nudum* was obtained from the Department of Botany greenhouse at Michigan State University; *Myriophyllum sp., Elodea sp., Marchantia sp., Selaginella apoda, Porella sp., Riccia sp., Pellia sp.,* and *Anthoceros sp.* were obtained from Carolina Biological Supply Co., Burlington, North Carolina; *Lemna minor* L., cultured on a mineral medium, was supplied by Paul Kindel of this department.

Preparation of Cell-free Extracts. Homogenization procedures varied according to the type of plant material, but the grinding medium generally used was 0.05 M K-phosphate, pH 7.0. Insoluble polyvinylpyrrolidone (10% w/w) was routinely added to this medium for *Spirogyra* and most of the nonalgal species.

Unicellular algae and *Coleochaete* were collected by centrifugation, washed several times with deionized water, and suspended in the grinding medium to a final volume 5 to 10 times that of the packed cells. The algal cells were then broken by passing the suspensions through a precooled French pressure cell at 8,000 to 16,000 p.s.i. one to three times, depending upon the ease of breakage.

Filamentous algae were collected by gentle suction filtration and washed by passing a large volume of deionized water through the filter. The filaments were fragmented by grinding for a short time with a chilled mortal and pestle, and the cells were ruptured by passing the suspension through a chilled French pressure cell. An exception to this procedure was *Spirogyra,* which was homogenized by grinding for several minutes with sand in a chilled mortar.

The larger algae (*e.g. Ulva, Codium,* and *Nitella*) and green tissues of land plants and aquatic angiosperms were washed in running tap water and in several changes of deionized water, then were blotted on filter paper and cut into small pieces. They were ground with or without sand in a chilled mortar and preparations were passed through four layers of cheesecloth.

All homogenates were centrifuged at 300*g* or 500*g* for 10 min to remove cell debris, and these supernatants were used in the enzyme assays. Exceptions were *Equisetum* and *Osmunda,* in which the 500*g* supernatants proved unsatisfactory for the glycolate oxidase assay because of extremely high endogenous rates. For these species, the 500*g* supernatants were centrifuged at 10,000*g* for 10 min, and the pellets, resuspended in grinding medium, were used in the glycolate oxidase assay.

Enzyme Assays. Glycolate dehydrogenase and glycolate oxidase were assayed by following the anaerobic reduction of DCPIP[3] at 600 nm at 25 C with a Gilford recording spectrophotometer (33). The 2.5-ml volume contained 0.08 Na-pyrophosphate buffer, pH 8.5, 0.12 mM DCPIP, 0.01% Triton X-100, 0.1 mM FMN, and either 8 mM glycolate (Na salt), 20 mM D(−)-lactate (Li salt), or 20 mM L(+)-lactate (Li salt). Reactions were initiated by addition of the substrate, after reading the endogenous rates for 5 min. To test for sensitivity to O_2 and to cyanide, assays were run aerobically or in the presence of 2 mM KCN, respectively. Activities are generally reported relative to the rates observed with glycolate. Specific activities were also determined, but these values are only moderately instructive since extraction efficiency differed from species to species, since the concentration of DCPIP may not have been saturating (48), and since varying amounts of enzyme inactivation likely occurred during grinding.

Manometric assays for glycolate oxidase were carried out in Warburg respirometers. Reaction vessels contained 2.5 ml of algal homogenate (in 0.05 M K-phosphate at pH 8.0 to 8.5), 0.05 ml of 5 mM FMN (final concentration of approximately 0.1 mM), wicks saturated with 30% KOH in the center well, and 0.2 ml of either 0.2 M glycolate, 0.5 M D-lactate, 0.5 M L-lactate, or deionized water (control) in the side arm. Flasks were shaken in the dark in a 25 C water bath. The reaction was initiated by tipping in the substrate 10 min after the system was closed to the atmosphere. Readings were taken at 10-min intervals, and rates of O_2 uptake were computed for the first 30 min of reaction time.

Catalase was assayed spectrophotometrically at 25 C by following the disappearance of H_2O_2 at 240 nm (30). Protein was determined by the method of Lowry *et al.* (29).

RESULTS

Spectrophotometric Detection of Glycolate Oxidase and Glycolate Dehydrogenase. Crude homogenates of the various plant species were assayed anaerobically for activity of glycolate oxidase and glycolate dehydrogenase by utilizing DCPIP as an artificial electron acceptor. A preparation was judged to contain glycolate oxidase if it oxidized glycolate and L-lactate but not D-lactate, and if this activity was not inhibited by 2 mM KCN. The presence of glycolate dehydrogenase was indicated by the oxidation of glycolate and D-lactate, and an inhibition by 2 mM KCN. A useful but less rigorous distinguishing criterion was whether or not inhibition of initial DCPIP reduction occurred when the assay was carried out aerobically. When glycolate oxidase is present, a greatly decreased rate of DCPIP reduction occurs under aerobic conditions, because this enzyme utilizes to some extent O_2 instead of DCPIP as electron acceptor and the product, H_2O_2, in turn reoxidizes the reduced DCPIP (48). In the case of glycolate dehydrogenase, which does not transfer electrons directly to molecular O_2, the anaerobic rate is largely retained when the reaction is run aerobically.

All of the various groups of lower land plants examined, including leafy and thallose liverworts, mosses, ferns, club mosses, horsetails, and psilopsids, contained glycolate oxidase, as judged by the criteria outlined above. These land plants showed maximal activity with glycolate as substrate (Table I), with specific activities ranging from 5 nmoles/min·mg protein in *Marchantia* to 12 nmoles/min·mg protein in *Leptodictyum.* Activity with L-lactate was also present in these plants, and varied from 32 to 100% of that with glycolate. Activity with D-lactate was detectable only in four of the land plants tested

[3] Abbreviations: DCPIP: 2,6-dichlorophenolindophenol; DAB: diaminobenzidine; FMN: flavin mononucleotide.

Table I. *Characteristics of the Glycolate-oxidizing Enzymes of Lower Land Plants, Aquatic Angiosperms, and Green Algae*

Taxonomic Group and Species	Type of Glycolate-oxidizing Enzyme	Activity Relative to Glycolate			Inhibition by 2 mM KCN			Inhibition by O_2
		Glycolate	L-Lactate	D-Lactate	Glycolate	L-Lactate	D-Lactate	Glycolate
		%			%			%
Hepaticae (liverworts)								
Marchantia sp.	Oxidase	100	100	0[1]	12	6	. . .	100
Porella sp.	Oxidase	100	100	19	0	0	. . .	>60
Pellia sp.	Oxidase	100	89	0	—[2]			—[2]
Musci (mosses)								
Leptodictyum riparium	Oxidase	100	67	0	0	0	. . .	100
Polytrichum sp.	Oxidase	100	62	18	0	9	. . .	>60
Psilopsida								
Psilotum nudum	Oxidase	100	33	0	10	10	. . .	>60
Lycopsida								
Selaginella apoda	Oxidase	100	97	18	0	0	. . .	100
Sphenopsida (horsetails)								
Equisetum sp.	Oxidase	100	78	19	0	0	. . .	>60
Pteropsida (ferns)								
Osmunda sp.	Oxidase	100	75	0	30	32	. . .	>60
Athyrium sp.	Oxidase	100	71	0	0	10	. . .	>60
Onoclea sensibilis	Oxidase	100	85	0	0	0	. . .	100
Aquatic angiosperms								
Elodea sp.	Oxidase	100	100	0	0	0	. . .	100
Myriophyllum sp.	Oxidase	100	72	0	0	0	. . .	100
Lemna minor	Oxidase	100	32	0	0	0	. . .	>60
Chlorophyta (green algae)								
Chlorella pyrenoidosa	Dehydrogenase	100	0	100	100	. . .	100	0–40
Chlorella vulgaris	Dehydrogenase	100	0	98	96	. . .	97	0–40
Dunaliella tertiolecta	Dehydrogenase	100	0	347	96	. . .	95	0–40
Eremosphaera viridis	Dehydrogenase	100	0	180	100	. . .	94	0–40
Oocystis polymorpha	Dehydrogenase	100	20	94	91	. . .	90	0–40
Protosiphon botryoides	Dehydrogenase	100	0	290	83	. . .	100	0–40
Codium sp.	Dehydrogenase	100	0	135	100	. . .	100	0–40
Microspora sp.	Dehydrogenase	100	18	190	100	. . .	100	0–40
Stigeoclonium helveticum	Dehydrogenase	100	10	126	95	. . .	92	0–40
Coleochaete scutata	Oxidase	100	67	0	8	6	. . .	100
Klebsormidium flaccidum	Oxidase	100	54–100	0	. . .	. . .	. . .	100
Nitella sp.	Oxidase	100	75	0	2	8	. . .	100
Spirogyra varians	Oxidase	100	59	10	5	6	. . .	>60
Netrium digitus	Oxidase	100	92	18	0	11	. . .	100

[1] "0" indicates activity absent or too low to be measured accurately.
[2] Not examined.

and in these it was low, being less than 20% of the activity with glycolate and less than 30% of that with L-lactate. It is possible that part of the activity measured with D-lactate resulted from contaminating microorganisms, which may contain a D-lactate dehydrogenase (7, 23, 24).

In almost all of the lower land plants listed in Table I, activities with either glycolate or L-lactate showed no significant inhibition by 2 mM cyanide. In some species (including *Marchantia, Psilotum, Equisetum,* and *Osmunda*) the presence of cyanide interfered with the assays of crude homogenates by causing abnormally high endogenous rates; these data are therefore less reliable than those from other species. Oxygen greatly inhibited the activity with DCPIP in all of the lower land plants. In several of these samples, DCPIP reduction could not be measured at all under aerobic conditions, whereas in the others, the initial rates were quite low (generally less than 25% of the anaerobic rates) and usually decreased to zero within the first 5 min. In a few of the homogenates tested (*Anthroceros, Riccia, Mnium*), activity with neither glycolate oxidizing enzyme could be detected, but it is probable that these negative results reflect an inactivation of enzymes during grinding.

In addition to lower land plants, two submerged aquatic angiosperms, *Elodea* and *Myriophyllum,* and one floating aquatic angiosperm, *Lemna,* were found to contain glycolate oxidase (Table I). Homogenates of *Myriophyllum* and *Elodea* had low specific activities (3 and 5 nmoles/min·mg protein, respectively), while that of *Lemna* had a specific activity about 10-fold higher.

It is apparent that the green algae differ among themselves with respect to the type of glycolate oxidizing enzyme (Table I). Some species, including both *Chlorella pyrenoidosa* and *Chlorella vulgaris, Dunaliella, Eremosphaera, Oocystis, Protosiphon, Codium, Microspora,* and *Stigeoclonium,* seemed to possess glycolate dehydrogenase in that they oxidized D- but not L-lactate (in addition to glycolate), and the activities with both of these substrates were inhibited severely by 2 mM cyanide. In all of these species, initial rates of the aerobic DCPIP assays were at least 60% of the anaerobic rates. Other algae contained glycolate oxidase since they oxidized L- but not

D-lactate and these activities were not inhibited by 2 mM cyanide; these included *Nitella* (in agreement with Downton and Tregunna [8]), *Spirogyra, Coleochaete,* and *Netrium. Klebsormidium* probably belongs to this group as well, although data on cyanide inhibition could not be interpreted in this species because of high endogenous rates in the presence of cyanide. All five of the latter species showed pronounced or complete inhibition of DCPIP reduction in the presence of O_2. Of all the algae tested, only *Oocystis, Microspora, Stigeoclonium, Spirogyra,* and *Netrium* showed measurable activity with both D- and L-lactate. In each alga, however, one of the two substrates was much preferred to the other, and additional experiments would be necessary to assess the significance of the lesser activity.

For most algae, specific activities of the glycolate oxidizing enzyme ranged from 2 to 10 nmoles/min·mg protein, but in *Chlorella* they were usually 15 to 20 nmoles/min·mg protein, and in *Spirogyra* they were 40 to 60 nmoles/min·mg protein. Of the 17 algal species tested for the ability to oxidize glycolate, only three (*Ulothrix fimbriata, Ulva,* and *Enteromorpha*) failed to show this activity at levels required for distinguishing glycolate oxidase from glycolate dehydrogenase by the assay employed.

Manometric Detection of Glycolate Oxidase. Four of the algae assayed spectrophotometrically for capacity to oxidize glycolate were assayed manometrically for glycolate dependent O_2 uptake. *Nitella* and *Spirogyra* were included to prove that their activities with DCPIP were indeed mediated by an enzyme which can link directly to O_2. Crude homogenates of both showed a definite glycolate-stimulated O_2 uptake (Table II). The rate of this uptake was in the same order of magnitude as that of DCPIP reduction in both species, although there was not an exact correspondence. There is no reason to expect, however, that the rates of electron transfer to O_2 and DCPIP should be the same. These results confirm that the enzyme in these multicellular algae was in fact glycolate oxidase and are consistent with the concept that on the basis of the DCPIP assay, specificity for the L-isomer of lactate and insensitivity to cyanide are reliable criteria for identifying this enzyme.

The homogenates of two species of *Chlorella* were assayed manometrically in order to resolve apparent differences between results of the present spectrophotometric assays, which indicated a typical glycolate dehydrogenase for both species, and reports in the literature (6, 26) that one of these, *Chlorella vulgaris,* possesses glycolate oxidase. Homogenates of both *Chlorella pyrenoidosa* and *Chlorella vulgaris* showed only barely detectable rates of glycolate-dependent O_2 uptake on the several occasions when they were assayed for such activity. In each species, addition of D-lactate also promoted O_2 uptake slightly, but in neither species did the uptake caused by either substrate approach the rate of DCPIP reduction. L-Lactate, on the other hand, failed to stimulate O_2 uptake.

Table II. *Comparison of Glycolate- or Lactate-dependent O_2 Uptake with DCPIP Reduction by Selected Green Algae*

Crude, cell-free homogenates of four green algae were assayed manometrically, and the rates of O_2 uptake were compared with the rates of anaerobic DCPIP reduction by the same preparations.

Species	Substrate	O_2 Uptake	DCPIP Reduction
		nmoles/min·mg protein	
Spirogyra varians	Glycolate	22	64
Nitella sp.	Glycolate	12	8.3
Chlorella pyrenoidosa	Glycolate	1.2	14
	D-Lactate	1.1	11
	L-Lactate	0	0
Chlorella vulgaris	Glycolate	1.3	26
	D-Lactate	1.4	26
	L-Lactate	0	0

Table III. *Catalase Activity in Various Green Plants*

Species	Catalase Activity
	μmoles H_2O_2 decomposed/min·mg protein
Algae	
Chlorella pyrenoidosa	11
Chlorella vulgaris	17
Dunaliella tertiolecta	11
Eremosphaera viridis	13
Oocystis polymorpha	12
Protosiphon botryoides	23
Codium sp.	trace
Microspora sp.	trace
Stigeoclonium helveticum	22
Coleochaete scutata	36
Klebsormidium flaccidum	57
Nitella sp.	32
Spirogyra varians	27
Lower land plants	
Marchantia sp.	64
Porella sp.	45
Pellia sp.	77
Leptodictyum riparium	61
Polytrichum sp.	92
Psilotum nudum	21
Selaginella apoda	15
Equisetum sp.	73
Osmunda regalis	85
Athyrium sp.	90
Onclea sensibilis	48
Aquatic angiosperms	
Elodea sp.	44
Lemna minor	56

Catalase Activity. Specific activities of catalase were determined in order that possible correlations between the levels of this enzyme and the presence of a particular type of glycolate oxidizing enzyme might be discerned. While there is considerable variation in the values (Table III), both among the green algae and among the land plants and aquatic angiosperms, some generalizations may be made. Catalase activities in the green algae, generally between 10 and 50 μmoles/min·mg protein, were lower on the average than those in the lower land plants and aquatic angiosperms, where they ranged for the most part between 50 and 100 μmoles/min·mg protein. Activities in all of these groups were somewhat lower than in leaves of higher plants, where values of 300 to 500 μmoles/min·mg protein are common (49). Within the algae, there was a tendency for species possessing glycolate oxidase (*e.g. Nitella*) to show higher levels of catalase than those which contain glycolate dehydrogenase (*e.g. Chlorella*). On the other hand, of the green algae which had glycolate oxidase activity, *Spirogyra* had by far the highest specific activity of this enzyme but had an activity of catalase which was relatively low and not substantially higher than that in species which possessed glycolate dehydrogenase.

DISCUSSION

The recognition that certain green algae contain a glycolate-oxidizing enzyme which differs distinctly from that of higher

plants (5, 33) raises questions of both physiological and evolutionary import. For example, it can be asked what factors determine whether a given plant species contains glycolate dehydrogenase or glycolate oxidase. It appears from the present survey that all green plants considered to be more advanced than the green algae have glycolate oxidase, whether their habitat is terrestrial or aquatic. The situation in the green algae is more intriguing, as some species apparently contain glycolate oxidase while others contain glycolate dehydrogenase. It may be pointed out that glycolate dehydrogenase is not confined to the unicellular forms in which it was first described, but occurs also in some of the multicellular and morphologically more complex algae such as *Stigeoclonium, Microspora,* and *Codium.* Moreover, glycolate oxidase is not restricted to multicellular algae, as it also occurs in *Netrium,* a unicellular species. Taken together, these results indicate that the distribution in green plants of the two enzymes which oxidize glycolate is not determined immediately by such considerations as whether the environment is aquatic or terrestrial or whether the organism is unicellular or multicellular. Rather, it seems more reasonable to conclude that species contain one or the other enzyme as a result of their development along separate evolutionary lines.

Our suggestion that the distribution of the two glycolate-oxidizing enzymes is determined phylogenetically is strengthened considerably by recent findings on certain cytological features of green algae (37, 39, 40). Through comparative studies of a large number of green algae, Pickett-Heaps and Marchant (40) have arrived at a phylogenetic scheme based on the mechanisms of mitosis and cytokinesis of these various species. Significantly, those algae in their scheme which show tendencies toward the higher plant type of organization of the mitotic and cytokinetic apparatus would appear from our studies to possess glycolate oxidase, as do the higher plants. In the light of this finding and of the generally accepted theory that all groups of land plants arose from one or more groups of green algae, it seems likely that the algae which possess glycolate oxidase show closer affinities to land plants than do those which possess glycolate dehydrogenase.

A report incompatible with the concept that the distribution of the glycolate-oxidizing enzymes follows taxonomic or phylogenetic patterns is that of Kowallik and Schmid (26). They contend that the parent strain and a mutant of *Chlorella vulgaris* contain glycolate oxidase, whereas other closely related species such as *Chlorella pyrenoidosa* do not. In our studies, homogenates of both *Chlorella vulgaris* (strain 211-11h) and *Chlorella pyrenoidosa* showed identical patterns of enzymatic activity. Contrary to the claim of Codd *et al.* (6), activity with DCPIP occurred with D-lactate and glycolate, but not with L-lactate, and this activity was inhibited by 2 mM cyanide. A low level of glycolate-dependent O_2 uptake of about 1 nmole/min·mg protein was detected with homogenates, but this phenomenon was demonstrable with *Chlorella pyrenoidosa* as well as with *Chlorella vulgaris.* The rates of O_2 uptake observed by Kowallik and Schmid (26), who were employing partially purified preparations, were of the same order of magnitude, being less than 1 nmole/min·mg protein. Such levels of activity are low relative to the rates of glycolate oxidation measurable in the same preparations by DCPIP reduction. By contrast, the glycolate-dependent O_2 uptake observed with homogenates of *Spirogyra* and *Nitella* was at least 10-fold higher. Our interpretation of the data concerning O_2 uptake in *Chlorella* is that the levels of this activity are too low to serve as proof that the enzyme involved is glycolate oxidase. On the contrary, substrate specificity and cyanide sensitivity indicate that the enzymes from both species of *Chlorella* resemble glycolate dehydrogenase.

Another question concerns the extent to which the glycolate-oxidizing enzymes in the various groups of green plants participate in peroxisomal metabolism. In green leaves of angiosperms, glycolate oxidase is associated with peroxisomes (microbodies); its oxidation of glycolate produces H_2O_2, which in turn is destroyed by peroxisomal catalase. The lower land plants have been studied less extensively, but microbodies have been observed with the electron microscope in green tissues of several bryophytes and lower vascular plants (34). Representatives of these groups were found in the present studies to contain glycolate oxidase and relatively high activities of catalase. Therefore, it would be reasonable to expect that they contain peroxisomes which house these enzymes and which resemble in other ways the peroxisomes of angiosperms.

The possible role of the glycolate-oxidizing enzymes in peroxisomal metabolism in green algae is harder to assess, mainly because peroxisomes have not yet been isolated in a convincing manner from autotrophically growing species. It is true that organelles resembling microbodies have been observed with the electron microscope in a number of unicellular green algae including *Chlorella* (14, 42), *Euglena* (1, 18), and *Chlamydomonas* (17), as well as several more complex species such as *Micrasterias* (25, 51), *Spirogyra* (Figs. 51 and 52, ref. 11), *Klebsormidium, Stigeoclonium,* and *Ulothrix* (9, 10). It is also true that microbodies, perhaps glyoxysomes, have been isolated from algae cultured heterotrophically on 2-carbon compounds (15, 20), and that there have been some reports indicating a particulate nature of glycolate dehydrogenase (19, 20, 28). However, these accomplishments have not established whether glycolate dehydrogenase and the other enzymes of the glycolate pathway in algae grown under autotrophic conditions are localized within peroxisomes. In fact, since glycolate dehydrogenase differs in several distinct ways from glycolate oxidase, one of these being that it does not produce H_2O_2, there is no *a priori* reason to assume that it is compartmentalized with catalase in a peroxisomal type of organelle. As a corollary, it can be suggested that if peroxisomes do exist generally in autotrophically growing green algae, their roles may vary depending on whether the algae possess glycolate dehydrogenase or glycolate oxidase.

That there are two types of glycolate-oxidizing enzymes in the green algae may be related to and may aid ultimately in explaining differences observed among algal species in various cytological and physiological features. For example, several algae—including one known to have glycolate dehydrogenase—possess microbodies which, unlike those of higher plants (12, 13, 21), do not show DAB staining when treated cytochemically for catalase (16, 17). Noteworthy exceptions are *Micrasterias* (51) and *Klebsormidium* (46), the latter of which was found in the present study to possess glycolate oxidase. Although the levels of catalase activity in the algae with glycolate oxidase did not differ greatly from levels in species with glycolate dehydrogenase, it is possible that in the latter species the catalase activity is mediated by a molecular form which is unable to oxidize DAB peroxidatically.

Another apparent dichotomy among the green algae involves photorespiration. Many green algae show low CO_2 compensation points (2, 4), whereas *Nitella* has a CO_2 compensation point of around 32 μl/l, near that of higher plants (2). Consistent with these observations is the fact that *Nitella* contains glycolate oxidase, while some (and perhaps all) of the species known to have low compensation points contain glycolate dehydrogenase. The glycolate-oxidizing enzymes, in short, may serve as markers for fundamental differences in the glycolate

pathway or other photorespiration-linked processes or both. It also seems reasonable to anticipate that these two enzymes, when considered in conjunction with other cytological and biochemical features of the algae, will serve as useful phylogenetic tools for determining affinities among existing groups and species, as well as for creating plausible hypotheses about their evolutionary histories.

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