EFFECT OF POLYAMINES ON PHOSPHORYLATION OF NON-HISTONE CHROMATIN PROTEINS FROM HOG LIVER

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SUMMARY

The effects of polyamines on the *in vitro* phosphorylation of non-histone chromatin proteins from hog liver has been found to be dose dependent. Maximal increase occurred at 0.2 mM spermine and 2 mM spermidine, respectively. These results suggest that spermine and spermidine may have a regulating function for phosphorylation of non-histone chromatin proteins in hog liver.

INTRODUCTION

It has been reported that spermine and spermidine accumulate extensively in such developing organs as regenerating liver and mammary gland (1, 2). Although these polyamines are known to be involved in vital cell functions as a synthesis of RNA (3, 4) and protein (5-8), no clear information is available as to their biological role in the liver.

On the other hand it has been reported that the extent of phosphorylation of tissue-specific non-histone chromatin phosphoproteins correlates to changes in rates of *in vitro* RNA synthesis (9-11).

During our study on the regulation of chromatin proteins phosphorylation, we observed that spermine and spermidine enhance phosphorylation of non-histone chromatin proteins. In this work we studied the effect of polyamines on the *in vitro* phosphorylation of non-histone chromatin proteins from hog liver.

MATERIALS AND METHODS

Hog liver was obtained from Otsu slaughter-house and kept at -20° C before use. Spermine and spermidine were obtained from Nakarai Chemicals, Kyoto. Histone (type II) was a product of Sigma. [γ-^{32}P]ATP was partly obtained from New England Nuclear and partly donated by Dr. M. Kobayashi, Department of Biochemistry, Nagoya University Faculty of Medicine, Nagoya. The other chemicals were obtained from commercial sources.

Preparation of protein fractions

Nuclei were prepared from hog liver by a dense sucrose technique (12) and the non-histone chromatin proteins were isolated by the method of salt extraction and purification on calcium phosphate gel reported by Gershey and Kleinsmith (13). Protein was determined by the method of Lowry et al.(14).

Assay of phosphorylation of non-histone chromatin proteins

The standard incubation mixture contained an appropriate concentration of non-histone chromatin proteins, 4 μmoles $MgCl_2$, 2.5 μmoles phosphate buffer (pH 7.2), 2.5 nmoles [γ-^{32}P]ATP, and varying concentrations of polyamines in a final volume of 0.25 ml. The samples were incubated from 5 min at 30° C, and the reaction terminated by the addition of cold 10 % trichloroacetic acid. Acid-insoluble material was collected on an HA millipore filter and washed with a total of 40 ml of 10 % trichloroacetic acid. The radioactivities of the [^{32}P]-samples were determined by using a Packard liquid scintillation spectrometer with Bray's solution (15).

RESULTS AND DISCUSSION

Spermine and spermidine exhibit dose dependent effects on the in vitro phosphorylation of hog liver non-histone chromatin proteins (Fig. 1). Maximal stimulation of phosphorylation by polyamines occured at 0.2 mM spermine and 2.0 mM spermidine, respectively. At higher concentrations, spermine and spermidine did not inhibit phosphorylation under the conditions used here.

To make certain that spermine would not in some unknown manner influence

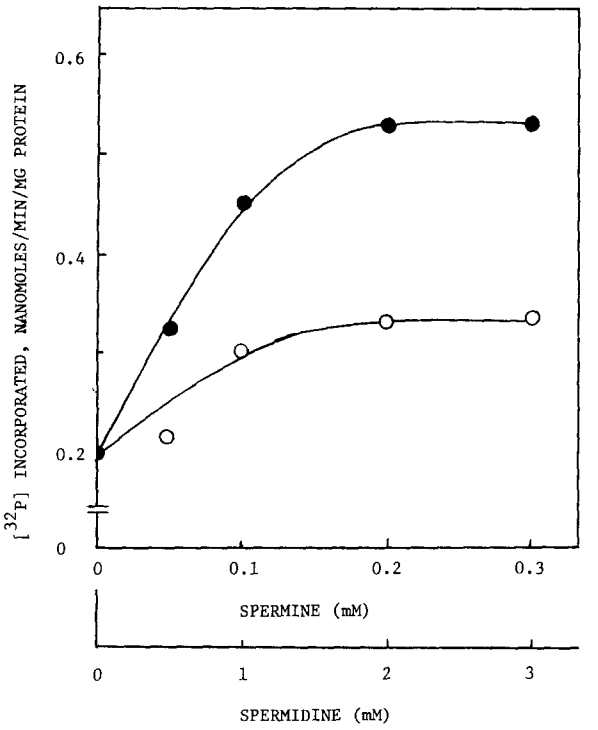

Fig. 1. Effect of increasing concentrations of polyamines on the rate of phosphorylation of non-histone chromatin protein. The phosphorylation was assayed by determining the incorporation of [γ-^{32}P]ATP into acid-insoluble protein during a 5 min incubation at 30° C. Polyamines were adjusted to pH 7.2 before use. Each tube contained 5 μg non-histone chromatin protein and 16 mM Mg^{2+}; the other assay conditions are as described under "Methods". ●——●, spermine; o——o, spermidine.

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the phosphorylation, it was added after competition of the assay. The phosphorylation was not affected (data not shown).

Fig. 2 A shows the rate of phosphorylation in the presence and in the absence of spermine with varying concentrations of non-histone chromatin proteins. We found that the degree of stimulation by spermine depends critically on the ratio of spermine to non-histone proteins. Furthermore, it was found that the reaction rate is lineally dependent on the incubation time (Fig. 2 B).

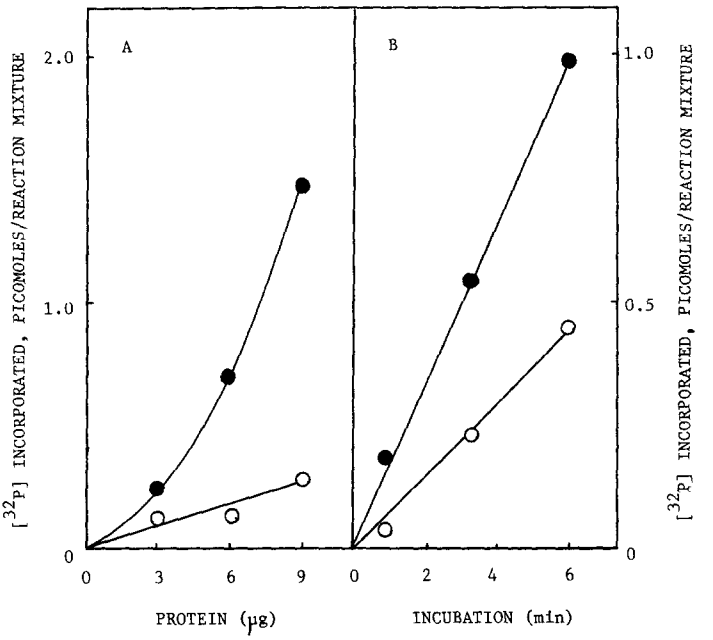

Fig. 2. The effect of spermine on the rate of phosphorylation of non-histone chromatin proteins as a function of protein amount and incubation time. Each tube contained 16 mM Mg^{2+}. The assays were carried out both in the presence (●——●) and in the absence (o——o) of 0.2 mM spermine. A, phosphorylated (per 0.25 ml reaction mixture) when the indicated amount of non-histone proteins were incubated for 5 min. B, phosphorylated (per 0.25 ml reaction mixture) when 5 μg non-histone proteins were incubated for indicated time.

Kolowitz et al. have reported that histone and other polycations, such as poly-L-lysine, cause an increase in the rate and extent of the phosphorylation of nuclear non-histone proteins prepared from calf thymus (16). We compared the effect of histone and spermine on the rate of phosphorylation with hog liver non-histone proteins. As shown in Table I, spermine is almost as effective as the histone in the stimulating the reaction, when agents are added at concentrations which maximally stimulate phosphorylation. However, the combined effects of both agents were not observed.

We tested the combined effects of various Mg^{2+} concentrations and of 0.2 mM spermine, a concentration which stimulate maximally phosphorylation at 16

Ingredients added	Activity
	[^{32}P] incorporated nmoles/min/mg protein
None	0.20
Spermine (0.2 mM)	0.57
Histone (20 μg)	0.67
Spermine and histone	0.65

Table 1. Effect of various cations on the rate of phosphorylation. Each ingredient was adjusted to pH 7.2 before use. The mixtures containing 5 μg non-histone chromatin proteins were incubated at 30° C for 5 min. Each tube contained 16 mM $MgCl_2$; the other conditions are described under "Methods".

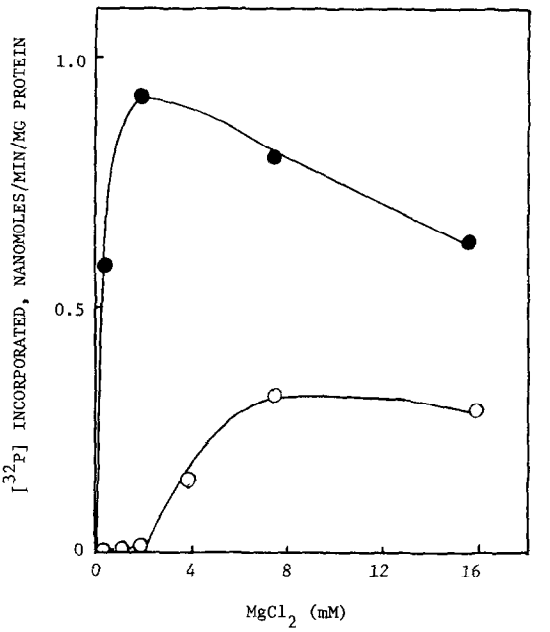

Fig. 3. Effect of 0.2 mM spermine (●——●) on the rate of phosphorylation at various Mg^{2+} concentrations. (o——o) no spermine. Each tube contained 5 μg non-histone chromatin proteins. The other conditions are as described under "Methods".

mM Mg^{2+}. As can be seen from Fig. 3, the omission of Mg^{2+} in the incubation assay leads almost to zero phosphorylation values. 0.2 mM spermine can not completely substitute for Mg^{2+} but cause a shift of the Mg^{2+} concentration

required for maximal phosphorylation from 8 mM to 2 mM. This is in accordance with results obtained from studies with rat brain (17), and with mammalian and bacterial ribosomes (3, 4, 18).

Spermine and spermidine concentrations required for the stimulation of non-histone proteins phosphorylation in hog liver (Fig. 1) are approximately within the range in several tissues. Therefore, our findings suggest that liver nuclei do respond to physiological polyamine concentrations.

Recently, Igarashi et al. (8) reported that polyamines play an important role in protein synthesis by regulating formation and binding of aminoacyl-tRNA to ribosome. Most recently, it was reported that the binding of spermine and spermidine to tRNA are preferetial to that of Mg^{2+}, and that these agents may have a regulating function for nuclear amino acid incorporation in rat brain (19, 17). Furthermore, we observed that polyamines stimulate the in vitro phosphorylation of non-histone chromatin proteins as described here.

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REFERENCES

1. Russel, D. H., Medina, V. J. and Synder, S. H. (1970) J. Biol. Chem. 245, 6732.
2. Russel, D. H. and McVicker, A. T. (1972) Biochem. J. 130, 71.
3. Tabor, H. and Tabor, C. W. (1972) Advances in Enzymology 36, 203.
4. Galdarera, C. M, Moruzzi, M. S., Rossoni, C. and Barbiroli, B. (1969) J. Neurochem. 16, 309.
5. Giorgi, P. P. (1970) Biochem. J. 120, 643.
6. Igarashi, K., Hikami, K., Sugawara, K. and Hirose, S. (1973) Biochem. Biophys. Acta 299, 325.
7. Igarashi, K., Takahashi, K. and Hirose, S. (1974) Biochem. Biophys. Res. Commun. 60, 234.
8. Igarashi, K., Sugawara, K., Izumi, I., Nagayama, C. and Hirose, S. (1974) Eur. J. Biochem. 48, 495.
9. Komiyama, M., Dastugue, B., Defer, N. and Kruh, J. (1972) Biochim. Biophys. Acta 277, 576.
10. Kamiyama, M., Dastsuge, B. and Kruh, J. (1971) Biochem. Biophys. Res. Commun. 44, 1345.
11. Fleischer-Lambropoulos, H., Sarkander, H-I. and Brade, W. P. (1974) FEBS Letters 45, 329.

12. Pogo, A. O., Allfrey, V. G., and Mirsky, A. E., (1966) Proc. Nat. Acad. Sci. U. S., 56, 550.
13. Gershey, E. L. and Kleinsmith, L. J. (1969)Biochim. Biophys. Acta 194, 331.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem., 193, 265.
15. Bray, G. A. (1955) Methods Enzymol. 1, 90.
16. Kaplowitz, P. B., Platz, R. D. and Kleinsmith, L. J. (1971) Biochim. Biophys. Acta 229, 739.
17. Fleischer-Lambropoulos, H., Sarkander, H.-I. and Brade, W. P. (1975) Biochem. Biophys. Res. Commun. 63, 792.
18. Takeda, Y. (1969) Biochem. Biophys. Acta 179, 232.
19. Takeda, Y. and Ohnishi, T. (1975) Biochem. Biophys. Res. Commun. 63, 611.