L-α-HYDROXY ACID OXIDASE ACTIVITY IN *TETRAHYMENA*

Change With Physiological State

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INTRODUCTION

Most of the L-α-hydroxy acid oxidase in *Tetrahymena* is localized on a particulate fraction (4) whose sedimentation properties differ from those of lysosomes and mitochondria (1). The association of this enzyme (or family of enzymes; see reference 3) with D-amino acid oxidase and catalase in density gradient centrifugation has provided evidence for the existence of peroxisomes in *Tetrahymena* (1, 2). The function of this organelle is unknown, but a role as a participant in an electron-shuttle system, in which it might aid in the regulation of carbohydrate metabolism, has been proposed by de Duve and Baudhuin (2). These workers have also proposed a role in glyconeogenesis based partly on the possibility that keto acids, produced in this organelle under the influence of the α-hydroxy acid oxidases, could serve as precursors for carbohydrate synthesis, and partly on the tentative localization (now verified; see reference 13) of the glyconeogenic enzyme isocitrate lyase in this particle.

The role of the peroxisome could perhaps be elucidated by determining variations in the levels of its constituent enzymes under growth conditions that lead to differences in cell metabolism. We have found that the level of α-hydroxy acid oxidase was highest in oxygen-deficient cultures of *Tetrahymena*, especially when glucose was present in the culture medium.

MATERIALS AND METHODS

Tetrahymena pyriformis (E) was grown on 2% (w/v) proteose peptone with 0.1% each of liver fraction and K_2HPO_4. The initial concentration of glucose, when added, was 0.1%. Cultures were grown, with or without shaking, either in 300-ml portions in 2l penicillin flasks (surface area = 225 cm^2) or 100-ml portions in 500-ml Erlenmeyer flasks.

For determination of L-α-hydroxy acid oxidase, reaction mixtures of 0.5 ml contained cell homogenate, 20 μmoles of the sodium salt of either L-(+)-lactate or glycolate, or 40 μmoles of the sodium salt of D,L-hydroxybutyrate or D,L-glycerate, and 40 μmoles of PO_4 buffer, pH 7.5 (except for lactate oxidase, which was tested at pH 9.0 in Tris) (5). Since homogenates were prepared in dilute EDTA, reaction mixtures also contained 0.1 μmole of the disodium salt of this compound. Homogenates, prepared from frozen-thawed cells, were preincubated with buffer for 5 min at 29°, and reactions were started by addition of substrate. All tests were performed at two enzyme levels, and a doubling of the amount of homogenate usually resulted in a 1.8–2-fold increase in the reaction rate. For lactate and α-hydroxybutyrate, 0.1-ml samples were removed at either 3- or 4-min intervals and added to 2,4-dinitrophenylhydrazine in HCl. For glycerate and glycolate, 0.2-ml samples were withdrawn at 6- or 8-min intervals. Reaction rates were usually linear for the first 12 min. Dinitrophenylhydrazones were measured at 510 mμ (14). Total homogenates were used in these experiments, but we have found that about 80% of the activity sediments upon centrifu-

gation in 0.25 M sucrose for 10 min at 2000 *g* in cells from both shaken and static cultures.

Glyconeogenesis from lipids in *Tetrahymena* can proceed by way of the glyoxylate bypass of the tricarboxylic acid cycle (7). This process was measured, as described previously (10), by shaking washed portions of cells for 1 hr in the presence or absence of 1.25 mg/ml sodium acetate. Other analytical procedures and methods for handling cells have been given (10, 11).

RESULTS

The specific activity of α-hydroxy acid oxidase in cells from static cultures was three to five times higher than in cells from shaken cultures, depending on whether or not glucose had been added to the medium (Table I). At 24 hr, all cultures exhibit equal growth (protein basis), but static cultures are nearing the stationary growth phase as oxygen becomes severely limiting at about this

TABLE I

Effects of Culture Conditions on α-Hydroxy Acid Oxidase and Glyconeogenesis

Cultures were grown for 24 hr under the conditions shown. Activities of α-hydroxybutyric and lactic acid oxidases, and of isocitrate lyase are given as μmoles product formed/hr/mg protein; those of glycolic and glyceric acid oxidases as change of absorbance/hr/mg protein. Glycogen content at time of harvesting is given as % of cell protein. Rates of glyconeogenesis are given as μmoles glucose formed/hr/mg protein.

	Culture conditions			
	Shaken	Shaken + glucose	Static	Static + glucose
α-OH acid oxidase				
D,L-α-OH butyrate	0.80	0.88	2.4	4.1
L-(+)-lactate	0.63	0.57	2.1	2.9
D,L-glycerate	0.79	0.60	1.8	2.5
Glycolate	0.45	0.57	1.4	2.1
Isocitrate lyase	0.67	0.84	4.1	2.3
Glycogen content	2.0	0.9	9.8	63.5
Rates of glyconeogenesis				
Endogenous	0.105	0.14	0.28	<0
+1.25 mg/ml Na Acetate	0.078	0.14	0.45	<0

TABLE II

Summary of Effects of Culture Conditions on α-Hydroxy Acid Oxidase Activity

Specific activities are given as described in the legend to Table I, with the standard deviation and number of experiments included. All cultures were harvested approximately 24 hr after inoculation.

	Culture condition			
	Shaken	Shaken + glucose	Static	Static + glucose
Substrate				
D,L-α-hydroxybutyrate	0.86 ± 0.28 (n = 8)	0.98 ± 0.37 (n = 4)	2.45 ± 0.31 (n = 4)	4.47 ± 0.82 (n = 3)
L-(+)-lactate	0.67 ± 0.17 (n = 9)	0.68 ± 0.23 (n = 4)	2.04 ± 0.12 (n = 4)	2.93 ± 0.56 (n = 3)
D,L-glycerate	0.57 ± 0.22 (n = 4)	0.75 ± 0.31 (n = 3)	1.7 ± 0.10 (n = 3)	2.6 ± 0.26 (n = 3)
Glycolate	0.32 ± 0.11 (n = 3)	0.52 ± 0.11 (n = 3)	1.2 ± 0.17 (n = 3)	1.8 ± 0.26 (n = 3)

time (10) (See also Table III). Oxidase levels did not correlate with the ability to perform glyconeogenesis, as cells with the highest specific activity were unable to convert either acetate or endogenous precursors to glycogen. For reference, the specific activity of isocitrate lyase, a glyconeogenic enzyme in *Tetrahymena*, is given.

Table II summarizes the effects of culture conditions on the specific activity of the oxidase towards several substrates. The presence of glucose in shaken cultures had little or no effect on the enzyme level, while its presence in static cultures led to levels somewhat greater than those found in static controls. The ratio of the activity of α-hydroxybutyrate to that of lactate is somewhat lower than that reported by other workers (2, 3).

Short-term environmental changes also affected the specific activity of the oxidase (Fig. 1). Cultures were grown for 24 hr with shaking and then transferred to static conditions, with or without glucose. Such a change leads to an increase of 2-fold or more in the rate of glyconeogenesis, either in peptone or in washed cells shaken with acetate, and to a 5–10-fold increase in the specific activity of isocitrate lyase. The presence of glucose eliminates all capacity for glyconeogenesis (9). The specific activities of the oxidase in the static cultures began to approach those found in cells grown under these conditions from the time of inoculation. The levels in the shaken controls showed little change, with perhaps a slight increase occurring in the presence of glucose. Again, there was no correlation with the ability to perform glyconeogenesis, as the greatest increase occurred in cells lacking this capacity. Also, during the first 3 hr after transfer, in the static cultures lacking glucose, the glyconeogenic enzyme isocitrate lyase increased more than 6-fold, while the oxidase increased by less than 50%. It should be noted that glucose, which had an inductive effect

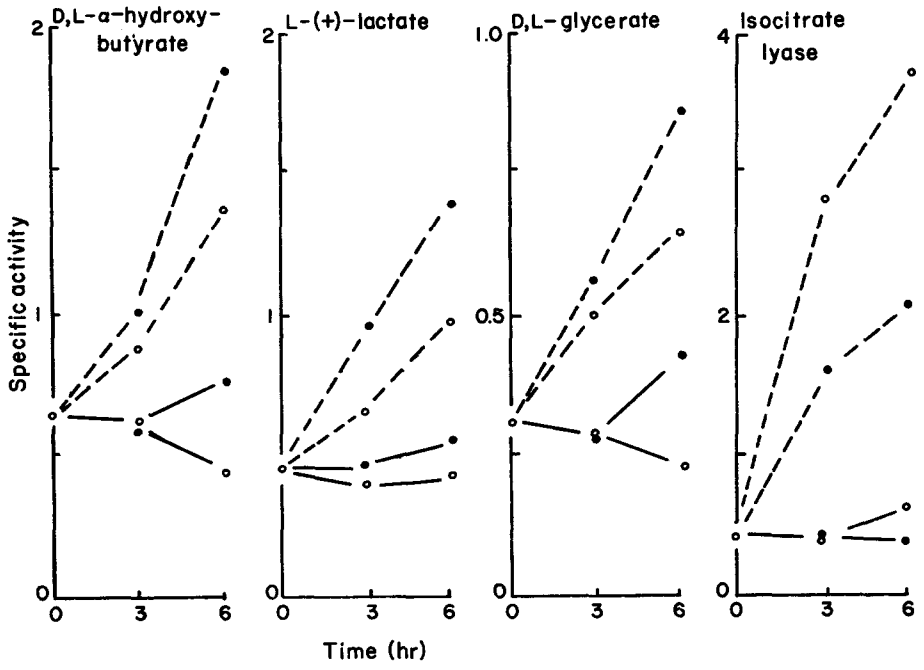

FIGURE 1 L-α-hydroxy acid oxidase and isocitrate lyase activities after transfer to static conditions.

Cultures were grown with shaking for 24 hr. Test cultures were then transferred to static conditions (130 ml/500 ml Erlenmeyer flask) with or without 1 mg/ml D-glucose. Shaking was continued in the controls (130 ml/penicillin flask). Initial protein concentration was 0.17 mg/ml of culture. Samples were taken at 3 and 6 hr after transfer. Broken lines, static cultures; solid lines, shaken cultures; open circles, no additions; solid circles, + 1 mg/ml D-glucose. Specific activities of glyceric acid oxidase are given as the change in absorbance at 510 mμ/hr/mg protein. Others are given as μmoles product formed/hr/mg protein.

TABLE III

Changes in α-Hydroxy Acid Oxidase Activity with Culture Age

Cultures were grown with or without shaking. Cultures treated with 1% O_2 were grown first for 14 hr under air. A water-saturated atmosphere of 1% O_2–1% CO_2–98% N_2 was then passed continuously over the culture.

Culture age	Culture growth	α-Hydroxy acid oxidase specific actvity	
	mg protein/ ml culture	α-Hydroxy butyrate	Lactate
hr			
Shaken			
14	0.038	1.0	0.7
24	0.146	1.4	1.05
34	0.457	1.05	0.88
48	0.733	0.38	0.43
Static			
24	0.175	2.3	1.85
34	0.190	2.6	2.15
48	0.322	2.0	1.8
72	—	<0.1	<0.1
Shaken, 1% O_2			
14 hr air, 20 hr 1% O_2	0.108	2.15	2.15
14 hr air, 34 hr 1% O_2	0.130	1.75	1.65

on the oxidase, acted to inhibit synthesis of isocitrate lyase.

Table III shows changes in α-hydroxy acid oxidase activity with culture age, as well as the effects of incubation under an atmosphere of 1% oxygen. The latter treatment led to specific activities similar to those found in cells from static cultures. The low activity in cells from the older cultures might be due to the presence of an inhibitor of the enzyme, since mixing of homogenates of these with homogenates of cells from young cultures led to a loss of activity. Other workers (5) have found substantial oxidase activity in 6-day cultures. This could be caused by differences in culture medium or growth conditions.

DISCUSSION

These results show that L-α-hydroxy acid oxidase, an enzyme (or family of enzymes) localized largely on the peroxisome in *Tetrahymena*, can vary with culture conditions, the specific activity being highest in cells grown under static conditions in the presence of glucose. The oxidase level does not seem to correlate with the capacity for glyconeogenesis, the specific activity being highest in cells that lack this capacity. In addition, glucose, which acts to prevent glyconeogenesis and repress the synthesis of the glyconeogenic enzyme, isocitrate lyase, seems to induce the oxidase. However, a role of the peroxisome in glyconeogenesis, as suggested by de Duve and Baudhuin (2) now seems likely, as isocitrate lyase and malate synthase, which serve to convert acetate to carbohydrate in *Tetrahymena*, have recently been reported to be localized on this organelle (13).

Another proposed role of the peroxisome is as a part of an electron-shuttle system in which it would serve to oxidize extra-mitochondrial DPNH and in this way help to regulate the breakdown of carbohydrate (2). In recent experiments, we have found that in cells starved for 24 hr under static conditions in inorganic medium supplemented with glucose, there is considerable net synthesis of α-hydroxy acid oxidase (more than a doubling) despite a net loss of cell protein. These results, together with those presented here, support the suggestion (2) of a role of the peroxisome in carbohydrate metabolism.

The L-(+) lactate oxidase of *Tetrahymena* has a low affinity for oxygen (5). This general property of flavin-containing oxidases led to the suggestion that the peroxisome might not compete successfully for oxygen with cytochrome oxidase under hypoxic conditions (2). However, it has been shown that the K_m for substrate for several of these enzymes decreases with a lowering of the oxygen concentration and that the rate of primary substrate oxidation under such conditions was much greater in the presence of catalase alone than in the presence of catalase and secondary substrate (8). It was suggested that, at low oxygen tensions, catalase and H_2O_2 lead to an increased rate of substrate oxidation. Also, under conditions of oxygen limitation, intracellular diffusion may well be the primary factor determining the apparent K_m for oxygen utilization (6), and this value can be several hundred times greater than that obtained when the terminal oxidase is measured in cell free preparations (12). Therefore, the location of mitochondria and peroxisomes, in relation to the cell membrane, could influence the amount of oxygen that each would receive, as could the relative amount of their respective oxidases. (The

terminal cytochrome oxidase in *Tetrahymena* has not as yet been determined; see reference 15.)

While the syntheses of α-hydroxy acid oxidase and of the enzymes of the glyoxylate bypass in *Tetrahymena* seem to react differentially to glucose, the specific activities of all of these enzymes are highest in static cultures of this organism. Since these enzymes all seem to be localized largely on the peroxisome, it is possible that this organelle functions in metabolic processes occurring under conditions of oxygen deficiency.

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