EFFECT OF POLYAMINES ON ADP-RIBOSYLATION OF NUCLEAR PROTEINS FROM RAT LIVER

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SUMMARY

The effects of polyamines on the in vitro ADP-ribosylation of rat liver nuclei have been found to be dose dependent. These effects were detected in the absence of Mg^{2+}. Maximal increases occurred at 1 mM spermine, 5 mM spermidine, and 7.5 mM putrescine, respectively. The rate of ADP-ribosylation in the presence of 1 mM spermine was 2-fold higher than that in the presence of 10 mM Mg^{2+}, a concentration which maximally stimulates ADP-ribosylation. When the nuclei were incubated with NAD in the presence of spermine, ADP-ribosylation occurred predominantly in the non-histone protein fractions, but in the presence of Mg^{2+}, it occurred predominantly in the histone fractions. These results suggest that polyamines may have some regulatory effect on the ADP-ribosylation of non-histone chromatin proteins in rat liver.

INTRODUCTION

Poly(ADP-ribose) polymerase, the chromatin bound enzyme, catalyzes the formation of the homopolymer by the successive elongation of ADP-ribose units deriving from the substrate NAD(1-4). Although the biological significance of poly(ADP-ribose) formation is not yet fully understood, there is considerable evidence that one function of this reaction is to modify histone and other nuclear proteins by the attachment of poly(ADP-ribose)(5-7).

Since a number of recent reports have indicated a close relationship between poly(ADP-ribose) synthesis and DNA metabolism(8-12), it would seen that attention would be focussed on the investigation of factors affecting this enzyme activity, but very little has been reported on this topic other than that histones appear to stimulate chain elongation(13) and to decrease the Km for NAD(14-16).

During our study on the regulation of ADP-ribosylation, we observed that polyamines enhance the ADP-ribosylation of nuclear proteins. In this work, we studied the effect of polyamines on the in vitro ADP-ribosylation of rat liver nuclear proteins.

MATERIALS AND METHODS

White male rats of the Wistar strain, weighing between 180-200 g, were used in all experiments. The [adenine-2,8-^{3}H]NAD was obtained from New England Nuclear. NAD and spermine were obtained from the Sigma Chemical Company. All other reagents were purchased from the Wakenyaku Co., Ltd., Kyoto.

Purification of rat liver nuclei

The isolated livers were washed with a homogenizing medium which contained 0.25 M sucrose, 5 mM Tris-Cl^- buffer(pH 7.5), 3 mM $CaCl_2$, 1 mM ethylenediamine tetraacetic acid(EDTA), and 0.5 mM ethyleneglycol-bis(β-aminoethylether)N,N'-

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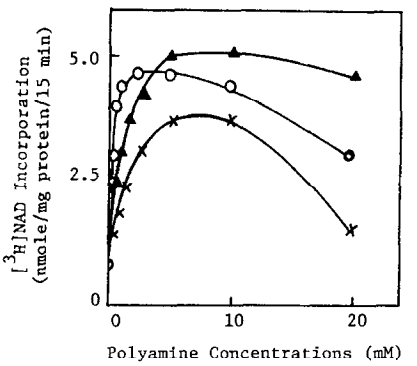

Fig. 1.
Effect of increasing concentrations of polyamines on the rate of ADP-ribosylation. The ADP-ribosylation was assayed by determining the incorporation of [adenine-^{3}H]NAD into acid-insoluble fractions during a 15 min incubation at 25° C. The polyamines were adjusted to pH 7.5 before use. Each tube contained 120 μg nuclear protein. The other assay conditions are as described under "Methods". o—o, spermine; ▲—▲, spermidine; ×—×, putrescine.

tetraacetic acid(EGTA); they were then cut into small pieces in 4 vols of the same medium and homogenized in a Teflone Homogenizer. All subsequent procedures were carried out at 0-4°C. The homogenate was centrifuged at 3,000 X g for 15 min. The pellet was resuspended in 5 vols of heavy sucrose solution containing 2.2 M sucrose, 5 mM Tris-Cl$^-$ buffer(pH 7.5), 3 mM $CaCl_2$, 1 mM EDTA, and 0.5 mM EGTA, homogenized and centrifuged at 70,000 X g for 1 hour. The sedimented nuclei were washed 3 times with a homogenizing medium, and then 3 times with 30% glycerol containing 50 mM Tris-Cl$^-$ buffer(pH 7.5), 1 mM EDTA, and 0.5 mM EGTA, in which medium, the final pellet was suspended.

Enzyme assay

The standard assay mixture of poly(ADP-ribose) polymerase activity contained 100 μM [^{3}H]NAD(0.5 μCi/reaction mixture), 50 mM Tris-Cl$^-$ buffer(pH 7.5), 1 mM dithiothreitol, and an appropriate concentration of nuclei in a total volume of 0.2 ml. After incubation for 15 min, the reaction was stopped by the addition of 3 ml of ice cold 10% trichloroacetic acid(TCA). The acid-insoluble material was collected on a millipore filter and washed with a total of 30 ml of 10% TCA. The radioactivities of the [^{3}H]-samples were determined by using a Packard liquid scintillation spectrometer with Bray's solution(17). Protein was determined by the method of Lowry et al.(18).

RESULTS AND DISCUSSION

Spermine, spermidine, and putrescine exhibit dose dependent effects on the in vitro ADP-ribosylation of rat liver nuclei(Fig. 1). Note that these experiments were carried out in the absence of Mg^{2+}. Maximal stimulation of ADP-ribosylation by polyamines occurred at 1.0 mM spermine, 5.0 mM spermidine, and 7.5 mM putrescine, respectively. At higher polyamine concentrations, the nuclear protein ADP-ribosylation was inhibited under the conditions used here. To make certain that polyamines would not in some unknown manner influence the

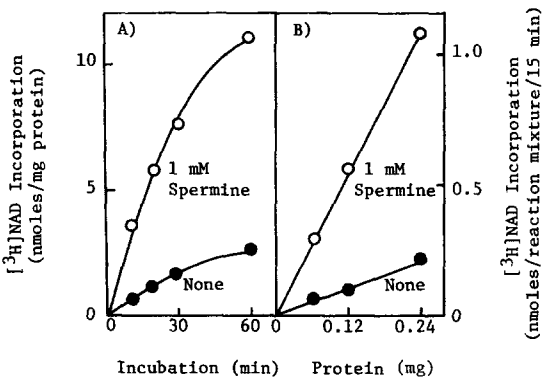

Fig. 2.
The effect of spermine on the rate of ADP-ribosylation of rat liver nuclei as a function of protein amount and incubation time. A) ADP-ribosylation when 120 μg nuclear proteins were incubated for indicated time. B) ADP-ribosylated when the incubated amount of nuclear proteins were incubated for 15 min at 25°C.

ADP-ribosylation, spermine was added after competition of the assay, but the ADP-ribosylation was not affected(data not shown). We confirmed that nicotinamide at 20 mM concentrations suppress the rate of incorporation of [^{3}H]NAD into nuclear protein in the presence of spermine by approximately 80% and that the radioactive materials incorporated into nuclear proteins in the presence of spermine are solubilized by treatment with venom phosphodiesterase, but pancreatic RNase and DNase do not hydrolyze(data not shown). These data are in good agreement with the general properties of poly(ADP-ribose) formed in nuclear fractions in the presence of Mg^{2+}(9,19,20).

With respect to the nuclear fraction, the ADP-ribosylation was proportional to the protein concentration up to 0.24 mg/0.2 ml and to the incubation time up to 30 min. It was stimulated by 1 mM spermine(up to 500%)(Fig. 2A and B).

It has been reported that the effects of polyamines on the activities of certain enzymes can only be detected in the presence of low concentrations of Mg^{2+} and that these effects may be attributed to their cationic nature(21-25). Again, our results indicate that polyamines stimulate ADP-ribosylation without Mg^{2+}(Figs. 1 and 2). To confirm this point further, EDTA was added into the reaction mixture. As shown in Table 1, basal and spermine stimulated poly(ADP-ribose) polymerase activities are not decreased, but are rather slightly increased by 1 mM EDTA.

We investigated the combined effects of various Mg^{2+} concentrations and of 1 mM spermine, a concentration which maximally stimulates ADP-ribosylation

Table I

Effect of EDTA on rat liver nuclei ADP-ribosylation in the presence and absence of spermine

Ingredients added	Activity
	pmoles/mg protein/15 min
None	758.0
EDTA (1 mM)	968.7
Spermine (1 mM)	4305.7
Both	4458.4

The assay was carried out with 120 μg nuclear proteins in the absence of Mg^{2+}; the other conditions are described under "Methods".

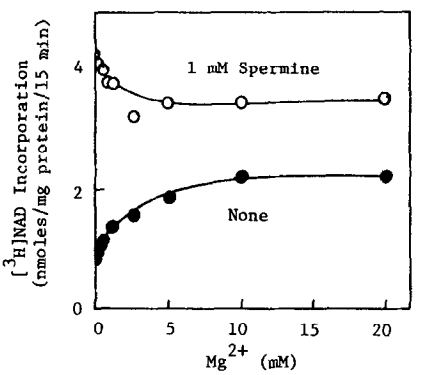

Fig. 3.
Effect of 1 mM spermine on the rate of ADP-ribosylation at various Mg^{2+} concentrations. Each tube contained 120 μg nuclear proteins. The other conditions are as described under "Methods".

in the absence of Mg^{2+}(Fig. 1). As depicted in Fig. 3, the ADP-ribosylation stimulated by 1 mM spermine is decreased by increasing concentrations of Mg^{2+}, but in the absence of spermine, Mg^{2+} leads to a dose dependent increase in the rate of ADP-ribosylation. The data of the Fig. 3 experiment also show that the rate of ADP-ribosylation in the presence of spermine at any of the concentrations of Mg^{2+} tested here is higher than that of the respective control.

Table II

Effect of Mg^{2+}, spermine, or both on the distribution of radioactivity in the different fractions of nuclear proteins obtained from rat liver

Nuclear protein fractions	Mg^{2+}(10 mM)		Mg^{2+}(10 mM) plus Spermine(1 mM)		Spermine(1 mM)	
	Activity (X 10^{-3}cpm)					
	Total	Specific	Total	Specific	Total	Specific
Whole nuclei	13.4	5.7	19.7	8.9	26.5	11.2
Globulin	2.2	2.3	3.0	1.9	4.2	2.7
Histones	7.6	14.3	9.1	14.5	4.3	7.2
Residue	3.7	9.2	8.6	21.4	17.7	44.3
Residue/Histones		0.6		1.5		6.6

Isolated nuclei(2.34 mg protein) were incubated for 15 min at 25°C in a volume of 1 ml which contained 100 μM [^{3}H]NAD(2 μCi/0.1 μmole), 50 mM Tris-Cl^- buffer, (pH 7.5), plus 10 mM Mg^{2+}, 1 mM spermine, or both. The nuclear proteins were then fractionated by the method of Steele and Busch(26). An aliquot of each fraction was precipitated with 10% TCA. The precipitate was collected on a millipore filter and washed 4 times with 20 ml of 10% TCA. The radioactivity was determined with a liquid scintillation spectrometer. The data are expressed in terms of total activity(cpm per each fraction) and specific activity(cpm per mg protein), respectively.

The distribution of labeled ADP-ribose in the various nucleoprotein fractions was determined by incubating isolated nuclei with [^{3}H]NAD in the presence of 1mM spermine, 10mM Mg^{2+}, and both combined, respectively(Table 11). The results show that in the presence of 1 mM spermine, the non-histone fractions contain the largest amount of labeled ADP-ribose, whereas in the presence of 10 mM Mg^{2+}, the histone fractions contain the largest amount of labeled ADP-ribose. If both Mg^{2+} and spermine were added into the reaction mixture, an increase in labeled ADP-ribose was observed in the non-histone protein, but not in the histone fractions as compared with the increase seen in the presence of Mg^{2+} alone. These results suggest that spermine promotes the ADP-ribosylation of non-histone proteins while Mg^{2+} promotes that of the histone.

Since polyamines accumulate extensively in such developing organs as regenerating liver and mammary gland, as well as in several malignant tissues (27-32), the present observations may contribute towards an understanding of the regulation of ADP-ribosylation, which probably has multiple functions

relative to certain large and small structural pertubations of chromatin. The reported increase in the ADP-ribosylation of the non-histone protein fractions from Novikoff hepatoma and from leukemic leucocyte nuclei(7,20) and increase in the poly(ADP-ribose) polymerase activity in SV40 transformation(14,33) would not be consistent with the data we have presented here.

We are now attempting to determine the differential effect of the ADP-ribosylation of non-histone protein and histone fractions in DNA metabolism.

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REFERENCES

1. Chambon, P., Weill, J. D., Doly, J., Strosser, M. T. and Mandel, P., Biochem. Biophys. Res. Commun., 25, 638 (1966).
2. Nishizuka, Y., Ueda, K., Nakazawa, K. and Hayaishi, O., J. Biol. Chem., 242, 3164 (1967).
3. Fujimura, S., Hasegawa, S., Shimizu, Y. and Sugimura, T., Biochim. Biophys. Acta, 145, 247 (1967).
4. Gill, D. M., J. Biol. Chem., 247, 5964 (1972)
5. Nishizuka, Y., Ueda, K., Honjo, T. and Hayaishi, O., J. Biol. Chem., 243, 3765 (1968).
6. Otake, H., Miwa,M., Fujimura, S. and Sugimura, T., J. Biochem., 65, 147 (1969).
7. Burzio, L. and Koide, S. S., FEBS Lett., 20, 29 (1972).
8. Haines, M. E., Johnston, J. R., Mathias, A. P. and Ridge, D., Biochem. J., 115, 881 (1970).
9. Burzio, L. and Koide, S. S., Biochem. Biophys. Res. Commun., 40, 1013 (1970).
10. Roberts, J. H., Stark, P. and Smulson, M., Proc. Nat. Acad. Sci., 71, 3212 (1974).
11. Ohtsuka, E., Tanigawa, Y. and Koide, S. S., Experientia, 31, 175 (1975).
12. Yoshihara, K., Tanigawa, Y., Burzio, L. and Koide, S. S., Proc. Nat. Acad. Sci., 72, 289 (1975).
13. Yamada, M. and Sugimura, T., Biochemistry, 12, 3303 (1973).
14. Smulson, M. and Shall, S., Nature, 263, 14 (1976).
15. Okayama, H., Edson, C. M., Fukushima, M. and Hayaishi, O., in Proceedings of the 4th International Symposium on Poly(ADP-Ribose) and ADP-Ribosylation of Protein, edited by Hilz, H., 1 (1976).
16. Yoshihara, K., Hashida, T., Yoshihara, H. and Kamiya, T., in Proceedings of the 4th International Symposium on Poly(ADP-Ribose) and ADP-Ribosylation of Protein, edited by Hilz, H., 1 (1976).
17. Bray, G. A., Methods Enzymol., 1, 90 (1955).
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
19. Hasegawa, S., Fujimura, S., Shimizu, Y. and Sugimura, T., Biochim. Biophys. Acta, 149, 369 (1967).
20. Burzio, L., Reich, L. and Koide, S. S., Proc. Soc. Exp. Biol. Med., 149, 933 (1975).
21. Takeda, Y., Biochim. Biophys. Acta, 179, 232 (1969).
22. Galdarera, C. M., Moruzzi, M. S., Rossoni, C. and Barbiroli, B., J. Neurochem., 16, 309 (1969).
23. Tabor, H. and Tabor, C. W., Advances in Enzymology, 36, 203 (1972).

24. Fleischer-Lambropoulos, H., Sakander, H.-I. and Brade, W. P., Biochem. Biophys. Res. Commun., 63, 792 (1975).
25. Imai, H., Shimoyama, M., Yamamoto, S., Tanigawa, Y. and Ueda, I., Biochem. Biophys. Res. Commun., 66, 856 (1975).
26. Steele, W. J. and Busch, H., Cancer Res., 23, 1153 (1963).
27. Raina, A., Janne, J. and Siimes, M., Biochim. Biophys. Acta, 123, 197 (1966).
28. Rosenthal, S. M. and Tabor, C. W., J. Pharmacol. Exp. Therap., 116, 131 (1956).
29. Siimes, M. and Janne, J., Acta Chem. Scand., 21, 815 (1967).
30. Russel, D. H., Medina, V. J. and Synder, S. H., J. Biol. Chem., 245, 6732 (1972).
31. Russel, D. H. and Levy, C. C., Cancer Res., 31, 248 (1971).
32. Russel, D. H. and McVicker, A. T., Biochem. J., 130, 71 (1972).
33. Miwa, M., Oda, K., Segawa, K., Tanaka, M., Sakura, H., Matsushima, T. and Sugimura, T., in Proceedings of the 4th International Symposium on Poly-(ADP-Ribose) and ADP-Ribosylation of Protein, edited by Hilz, H., 1 (1976).