doi:10.4149/gpb_2011_01_106

Short Communication

Evaluation of uridine 5'-eicosylphosphate as a stimulant of cyclic AMP-dependent cellular function

Masahiro Yutani[1], Akira Ogita[1,2], Ken-ichi Fujita[1], Yoshinosuke Usuki[1] and Toshio Tanaka[1]

[1] *Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan*
[2] *Research Center for Urban Health and Sports, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan*

Abstract. Sporulation of the yeast *Saccharomyces cerevisiae* is negatively regulated by cyclic AMP (cAMP). This microbial cell differentiation process was applied for the screening of a substance that can elevate the intracellular cAMP level. Among nucleoside 5'-alkylphosphates, uridine 5'-eicosylphosphate (UMPC20) selectively and predominantly inhibited ascospore formation of the yeast cells. We suppose the inhibitory effect of UMPC20 could indeed reflect the elevation of the cellular cAMP level.

Key words: cAMP — Sporulation — Nucleoside 5'-alkylphosphate — *Saccharomyces cerevisiae*

Abbreviations: AMPC16, adenosine 5'-hexadecylphosphate; UMPC16, uridine 5'-hexadecylphosphate; UMPC20, uridine 5'-eicosylphosphate; TMP, thymidine monophosphate.

Cyclic AMP (cAMP) is a signaling molecule involved in the control of cellular function, metabolism, and even cell death *via* protein kinase A-mediated phosphorylation of specific substrates (Dumaz and Marais 2005; Tamaki 2007). The cellular cAMP level is regulated by phosphodiesterase specific for its degradation in addition to adenylyl cyclase specific for its production (Sunahara and Taussig 2002; Houslay and Adams 2003). This cyclic nucleotide also participates in the control of cell cycle progression in the yeast *Saccharomyces cerevisiae*, as judged by its inhibitory activity on ascospore formation in response to nutrient starvation (McDonald et al. 2009). It seems possible to find a substance that can elevate the intracellular cAMP level by applying simple microscopic observation of the yeast cell differentiation process.

Adenosine 5'-hexadecylphosphate (AMPC16) inhibits phosphodiesterases from rat adipose and rat liver, but its inhibitory activity was more potently detected against adenylyl cyclase (Hynie and Smrt 1978). In *S. cerevisiae*, AMPC16 was rather evaluated as an inhibitor of acyl-CoA synthetase, which is involved in phospholipid metabolism, suggesting that AMP derivatives generally exhibit cytotoxic activities widely against organisms including mammals (Tanaka et al. 2000; Nakayama and Tanaka 2001; Nakayama et al. 2002). On the other hand, uridine 5'-hexadecylphosphate (UMPC16) exhibits unique activity such as inhibiting pheromone-triggered aggregation of **a** and α haploid cells which occurs at the initial step in the sexual reproduction of *S. cerevisiae* (Machida et al. 1997). UMPC16 is similarly effective in inhibiting mammalian cell-cell interaction system such as platelet aggregation induced with each of platelet-activating factors, arachidonic acid, and thrombin (Sugatani et al. 2000).

In this study, we examined the inhibitory activities of nucleoside 5'-alkylphosphates including thymidine 5'-alkylphosphates newly synthesized on the yeast sporulation, and confirmed their effects on the cellular cAMP level. Thymidine 5'-alkylphosphates were synthesized by incubating the mixture containing thymidine monophosphate (TMP) tetrabutylammonium (300 μmol) and each of lauryl bromide, hexadecyl bromide or eicosyl bromide (3 mmol) in 1.95 ml of dimethylacetamide at 80°C for 16 h. The reaction mixture was appropriately diluted with CH_2Cl_2 and the alkylated product was purified by a silica gel column chromatography. The elution was performed with CH_2Cl_2 containing an increasing ratio of methanol. The corresponding fractions were combined and evaporated *in vacuo*. The resulting precipitate was dissolved in 50% methanol and desalted with the addi-

Correspondence to: Toshio Tanaka, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan
E-mail: tanakato@sci.osaka-cu.ac.jp

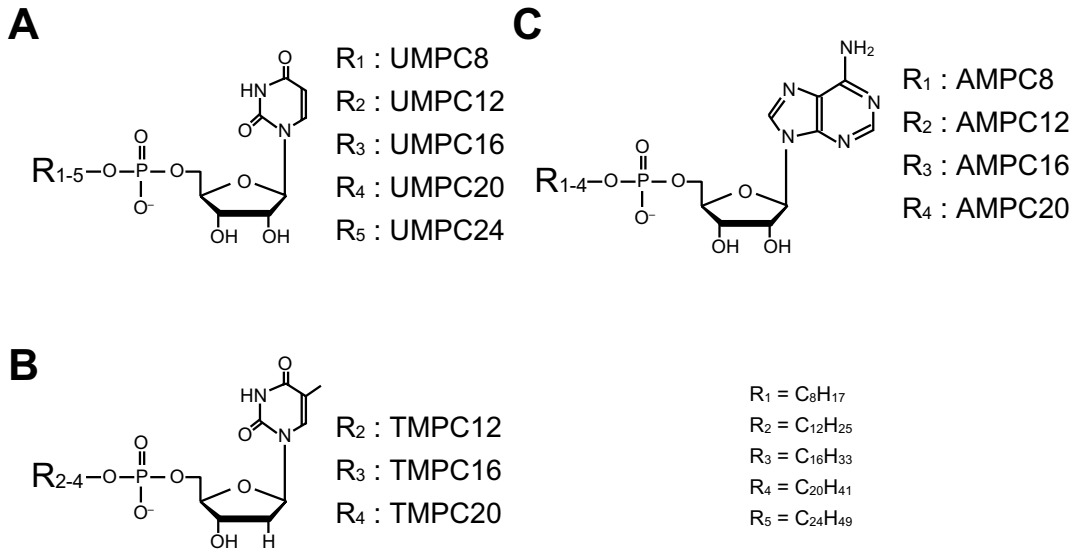

Figure 1. Structures of nucleoside 5'-alkylphosphates: uridine 5'-alkylphosphates (**A**), thymidine 5'-alkylphosphates (**B**), and adenosine 5'-alkylphosphates (**C**).

tion of Amberlite IR 120 (H^+ form). The chemical structures of TMP derivatives were confirmed on the basis of mass and NMR spectral data (Table 1), and were illustrated in Fig. 1 together with the structures of uridine 5'-alkylphosphates and adenosine 5'-alkylphopshates (Machida et al. 1997; Sugatani et al. 2000; Tanaka et al. 2000). The spectral data were recorded on a Jeol AX-500 mass spectrometer as well as JNM-400GX spectrometer operating at 400 MHz for ^{1}H-NMR at 50°C.

First we assayed the inhibitory activities of nucleoside 5'-alkylphosphates on the vegetative growth of *S. cerevisiae* cells by the serial broth dilution method according to our previously described method (Tanaka et al. 2000). Overnight-grown culture of *S. cerevisiae* AKU 4011 (Suizu et al. 1995) was inoculated into YPD medium at 10^6 cells/ml, and were grown at 30°C for 2 days with varying concentration of each nucleotide derivative. The nucleotide derivatives tested did not inhibit the vegetative growth even at the concentration of 100 μg/ml. This made it possible to examine whether the nucleotide derivatives can selectively inhibit the entry process for sporulation *via* meiosis by microscopic observation at this concentration. As shown in Fig. 2A, the yeast cells formed four-spored asci after 5-days of incubation in the nutrient-deprived medium containing acetic acid alone at 30°C. The sporulation frequency reached nearly 60% in untreated cells, and was not affected by the addition of any of TMP derivatives (Fig. 2B). AMP derivatives slightly reduced the sporulation frequency to 50% or less, but their inhibitory effects showed none of relations to the length of alkyl side chain. Although AMP derivatives were more likely to influence the metabolism of cAMP, the yeast sporulation

Table 1. FABMS and selected ^{1}H-NMR data for the TMP derivates

Compound	FABMS (negative mode) *m/z* ($[M-H]^-$)	^{1}H-NMR (DMSO d_6, ppm)
TMPC12	489	1.78 (d, *J* = 1.2, thymine ring CH_3) 7.15 (d, *J* = 1.2, thymine ring 6-H) 6.19 (d, *J* = 1.2, 1'-CH) 3.91 (br. 3'-CH) 4.24 (q, *J* = 4.4, 4'-CH) 3.88 (q, *J* = 6.6, CH_2-O-P) 0.85 (t, *J* = 6.8, CH_3)
TMPC16	545	1.78 (d, *J* = 1.2, thymine ring CH_3) 7.53 (d, *J* = 6.7, thymine ring 6-H) 6.19 (d, *J* = 6.7, 1'- CH) 3.90 (br. 3'-CH) 4.25 (q, *J* = 4.0, 4'-CH) 3.86 (q, *J* = 7.0, CH_2-O-P) 0.85 (t, *J* = 7.0, CH_3)
TMPC20	601	1.78 (d, *J* = 1.2, thymine ring CH_3) 7.53 (d, *J* = 6.7, thymine ring 6-H) 6.19 (t, *J* = 6.7, 1'-CH) 3.90 (br. t, *J* = 5.2, 3'-CH) 4.25 (q, *J* = 4.0, 4'-CH) 3.86 (q, *J* = 7.0, CH_2-O-P) 0.85 (t, *J* = 7.0, CH_3)

FABMS, fast-atom bombardment mass spectrometry; TMPC12, thymidine 5'-laurylphosphate; TMPC16, thymidine 5'-hexadecylphosphate; TMPC20, thymidine 5'-eicosylphosphate; J, coupling constant; d, doublet; q, quartet; t, triplet; br., broad.

was most predominantly inhibited by UMPC20 consisting of UMP and eicosyl side chain. The sporulation frequency was reduced nearly to 20% even if UMPC20 was added at 12.5 μg/ml (data not shown).

Next we examined the effects of UMPC20 on the intracellular levels of cAMP. As shown in Fig. 3, the intracellular cAMP level was reduced with increasing time of incubation in untreated cells, which were mostly transformed to asci carrying 4 spores at 5-days of incubation. In contrast, the intracellular cAMP level was gradually increased in the presence of UMPC20, and the fact agreed with the failure of most cells in provoking spore formation. As deduced from the structure of UMPC20 consisting of UMP, UMPC20 is not likely to inhibit phosphodiesterase, which specifically catalyzes the esterase activity on cAMP. UMPC20 may be evaluated as a novel class of stimulant in the cAMP synthetic reaction, as seen from its increase even under the nutrient-starved condition where phosphodiesterase is generally activated for its degradation.

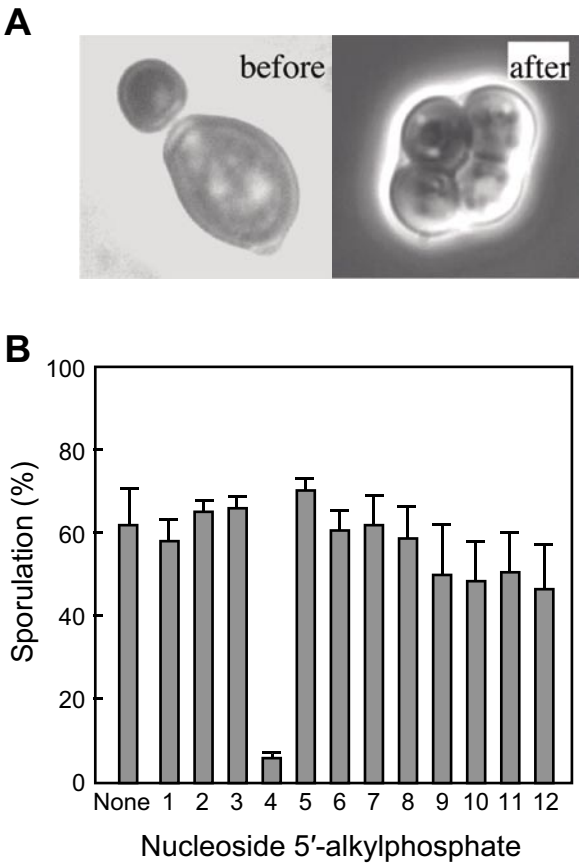

Figure 2. The inhibitory activities of nucleoside 5'-alkylphosphates on sporulation of *S. cerevisiae* cells. **A.** Photographs were taken before (left) and after 5-days of incubation (right) in the sporulation medium at 30°C when four-spored asci were formed. **B.** Cells were incubated in the sporulation medium without or with UMPC8 (1), UMPC12 (2), UMPC16 (3), UMPC20 (4), UMPC24 (5), TMPC12 (6), TMPC16 (7), TMPC20 (8), AMPC8 (9), AMPC12 (10), AMPC16 (11), and AMPC20 (12) at 100 μg/ml, and the sporulation frequency was determined by counting the number of asci to a total population of at least 200 at 5-days of incubation. Values are the means of triplicate assays.

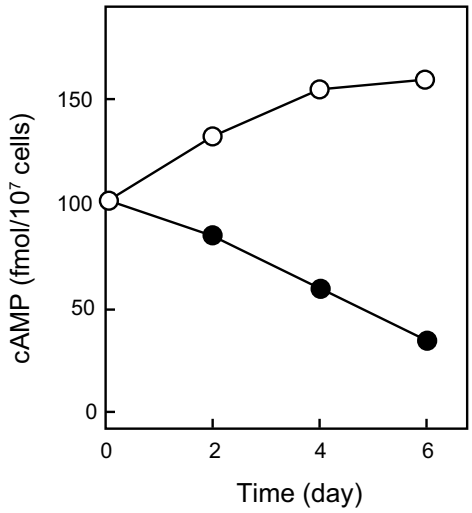

Figure 3. Changes in the intracellular levels of cAMP during the course of incubation with or without UMPC20. Cells were incubated in the sporulation medium without (●) and with 100 μg/ml UMPC20 (○), as described in the legend of Fig. 2. At the indicated times, 1 ml-portions of the cell suspensions were withdrawn and cells were washed three times with distilled water. Cells were then disrupted by repeated vortexing with glass beads (in diameter 0.45–0.55 mm) in a small volume of distilled water at 0°C. The supernatants obtained after centrifugation were used as samples for measurement of cAMP by cAMP Enzyme Immnunoassay System (Amersham Pharmacia Biotech., Braunschweig, Germany).

Acknowledgement. The authors are grateful to F. Horii for technical assistance.

References

Dumaz N., Marais R. (2005): Integrating signals between cAMP and the RAS/RAF/MEK/ERK signalling pathways. FEBS J. **272,** 3491–3504 doi:10.1111/j.1742-4658.2005.04763.x

Houslay M. D., Adams D. R. (2003): PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. Biochem. J. **370,** 1–18 doi:10.1042/BJ20021698

Hynie S., Smrt J. (1978): Effects of adenosine 5'-phosphate esters with lipoid hydroxy compounds (adenosine nucleolipids) on the activity of enzymes of cyclic AMP system. FEBS Lett. **94,** 339–341 doi:10.1016/0014-5793(78)80971-5

Machida K., Tanaka T., Shibata K., Taniguchi M. (1997): Inhibitory effects of nucleoside 5'-alkylphosphates on sexual agglutination in Saccharomyces cerevisiae. FEMS Microbiol. Lett. **147,** 17–22
doi:10.1016/S0378-1097(96)00496-X

McDonald C. M., Wagner M., Dunham M. J., Shin M. E., Ahmed N. T., Winter E. (2009): The Ras/cAMP pathway and the CDK-like kinase Ime2 regulate the MAPK Smk1 and spore morphogenesis in Saccharomyces cerevisiae. Genetics **181,** 511–523
doi:10.1534/genetics.108.098434

Nakayama K., Tanaka T. (2001): Evaluation of adenosine 5'-hexadecylphosphate as an inhibitor of acyl-CoA synthetase isozyme functional for phospholipid reconstitution in the yeast Saccharomyces cerevisiae. J. Biosci. Bioeng. **92,** 475–477
doi:10.1263/jbb.92.475

Nakayama K., Nakamura T., Taniguchi M., Tanaka T. (2002): Irreversible deacylation of plasma membrane phospholipids by the combined action of Mg2+ and a long-chain acyl-CoA synthetase inhibitor in Saccharomyces cerevisiae. J. Biosci. Bioeng. **94,** 258–263
doi:10.1263/jbb.94.258

Sugatani J., Iwai T., Watanabe M., Machida K., Tanaka T., Maeda T., Miwa M. (2000): Inhibition of rabbit platelet aggregation by nucleoside 5'-alkylphosphates: correlation with inhibition of agonist-induced calcium influx. Biochem. Pharmacol. **60,** 197–205
doi:10.1016/S0006-2952(00)00323-3

Suizu T., Tsutsumi H., Kawado A., Inose T., Suginami K., Murata K. (1995): Analysis of lysine-dependent yeast sporulation: a decrease in cyclic AMP is not required for initiation of meiosis and sporulation in Saccharomyces cerevisiae. Microbiology **141,** 2463–2469
doi:10.1099/13500872-141-10-2463

Sunahara R. K., Taussig R. (2002): Isoforms of mammalian adenylyl cyclase: multiplicities of signaling. Mol. Interv. **2,** 168–184
doi:10.1124/mi.2.3.168

Tamaki H. (2007): Glucose-stimualated cAMP-protein kinase a pathway in yeast Saccharomyces cerevisiae. J. Biosci. Bioeng. **104,** 245–250
doi:10.1263/jbb.104.245

Tanaka T., Nakayama K., Machida K., Taniguchi M. (2000): Long-chain alkyl ester of AMP acts as an antagonist of glucose-induced signal transduction that mediates activation of plasma membrane proton pump in Saccharomyces cerevisiae. Microbiology **146,** 377–384

Received: September 9, 2010
Final version accepted: December 2, 2010