



# Effect of 3-Amino-1-Propanol on the Phosphatidylinositol (PI) and Glycosyl Phosphatidylinositol (GPI) Systems of *Tetrahymena*

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**ABSTRACT.** 3-Amino-1-propanol (AP), a substance replacing ethanolamine in phosphatidylethanolamine (PE) significantly reduced  $^{32}\text{P}$  incorporation to phosphatidylinositol (PI) and glycosyl-phosphatidylinositol (GPI) in the unicellular organism *Tetrahymena pyriformis*. At 10 mM, AP completely inhibited the incorporation of  $^{32}\text{P}$  into PI.  $^3\text{H}$ -arachidonate incorporation into PI was also inhibited, while that into diacylglycerol (DAG) was high. The experiments indicate the presence and metabolism of inositol phospholipids and GPI in *T. pyriformis*. COMP BIOCHEM PHYSIOL 118C;1:83–87, 1997. © 1997 Elsevier Science Inc.

**KEY WORDS.** Phosphoinositol metabolism, *Tetrahymena*, PI, GPI, signaling

## INTRODUCTION

The unicellular *Tetrahymena* contains second messenger systems which are able to transmit information of environmental signals to the effectors. The presence of cAMP (5–8,10,14); cGMP (14) and guanylate cyclase-dependent calmodulin (13); and phosphatidyl inositol system (11) have been demonstrated in it.

Glycosyl-phosphatidylinositol (GPI)-anchored proteins also play important roles in the cell signaling. Simultaneously, with the release of anchored proteins by GPI-specific phospholipase C (GPI-PLC), runs the production of biological active lipids such as 1,2-diacylglycerol (DAG) and phosphatidate (PA). The signaling role of the GPI in *Tetrahymena* is indicated by the effect of insulin treatment (12).

One approach to gaining insight into the involvement of lipids in signal transduction systems is to manipulate the lipid composition of *Tetrahymena* cells by supplementation to the culture medium with various components. For example, about 50% of the ethanolamine in phosphatidyl-ethanolamine (PE) of *Tetrahymena* is replaced by 3-amino-1-propanol when this compound is added to the culture medium (18). The phosphatidyl-propanolamine formed cannot be converted into phosphatidyl-choline (PC) by methylation.

Since the PI system plays a cardinal role in signal transduction, and in some cell type, PE is a major source of members of the “lipid cycle” (e.g., PA, DAG, PI), (1), thus, the

modification of PE-content of *Tetrahymena* seems to be a good possibility for the examination of the lipid signal transduction systems in this unicellular organism. Since the donor of terminal phosphoethanolamine of GPI is PE (15), it can be supposed that the alteration of PE level will also exert an effect on the synthesis of GPI.

Arachidonic acid (AA) plays a central role in the signal transduction of mammals. AA was found to be esterified preferentially the sn-2 position of glycerophospholipids, and predominated in PI and PE (9). As AA is a widely used marker in the study of the phospholipid metabolism, it seemed reasonable to measure its incorporation into phospholipids.

The experiments could shed light on the similarities (or dissimilarities?) of signaling mechanisms at an early level of evolution related to data gained by experiments done in mammals.

## MATERIALS AND METHODS

The quantity of cells, culture medium (which was also used as solvent), experimental materials, scintillation fluid, etc., were strictly standardized. In each group and type of measurement, at least three separate experiments were done. The means of the experiments are given in the Tables. Significances (analyzed by Student's *t*-test) and SD values are based on individual measurements.

### Cells

*Tetrahymena pyriformis* GL were grown axenically in a 0.1% yeast extract containing 1% Bacto trypton (Difco, MI, U.S.A.) medium at 28°C. In the experiments, cells in the

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Received 19 February 1996; revised 3 January 1997; accepted 21 January 1997.

logarithmic phase of growth were used. Before the experiments the cells were washed with fresh medium, collected by gentle centrifugation, and were resuspended at a concentration of  $5 \times 10^6$  cells/ml.

### Chemicals

3-Amino-1-propanol (AP), and PI, PIP, PIP<sub>2</sub>, PA, PC standards were purchased from Sigma (St Louis, MO, U.S.A.). <sup>32</sup>P Na-orthophosphate (specific activity 7 GBq/mM) and <sup>3</sup>H-arachidonic acid (specific activity 7 TBq/mM) were obtained from Izinta (Budapest, Hungary).

### Parameters Studied

**ASSAY OF <sup>32</sup>P INCORPORATION INTO THE PHOSPHOLIPIDS OF *TETRAHYMENA*.** In these experiments, the following groups were tested:

- [a] control, untreated group;
- [b–d] groups treated with 1, 5, and 10 mM 3-amino-1-propanol, respectively.

*Tetrahymena* cultures (25 ml) were treated for 1 hr with AP, then 60 ml <sup>32</sup>P Na-orthophosphate (12 MBq) was added to the cells in the continued presence of AP. Five ml samples were taken from the cultures after 5, 15, 30, and 60 min, respectively. After centrifugation, the cell-pellet was extracted into 2 ml acidic chloroform/methanol (chloroform:methanol:HCl = 10:20:0.1 by vol.) at room temperature. After 15 min, the samples were made biphasic by adding of 0.66 ml chloroform and 0.66 ml water. The lower (chloroform) phase was dried under a stream of nitrogen (20). Phospholipids were separated by oxalate-pretreated silica gel G thin layer chromatography plates (Merck, Darmstadt, Germany) activated at 110°C. Solvent system: chloroform:acetone:methanol:acetic acid:water = 40:15:13:12:8 by vol. Following the development, the chromatograms were covered with Kodak TMG X-ray film. After exposure (~18 hr) and development of radiograms, the radioactivity of phospholipid spots were analysed by laser densitometry (Ultrosan XL, Pharmacia-LKB, Uppsala, Sweden). The individual <sup>32</sup>P labelled phospholipids were expressed in percent of total incorporated <sup>32</sup>P as 100%. Significances were evaluated by Student's *t*-test. The phospholipids were identified by parallel run of authentic standards of PI, PIP, PIP<sub>2</sub>, PA, PC, and PE.

Before the chromatographic analysis, 5-ml aliquots were taken from the redissolved lipid samples for liquid scintillation counting of absolute values of total incorporated <sup>32</sup>P.

**ASSAY OF THE GPI SYNTHESIS.** The following groups were tested:

- [a] control (untreated and [b–d] groups treated with 1, 5, and 10 mM AP, respectively.

Five ml *Tetrahymena* cultures were treated with AP. After 30 min, 12 ml <sup>32</sup>P Na-orthophosphate (2.4 MBq) were added to the cells for 60 min in continued presence of AP. Centrifugation at 4°C was followed by extraction of pellet with acetone to remove most of the lipids. After centrifugation, the dried pellet was extracted for 2 hr with chloroform:methanol:water = 10:10:3 by vol., and the dried samples were developed by this solvent on silica gel G thin layer chromatography plates (16); 0.5 cm strips of chromatograms were scraped into cuvettes, and the radioactivity was determined by liquid scintillation counting in Optiphase HiSafe fluid (Wallac, U.S.A.; 4 ml/vial). Quench was identical for all samples.

**ASSAY OF THE ARACHIDONIC ACID (AA) INCORPORATION.** In this series of experiments,

- Control cultures; and groups treated with 10 mM AP were tested.

Thirty minutes from the beginning of AP treatment, <sup>3</sup>H-arachidonic acid (7.4 KBq/ml) were added to the cultures in continued presence of AP for 60 min. After treatments, the lipid content was extracted with ethyl acetate and dried under nitrogen stream (slightly modified after ref. 2). The redissolved samples were separated on silica gel G thin layer chromatography plates by benzene:dioxane:acetic acid = 20:10:1 (by vol.) solvent system, then 0.5–0.5 cm strips of chromatograms were scraped into cuvettes, and the radioactivity was determined in a Beckman liquid scintillation counter, as in the GPI-assay.

**MORPHOMETRIC ANALYSIS OF THE CELLS.** Before analysis, samples were stained with 0.1% toluidine blue for 2 min. The gently washed cells were applied to microscopic slides and were photographed with a light microscope (magnification: 800 X). The prints developed were scanned by Hewlett Packard HP-ScanJet IIc scanner and the data obtained were analysed by a computer programme (Biomorph 1.1). The interactive morphometry programme made it possible to determine area, outline, and ratio of the shortest and longest axis (w/l). Over these, some other special ratios and the histograms were also calculated. The statistical analysis of data, e.g., variance, standard deviation were given by the program.

## RESULTS

### Assay of <sup>32</sup>P Incorporation into the Phospholipids of *Tetrahymena*

Treatments with AP significantly reduced the incorporation of <sup>32</sup>P into the phospholipids of *Tetrahymena*. The total incorporated <sup>32</sup>P was 88% (*p* < 0.01) in the 5 mM, 90.3% (*p* < 0.01) in the 1 mM AP treated groups compared to the control groups as 100%. In the presence of 10 mM AP, no activity in the phospholipid spots was found. The 10 mM AP concentration proved to be somewhat toxic since the

**TABLE 1. Incorporation of  $^{32}\text{P}$  into the phospholipids and inositol phospholipids of control and AP treated *Tetrahymena*. Values (%) are averages ( $\pm$  SD) of three separate experiments. Total incorporated  $^{32}\text{P}$  = 100%**

	PI	PIP	PIP <sub>2</sub>	PA	PC	PE
cont 5 min	0	0.4 $\pm$ 0.025	1.05 $\pm$ 0.04	0	0	0
cont 15 min	2.02 $\pm$ 0.12	3.4 $\pm$ 0.11	1.18 $\pm$ 0.08	4.73 $\pm$ 0.24	2.0 $\pm$ 0.17	15.3 $\pm$ 0.94
cont 30 min	6.17 $\pm$ 0.28	5.25 $\pm$ 0.41	3.6 $\pm$ 0.19	3.71 $\pm$ 0.1	16.9 $\pm$ 0.37	34.0 $\pm$ 0.19
cont 60 min	8.23 $\pm$ 0.33	4.28 $\pm$ 0.16	1.77 $\pm$ 0.07	4.3 $\pm$ 0.068	22.0 $\pm$ 0.9	28.4 $\pm$ 0.94
1 mM 5 min	0	0.42 $\pm$ 0.03	0	0	0	0
1 mM 15 min	4.0 $\pm$ 0.13*	3.62 $\pm$ 0.21	0.83 $\pm$ 0.06	4.44 $\pm$ 0.21	4.0 $\pm$ 0.18	11.3 $\pm$ 0.94
1 mM 30 min	5.62 $\pm$ 0.29	6.32 $\pm$ 0.34	1.27 $\pm$ 0.11*	5.8 $\pm$ 0.31*	15.2 $\pm$ 0.76	33.1 $\pm$ 1.3
1 mM 60 min	9.67 $\pm$ 0.39**	4.87 $\pm$ 0.16	1.36 $\pm$ 0.11	4.62 $\pm$ 0.34	23.1 $\pm$ 0.97	35.2 $\pm$ 1.38
5 mM 5 min	1.21 $\pm$ 0.09	0	0	0	0	0
5 mM 15 min	2.8 $\pm$ 0.13	4.4 $\pm$ 0.33	1.38 $\pm$ 0.1	3.58 $\pm$ 0.19	2.5 $\pm$ 0.14**	4.9 $\pm$ 0.2*
5 mM 30 min	8.25 $\pm$ 0.33*	5.8 $\pm$ 0.24	1.6 $\pm$ 0.09*	4.2 $\pm$ 0.21	2.8 $\pm$ 0.13*	15.2 $\pm$ 0.98*
5 mM 60 min	7.66 $\pm$ 0.39	4.75 $\pm$ 0.34	1.11 $\pm$ 0.14	3.68 $\pm$ 0.29	18.5 $\pm$ 1.13	28.1 $\pm$ 1.27

\* $p < 0.01$  to the control.

\*\* $p < 0.05$  to the control.

number of viable cells decreased after 60 min of treatment from  $1,75 \times 10^6/\text{ml}$  in the controls to  $0,75 \times 10^5/\text{ml}$  in the experimentals.

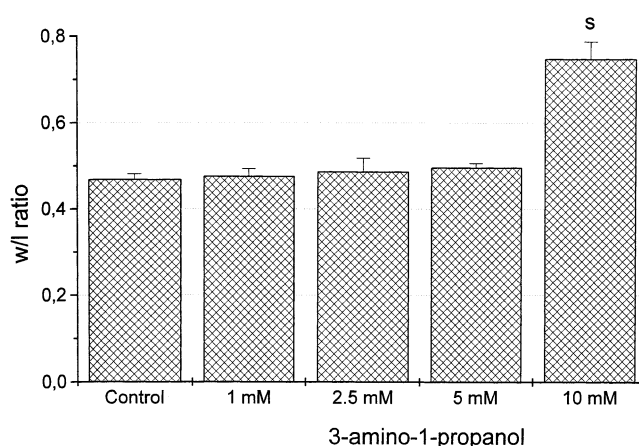
Thus, we separated the viable (swimming) cells from the immobile cells after the treatments with centrifugation, and only viable cells were examined for incorporation of  $^{32}\text{P}$ .

In addition, the relative, comparative data of the individual phospholipids expressed by laser densitometer diverged from the control values.

The proportion of labelled PE in the case of 1 mM AP concentration ran parallel with the control values; while in the case of 5 mM AP treated groups after the 15- and 30-min treatments, significantly lower activity was measured than in the control ones. After 60 min in these groups, the activity reached the controls (Table 1).

A similar situation was found in the case of PC, where the control and 1 mM AP treated groups showed similar radioactivity related to the total incorporated  $^{32}\text{P}$ . In the cells treated with 5 mM AP only after 30 min treatments elevated significantly the incorporated  $^{32}\text{P}$  content (Table 1). In the case of Pa, the values ran parallel in each of the experimental groups, and only after 30 min was a peak in the  $^{32}\text{P}$  incorporation in the 1 mM AP treated groups, while in 5 mM AP treated groups after 5-min treatments, the incorporation of  $^{32}\text{P}$  was significantly lower than in the controls (Table 1).

The ratio of radioactivity in the case of PI and PIP ran similarly in the AP treated and control groups in the course of the treatments, but the incorporated  $^{32}\text{P}$  content was moderately higher in the treated groups than in the control ones (Table 1). PIP<sub>2</sub> showed markedly different labelling from PI and PIP. In the AP treated groups, the proportion of the activity was significantly lower after 30-min treatments, but after 60 min the values of control and AP treated groups came closer (Table 1). These phospholipids (especially PA and PIP<sub>2</sub>) can be found in relatively low quantities, thus, some percent elevation or decrease mean significant alterations.



**FIG. 1. The ratio of the shortest and longest axis (w/l) of control and AP treated *Tetrahymena*. The values are averages ( $\pm$  SD) of three separate experiments. s =  $p < 0.01$  to the control.**

#### Assay of the GPI Synthesis

The  $^{32}\text{P}$  content GPI (at the 2 cm of chromatogram, i.e.,  $R_f = 0,16$ ) decreased significantly in the AP treated groups; AP reduced the GPI formation in a dose-dependent manner and  $^{32}\text{P}$  incorporation to GPI was totally inhibited in the 10 mM AP-treated groups (Table 2).

#### Assay of Incorporation of the $^3\text{H-AA}$ into the Lipids of *Tetrahymena*

Treatment with 10 mM AP significantly reduced the incorporation of  $^3\text{H-AA}$  into the phospholipids of *Tetrahymena*. The  $^3\text{H-AA}$  content of DAG were elevated significantly by the AP treatment, and AA content of the cells also showed a considerable increase (Table 3).

**TABLE 2. The incorporation of  $^{32}\text{P}$  into the GPI of the control and AP treated *Tetrahymena*. The GPI peak is at the  $R_f = 0.16$  on the chromatogram. Values are averages ( $\pm SD$ ) of three separate experiments**

	GPI (DPM)
Control	68000 $\pm$ 750
AP 1.0 mM	51000 $\pm$ 810*
AP 5.0 mM	800 $\pm$ 65***
AP 10.0 mM	150 $\pm$ 42*****

\* $p < 0.01$  to the control; \*\* $p < 0.01$  to the 1 mM AP treated; \*\*\* $p < 0.01$  to the 5 mM AP treated.

### Morphometric Analysis of the Cells

The 10 mM AP treatment for 60 min alter significantly the ratio of the shortest and longest axis (w/l) of the mobile (viable) cells (Fig. 1).

## DISCUSSION

The manipulation of lipid composition of the cells is a very useful method to determine the biochemical events connected with these molecules (17–19). For example, cultivation of the NG 108-15 cells in a medium containing different polyunsaturated fatty acids induced extensive and specific changes in the phospholipid fatty acid composition. In these cells, the activity of the protein kinase C-mediated phospholipase D activity is increased (3). It was supposed that in *Tetrahymena* the phospholipid composition may be altered by culturing the cells in presence of various phosphonic acid analogs of the phospholipid bases choline phosphate and ethanolamine phosphate (17). The ability to alter the lipid composition *in vivo* provides an opportunity to study the effects of such alterations—among others on the activity of membrane bound enzymes (17). When *Tetrahymena* were grown in ethanolamine or choline supplemented media this makes possible to examine the PE-PC conversions, the cholinephosphotransferase and ethanolamine-phosphotransferase pathways (19).

The supplementation of the culture medium of *Tetrahymena* with 3-amino-1-propanol resulted in an about 50% decrease in the PE content of the cells, but the PC content remained constant (18). Thus, this lipid manipulation gives

**TABLE 3. Incorporation of  $^3\text{H}$ -arachidonic acid (AA) into the lipids of control and 10 mM AP treated *Tetrahymena*. The data represent the mean ( $\pm SD$ ) derived from three separate experiments**

	Control (DPM)	10 mM AP treated (DPM)
AA	68000 $\pm$ 1800	250000 $\pm$ 2300*
DAG	177000 $\pm$ 2200	226000 $\pm$ 1900*
Phospholipids	82000 $\pm$ 1550	61800 $\pm$ 1100*

\* $p < 0.01$  to the control.

a possibility to examine the role of PE in different biochemical pathways, e.g., the role of PE in the signaling.

In *Tetrahymena*—among others the presence of the phosphatidylinositol signaling system (11) was demonstrated. In this signal transduction system, the lipid cycle, where DAG, derived from the hydrolysis of  $\text{PIP}_2$  plays a very important role, turning into—through PA and PI—back to  $\text{PIP}_2$  (with coexistence of inositol phosphate cycle which yields PI from  $\text{IP}_3$  again) (1). In the lipid cycle, one possible source of DAG and PA is PE. Another very important role of PE in the signaling is to be the donor of terminal phosphoethanolamine for GPI (15).

Smith and Barrows (18) found that 10 mM AP treated *Tetrahymena thermophyla* WH 14 cell populations grown in proteose-peptone/glucose/yeast extract medium at 37°C did not significantly differ from controls in cell density or morphology. In our system, using the *T. pyriformis* GL strain cultivated in trypton/yeast extract medium at 28°C 10 mM AP proved to be toxic. This toxicity indicated the large number of dead (immobile) cells, and the alteration of the shape of viable cells (elevation of W/L ratio) after the treatments. In accordance with our earlier experience, the rounding off of the *Tetrahymena* cells indicates unfavourable environmental conditions (unpublished). The AP treated viable cells inoculated into fresh culture medium for 24 hr showed no changes in shape compared to the controls, but the growth rate of the 10 mM AP-treated cultures was slower than that of the controls.

The disturbance of signaling machinery indicate the effect of AP treatments on the members and precursors of the PI system. The amount of incorporated  $^{32}\text{P}$  is markedly reduced in the 1 and 5 mM AP treated groups, and in the 10 mM AP treated groups was totally inhibited. This inhibitory effect of  $^{32}\text{P}$  incorporation was measurable in the case of GPI too. Similarly, the content of  $^3\text{H}$ -AA showed a very high rate, and also the AA content of the cells was very high. In human neuroblastoma cells, the patterns of incorporation and redistribution of AA were effectively regulated to favour lipids such as PI and PE (4). A similar situation is conceivable in *Tetrahymena* because the AP treatments might be inhibited, which is indicated by high level of AA and in DAG.

The lower amount of incorporated  $^{32}\text{P}$  indicate the effect of AP. The radioactivity was reduced in the PE and PC in the first 30 min of treatments with 5 mM AP, however, the proportion of labeled PE in the treated cells after 60 min reached to control level. The AP treatments caused no significant changes in the proportion of the  $^{32}\text{P}$ -labeled PI and PIP content, but the relative amount of  $\text{PIP}_2$  decreased significantly in the AP treated groups. This reduced  $^{32}\text{P}$  content may be the result of a disturbed inositol phosphate cycle and connection with this, a disturbed lipid cycle, too. The inhibited lipid cycle is indicated by an elevated  $^3\text{H}$ -AA level in the DAG parallel with a lower AA content in the phospholipids.

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