Use of 3-aminopropanol as an ethanolamine analogue in the study of phospholipid metabolism in *Tetrahymena*

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About 50% of the ethanolamine in phosphatidylethanolamine in *Tetrahymena* is replaced by 3-aminopropan-1-ol when the compound is added to the growth medium. The phosphatidylpropanolamine which is formed is not converted into the corresponding phosphatidylcholine analogue by methylation. There is an increase in phosphatidylcholine formed by the phosphotransferase pathway from free [³H]choline and a decrease in the phosphatidylcholine formed by the methylation pathway from [¹⁴C]methionine. The nature of the observed phospholipid alterations suggests that the regulation of phosphatidylcholine biosynthesis in *Tetrahymena* may be different from that found in higher eukaryotes.

INTRODUCTION

A variety of phosphonic acid analogues of ethanolamine have been used to modify the phospholipid composition of the ciliate protozoan *Tetrahymena thermophila* [1–3]. These compounds decrease the cellular contents of phosphatidylethanolamine without affecting phosphatidylcholine content. Since *Tetrahymena* has both the methylation and phosphotransferase pathways for phosphatidylcholine biosynthesis [4], the organism maintains homeostasis by increasing phosphatidylcholine formation via the phosphotransferase pathway to accommodate the decreased methylation from phosphatidylethanolamine [5]. However, neither cellular phosphatidylethanolamine or phosphatidylcholine contents change when the cell is grown with ethanolamine or choline, respectively [1].

In contrast, mammalian cells, in particular hepatocytes, increase cellular phosphatidylethanolamine

Table 1. Phospholipid composition of control and amino-propanol-grown *Tetrahymena*

Results are means $\pm$ S.D. ($n = 10$). Statistical analysis was carried out using Student's t test ($P < 0.001$ for phosphatidylethanolamine); none of the other differences was significant. Abbreviation: AEP, 2-aminoethyl-phosphonate.

Phospholipid	Content (% of total phospholipid)	
	Control	Amino-propanol-grown
Phosphatidylcholine	23.8 $\pm$ 3.2	20.7 $\pm$ 3.3
AEP-ceramide	10.7 $\pm$ 2.4	10.4 $\pm$ 5.0
Phosphatidylethanolamine	38.1 $\pm$ 3.4	17.5 $\pm$ 7.3
Phosphatidylaminopropanol	—*	22.2 $\pm$ 4.4
AEP-glyceride	24.4 $\pm$ 2.2	23.6 $\pm$ 4.3
Cardiolipin	3.4 $\pm$ 1.6	3.4 $\pm$ 1.2

* Not present in the control.

Fig. 1. Thin-layer chromatograms of phospholipids of (a) control and (b) 3-aminopropan-1-ol-grown *Tetrahymena*

First dimension (F_1), chloroform/methanol/ NH_3 (13:7:1, by vol.); second dimension (F_2), chloroform/acetic acid/methanol/water (375:125:25:11, by vol.) (O, origin). Phospholipid identification: 1, phosphatidylcholine; 2, AEP-ceramide; 3, phosphatidylethanolamine; 4, AEP-glyceride; 5, cardiolipin; P, phosphatidylpropanolamine (AEP, 2-aminoethylphosphonate).

when cultured with ethanolamine [6,7]. Mammalian cells have also been shown to use various amino alcohols in place of ethanolamine, replacing some of the ethanolamine in the phospholipid with the analogue, and in all cases lowering phosphatidylcholine contents [6–11]. Choline and ethanolamine analogues have also been shown to be incorporated into the phospholipids of, and to act as growth inhibitors of, the malarial parasite *Plasmodium* [12].

These differences between *Tetrahymena* and the mammalian cells in their responses to phospholipid base substitution suggest possible differences in the regulation of phospholipid biosynthesis. To clarify these differences further, we have begun to examine the effects of some of the amino alcohols in *Tetrahymena*, so that the comparison between the organisms may be expanded by using more of the same compounds.

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Table 2. Incorporation *in vivo* of [*Me*-³H]choline and [*Me*-¹⁴C]methionine into phosphatidylcholine of control and 3-aminopropan-1-ol-grown *Tetrahymena*

Phospholipid P and radioactivity were determined as described in the Experimental section. The results are means $\pm$ S.D. ($n = 4$) for a typical experiment.

Culture	Incorporation (d.p.m./ nmol of P in phosphatidylcholine)		
	[<i>Me</i> - ³ H]- Choline	[<i>Me</i> - ¹⁴ C]- Methionine	³ H/ ¹⁴ C
Control	18.6 $\pm$ 0.8	52.7 $\pm$ 1.0	0.37 $\pm$ 0.03
3-Aminopropan-1-ol-grown	29.9 $\pm$ 0.1	21.6 $\pm$ 1.9	1.39 $\pm$ 0.08

EXPERIMENTAL

Tetrahymena thermophila (WH-14) was cultured at 37 °C in proteose-peptone/glucose/yeast extract medium as previously described [5]. Both the control and experimental media contained 10 μ Ci each of [*Me*-³H]-choline and [*Me*-¹⁴C]methionine, and the experimental medium contained 10 mM-3-aminopropan-1-ol. After 24 h growth at 37 °C, the cells were harvested and the phospholipids isolated [5]. Individual phospholipids were prepared by two-dimensional t.l.c. in chloroform/methanol/NH₃ (13:7:1, by vol.) and chloroform/acetic acid/methanol/water (375:125:25:11, by vol.) [13] and quantified by the procedure of Snyder & Law [14]. Radioactivity was determined in Scintiverse II scintillation fluid (Fisher Scientific Co.) in a Beckman LS5801 liquid-scintillation counter. Glyceryl ether content was determined as previously described [15].

RESULTS AND DISCUSSION

Tetrahymena grown with 3-aminopropan-1-ol did not significantly differ from control cells in cell density or morphology as observed at $\times 40$ magnification under the phase-contrast microscope. The experimental cultures incorporated 3-aminopropan-1-ol into their phospholipids as shown in Fig. 1. The presence of a new phospholipid is consistent with the formation of phosphatidylpropanolamine by the cells.

This new phospholipid, phosphatidylpropanolamine, comprised about 20% of the total cellular phospholipid composition (Table 1). It was formed at the expense of phosphatidylethanolamine, which decreased in proportion to the amount of new lipid formed. There was no apparent methylation of the phosphatidylpropanolamine to its phosphatidylcholine analogue.

The decrease in phosphatidylethanolamine resulted in a decrease in formation of phosphatidylcholine via the methylation pathway (Table 2). However, the total amount of phosphatidylcholine in the cells was maintained (Table 1) by a corresponding increase in the incorporation of choline by the phosphotransferase pathway (Table 2). This represented a change in the ratio of the two pathways for phosphatidylcholine formation from the normal 1:1 to 3:1 in favour of the phosphotransferase pathway [5]. These results are consistent with the other phospholipid base substitutions which have been made in *Tetrahymena* (2-aminoethyl-

phosphonate [16], 3-aminopropylphosphonate [1] and dimethyl-2-aminoethylphosphonate [7]) in which cellular phosphatidylethanolamine contents are lowered: an increase in the phosphotransferase pathway and a decrease in methylation, but maintenance of cellular phosphatidylcholine contents. The 3-aminopropan-1-ol is apparently being incorporated into the phospholipid via the ethanolamine phosphotransferase pathway, since the phosphatidylpropanolamine produced has a low glyceryl ether content, comparable with that of phosphatidylethanolamine (less than 5%) rather than that of phosphatidylcholine (50%) (results not shown).

These results confirm that the regulation of phospholipid biosynthesis may be different in *Tetrahymena* from that in higher eukaryotes. An examination of the regulatory properties of the specific phospholipid-biosynthetic enzymes of *Tetrahymena* seems desirable.

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