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Inactivation of spermidine N^1-acetyltransferase with alkaline phosphatase

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Spermidine N^1-acetyltransferase in an extract from phytohemagglutinin-stimulated bovine lymphocytes was inactivated by preincubation with alkaline phosphatase. Inactivation of the acetylase with the phosphatase was totally inhibited by addition of pyrophosphate. These results suggest that spermidine N^1-acetyltransferase, the rate-limiting enzyme in the biodegradative pathway of polyamines, is inactivated by dephosphorylation. A similar effect of alkaline phosphatase on the acetylase in an extract from *Escherichia coli* was also observed. The acetylase has a rapid rate of turnover and the rapid loss of the enzyme activity may be to some extent regulated by the covalent modification.

Acetyltransferase *Spermidine* *Alkaline phosphatase* *Polyamine metabolism* *Lymphocyte* Escherichia coli

1. INTRODUCTION

The higher polyamines are converted back into putrescine by sequential action of two enzymes, spermidine/spermine N^1-acetyltransferase and polyamine oxidase [1–3]. The rate-limiting step in this conversion is the activity of the acetylase, and the enzyme is induced by a number of stimuli in mammalian cells and bacteria [4,5]. The acetylase has a very short half-life as indicated by the rapid loss of activity when protein synthesis is inhibited [6]. Although this rapid decline in acetylase activity may be attributed to decreased enzyme synthesis, regulation by a covalent modification of the enzyme molecule is also possible since there is much evidence showing that rate-limiting enzymes may be regulated by phosphorylation and/or dephosphorylation [7].

Here, we have studied the possibility of the regulation of spermidine N^1-acetyltransferase activity by a covalent modification and found that the acetylase is indeed inactivated by dephosphorylation with alkaline phosphatase.

2. MATERIALS AND METHODS

2.1. *Reagents*

Alkaline phosphatase (grade I) from calf intestine was obtained from Boehringer (Mannheim). Spermidine was from Sigma Chemical Co. (St Louis MO). Phytohemagglutinin P was purchased from Difco Labs. (Detroit MI). Horse serum was from Commonwealth Serum Labs. (Victoria). Oxoid 'Lab-Lemco' powder was from Oxoid Ltd. (Basingstoke). Sodium pyrophosphate and other chemicals were obtained from Wako Pure Chemicals Co. (Osaka). [*acetyl*-1-^{14}C]Acetyl CoA (52.3 mCi/mmol) was purchased from New England Nuclear (Boston MA).

2.2. *Lymphocyte culture*

Bovine pharyngenous lymph nodes were obtained from a local slaughterhouse. Lymphocytes were separated from other cells as in [8] and were suspended in Eagle's minimum essential medium containing 1% (v/v) horse serum at 1×10^7 cells/ml. Cell suspensions (3 ml) were cultured in a

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sterile glass petri dish (45 × 15 mm) in a humidified atmosphere of 5% CO_2 in air at 37°C. After treatment with phytohemagglutinin (15 μg/ml) for 24 h, the cells were harvested by centrifugation (400 × g for 5 min) and the pellet was washed once with cold phosphate-buffered saline. Subsequently, the cells were suspended in 0.15 ml 50 mM Tris–HCl (pH 7.8) and were disrupted by repeated (3 times) freeze-thawing. After centrifugation (30000 × g for 20 min), the supernatant was used for enzyme assay. Spermidine N^1-acetyltransferase activity was induced 2–3-fold under these conditions.

2.3. *Culture of* Escherichia coli

Escherichia coli B was cultured with aeration at 37°C in nutrient broth containing 1% polypeptone, 0.5% Oxoid 'Lab-Lemco' powder and 0.5% NaCl. The cells were collected by centrifugation (8000 × g for 10 min) 3 h after inoculation, and the pellets were washed with cold phosphate-buffered saline. After centrifugation (8000 × g for 10 min), the pellet was suspended in 50 mM Tris–HCl (pH 7.8). The cells were then disintegrated by sonication with a Branson Sonifier model W 185 (dial 3 for 2 min). The lysate was centrifuged at 30000 × g for 20 min, and the supernatant was used for enzyme assay.

2.4. *Assay of spermidine* N[1]*-acetyltransferase activity*

The acetylase activity was determined by following the incorporation of [*acetyl*-1-^{14}C]acetyl CoA into monoacetylspermidine [9]. The incubation time was 10 min at 37°C. Enzyme activity was strictly proportional to the time of incubation and the amount of extract under these conditions.

2.5. *Protein determination*

Protein was determined by the method in [10] using bovine serum albumin as a standard.

3. RESULTS AND DISCUSSION

When spermidine N^1-acetyltransferase in the extract from phytohemagglutinin-stimulated lymphocytes was preincubated with alkaline phosphatase, the activity of the acetylase was decreased in a dose-dependent fashion. The presence of 0.6 units of the phosphatase decreased the acetylase activity by 50% and the addition of 5 units of the phosphatase inactivated the acetylase activity to 10% of the control level (fig. 1). The effect of alkaline phosphatase was not observed when the phosphatase was inactivated at 100°C for 5 min (not shown).

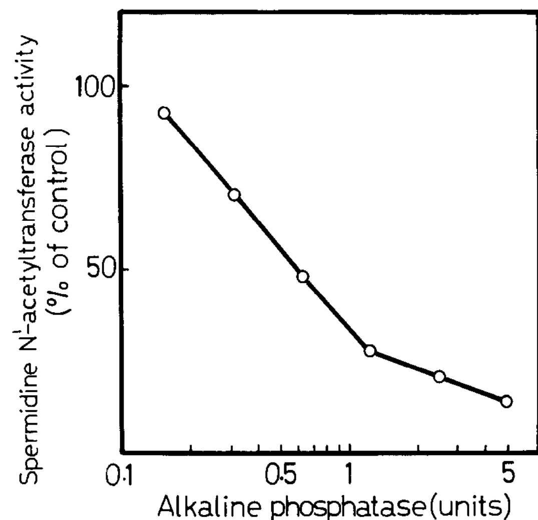

Fig. 1. Effect of addition of alkaline phosphatase on spermidine N^1-acetyltransferase activity. Spermidine N^1-acetyltransferase in the extract (35 μg protein) from phytohemagglutinin-stimulated lymphocytes was preincubated with 10 μmol Tris–HCl (pH 7.8), 300 nmol spermidine and various concentrations of alkaline phosphatase at 37°C for 5 min. The acetylation was initiated by the addition of 0.8 nmol [*acetyl*-1-^{14}C]acetyl CoA and the mixture was incubated at 37°C for 10 min. The activity for the control was 74.4 pmol/10 min.

To confirm the inactivation of the acetylase by dephosphorylation with alkaline phosphatase, the effect of pyrophosphate, an inhibitor of the phosphatase, was examined. Addition of 2 μmol pyrophosphate totally inhibited the inactivation of acetylase by alkaline phosphatase (fig. 2). A similar effect of alkaline phosphatase on the acetylase in the extract from *Escherichia coli* was observed as shown in table 1. Therefore, the inactivation of spermidine acetylase with alkaline phosphatase is not a specific phenomenon for the enzyme from mammalian cells.

This study shows that spermidine N^1-acetyltransferase is inactivated by preincubation with alkaline phosphatase. The acetylase is the rate-

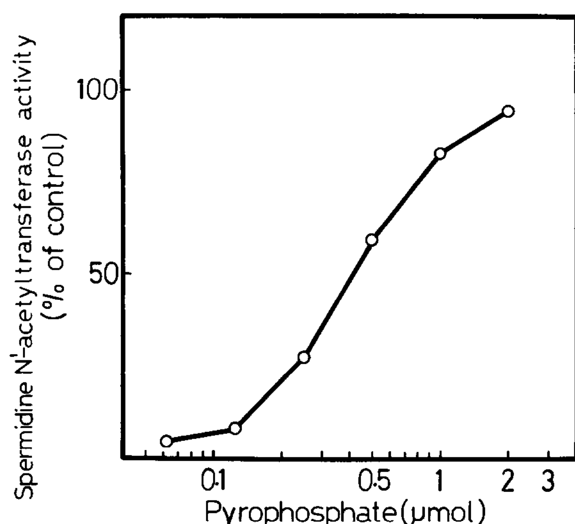

Fig. 2. Effect of pyrophosphate in inactivation of spermidine N^1-acetyltransferase activity by alkaline phosphatase. Spermidine N^1-acetyltransferase in the extract (47 μg protein) from phytohemagglutinin-stimulated lymphocytes was preincubated at 37°C for 5 min with 10 μmol Tris–HCl (pH 7.8), 300 nmol spermidine, 5 units of alkaline phosphatase which was treated with various concentrations of pyrophosphate at 37°C for 2 min. The acetylation was initiated by the addition of 0.8 nmol [*acetyl*-1-^{14}C]acetyl CoA. The activity for the control was 81.5 pmol/10 min.

Table 1

Effect of alkaline phosphatase on spermidine N^1-acetyltransferase from *Escherichia coli*

Treatment	Spermidine N^1-acetyltransferase activity	
	(pmol/10 min)	(% of control)
Control	42.6	100
Alkaline phosphatase	7.8	18
+ pyrophosphate	38.7	91

Spermidine N^1-acetyltransferase in the extract (15 μg protein) from *Escherichia coli* B was preincubated at 37°C for 5 min with 10 μmol of Tris–HCl (pH 7.8), 300 nmol spermidine, 0.3 units of alkaline phosphatase which was pretreated with 0.4 μmol pyrophosphate or distilled water at 37°C for 2 min. The acetylation was initiated by the addition of 0.8 nmol of [*acetyl*-1-^{14}C]acetyl CoA

limiting enzyme of biodegradative pathway of polyamines and has a rapid rate of turnover [4–6]. The half-life for the enzyme in the liver of thioacetamide-treated rat was 40 min [6] and that in phytohemagglutinin-treated lymphocytes was shorter (20 min) (unpublished). The rapid loss of the enzyme activity may be to some extent regulated by dephosphorylation.

In [11], ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis, is phosphorylated by a polyamine-dependent protein kinase and is thereby inhibited in its capacity for decarboxylation of ornithine. Considered together with our findings, it is possible that phosphorylation–dephosphorylation reactions play an important role in the regulation of polyamine metabolism.

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