POLY ADENOSINE DIPHOSPHATE RIBOSE SYNTHESIS ASSOCIATED WITH CHROMATIN*

Kunihiro Ueda**, Ronald H. Reeder+, Tasuku Honjo++,
Yasutomi Nishizuka, and Osamu Hayaishi

Department of Medical Chemistry
Kyoto University Faculty of Medicine
Kyoto, Japan

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Polymerization of the ADPR[1/] portion of NAD has been established in mammalian cell nuclei (Chambon et al., 1966; Sugimura et al., 1967; Nishizuka et al., 1967), and the product, poly ADPR, has been proposed to exist in vivo (Doly and Mandel, 1967). The enzymic activity to synthesize poly ADPR was strictly localized in nuclei and was destroyed by DNase, but not by RNase (Chambon et al., 1966; Nishizuka et al., 1967). The present study was undertaken to investigate the intranuclear localization of poly ADPR synthesis in an attempt to elucidate the role of DNA in this reaction. Evidence to

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** Recipient of Postgraduate Fellowship of California Foundation for Biochemical Research.

+ Present address: Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland, U. S. A.

++ Recipient of Postgraduate Fellowship of Sigma Chemical Company.

1/ Abbreviations used: ADPR, adenosine diphosphate ribose; DNase, deoxyribonuclease; RNase, ribonuclease.

be presented suggests that both enzyme and product are associated with DNA and that a certain polyanionic structure is required for the poly ADPR synthesis.

MATERIALS AND METHODS

Rat liver cell nuclei were prepared by the method of Chauveau et al. (1956). Chromatin was prepared from isolated nuclei by the use of saline-EDTA (Marushige and Bonner, 1966). The nuclei obtained from about 50 g liver (wet weight) were extracted successively with 20 ml each of Tris-Cl buffer (0.01 M, pH 7.4, and 0.05 M, pH 7.8), NaCl (0.075 M)-EDTA (0.024 M, pH 8.0), and further Tris-Cl buffer (0.05 M and 0.01 M, pH 8.0), each time by centrifugation for 10 min at 20,000 x g. The final sediment was stirred overnight with 10 ml of water at 0° and centrifuged for one hour at 105,000 x g. The clear viscous supernatant solution contained approximately 80% of DNA in the original nuclei, and was referred to as chromatin fraction.

The assay of poly ADPR synthesis was essentially identical with that used in our earlier experiments (Nishizuka et al., 1967) except that the standard reaction mixture contained 16 mμmoles of NAD-(adenine-8)-^{14}C (2,260 cpm/mμmole), 7.5 μmoles of $MgCl_2$, 25 μmoles of Tris-Cl buffer, pH 8.0, and the chromatin fraction in a total volume of 0.25 ml. The RNA polymerase activity was assayed by incubating the following reaction mixture for 20 min at 37°, followed by measuring the acid-insoluble radioactivity with a Millipore filter. The reaction mixture contained 3.6 mμmoles of ATP-8-^{14}C (11,000 cpm/mμmole), 100 mμmoles each of GTP, CTP and UTP, 5 μmoles of $MgCl_2$, 2.5 μmoles of $MnCl_2$, 25 μmoles of Tris-Cl buffer, pH 8.0, and the chromatin preparation in a total volume of 0.25 ml. Protein was

determined by the method of Lowry et al. (1951).

RESULTS

The chromatin prepared as mentioned above contained 80 to 90% of the poly ADPR polymerase activity present in the original nuclei, while about 20% of protein was recovered in this fraction[2/]. The chromatin contained DNA and protein in a ratio of 1:1.25 and showed a similar solubility pattern in a salt solution to that observed with calf thymus deoxyribonucleoprotein (Oth and Desreux, 1957).

The dissociation of protein from DNA was accomplished by gel filtration in the presence of high concentrations of salt as described by Georgiev et al. (1967). Ammonium sulfate was chosen as a dissociating agent because of its profitable property to be described later. As seen in Fig. 1, the bulk of protein was released from DNA and eluted in the later fractions, however the activity to synthesize poly ADPR was recovered exclusively in the void volume which consisted mainly of DNA. Even on the addition of rat liver DNA the activity was not detected in the dissociated protein fractions. An association of the poly ADPR polymerase activity with DNA was evident also on equilibrium cesium sulfate density gradient centrifugation of chromatin. Although approximately 70% of protein was dissociated from DNA, the polymerase activity was not recovered in it, but was found in a minor part of protein bound to DNA.

2/ The preparation of chromatin used in this study contained a part of so-called "nucleoli-associated chromatin". However, Drs. M. Muramatsu and T. Sugimura observed that the poly ADPR polymerase activity was not detectable in purified nucleoli (personal communication).

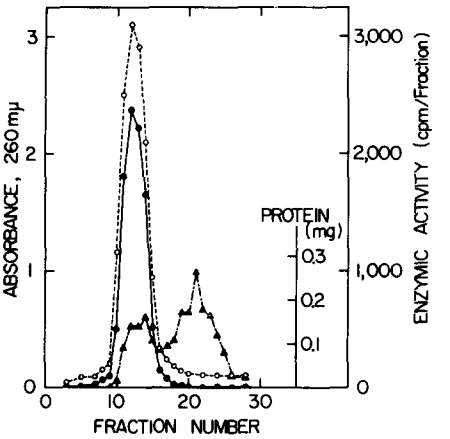

Fig. 1. Gel filtration of chromatin in the presence of ammonium sulfate. Chromatin containing about 10 mg of protein was applied on a Sephadex G-200 column (1 x 40 cm), which had been equilibrated with 1.7 M ammonium sulfate buffered with Tris (final 0.1 M) at pH 8.0, and eluted with the same salt-buffer solution. Aliquots from each fraction (5 ml) were assayed for poly ADPR synthesis under the standard conditions except the addition of ammonium sulfate to the final concentration of 1.7 M. ●——● Enzymic activity, o----o absorbance at 260 mμ, ▲-·-·-▲ protein.

It has been noted that ammonium sulfate increases RNA synthesis by either an enzyme "aggregate" (Goldberg, 1961), a DNA-histone-RNA polymerase complex (Chambon et al., 1965), or isolated mammalian cell nuclei (Widnell and Tata, 1966). The stimulation has been tentatively ascribed to the removal of histone from deoxyribonucleoprotein. Fig. 2A shows the initial velocity of poly ADPR synthesis in the presence of various concentrations of ammonium sulfate. Low concentrations (0.1 - 0.5 M) of ammonium sulfate depressed markedly the poly ADPR synthesis in contrast with a remarkable increase in RNA synthesis. However, at higher concentrations, the poly ADPR synthesis increased again and reached the maximum at 1.7 M which was 40% as active as at the salt-free state. The decrease in poly ADPR synthesis at lower ionic strength, together with the stimulation of RNA polymerase activity, was observed with many other

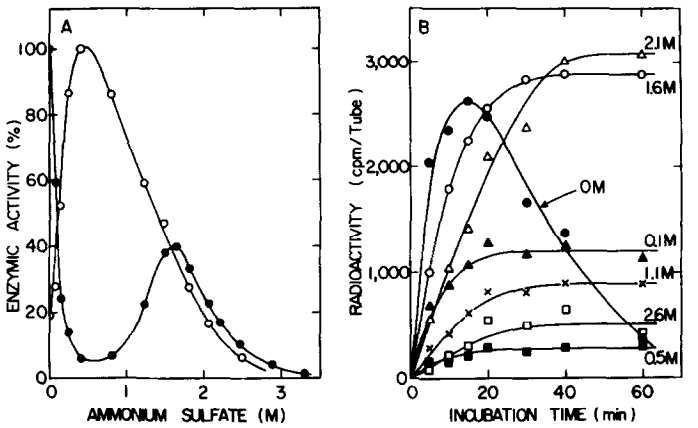

Fig. 2A. Effect of varying concentrations of ammonium sulfate on poly ADPR synthesis and RNA synthesis by chromatin. Assays were performed under the standard conditions except the addition of ammonium sulfate as indicated, and the incubation period for poly ADPR synthesis being 7 min. ●——● Poly ADPR synthesis, o——o RNA synthesis.

B. Time course of poly ADPR synthesis by chromatin in the absence and presence of ammonium sulfate. Aliquots (0.05 ml) were taken out of the reaction mixture (0.50 ml) at various intervals and assayed under the standard conditions. The concentration of ammonium sulfate was given in each line.

salts, while the increase in poly ADPR synthesis at higher ionic strength was observed only with sulfate and phosphate ions, irrespective of the pairing cations.

The radioactivity once incorporated into the acid-insoluble material rapidly disappeared during the incubation in the absence of salt. However, as shown in Fig. 2B, the disappearance of acid-insoluble radioactivity was completely inhibited by ammonium sulfate at concentrations of 0.1 M or higher. This seems to indicate the inhibition of degradation of the polymer by the salt. The enzyme responsible for the degradation appears to be a new kind of phosphodiesterase[3].

3/ The details will be reported elsewhere. During the preparation of this manuscript a new type of phosphodiesterase which reacts also with poly ADPR has been briefly reported by Futai et al. (1967).

In addition, as shown in Fig. 2B, the final amount of radioactivity incorporated into acid-insoluble material increased with higher concentrations of ammonium sulfate (0.5 M to 2.1 M). The product made in the presence of high concentrations of salt had a larger molecular size than that made with lower concentrations of salt, as judged by a sucrose density gradient analysis.

The product, when examined with gel filtration in the presence of salt, showed an association with DNA fraction just like the enzyme activity. But it dissociated from DNA in cesium sulfate density gradient, implying that the binding was not covalent. In addition, sodium dodecyl sulfate or proteinase promoted the release of poly ADPR from DNA. It seems plausible, therefore, that the polymer links to DNA through some protein molecule.

DISCUSSION

The sensitivity to DNase has been noted as one of the characteristics of poly ADPR synthesizing activity (Chambon et al., 1966; Nishizuka et al., 1967) in spite of some discrepancy of results by others (Fujimura et al., 1967). Although the significance of this phenomenon has not been clarified yet, Chambon et al. described that the activity lost by DNase was restored partially by the addition of DNA or other polyanions (1966). The present study shows that chromatin is the site for poly ADPR synthesis and that salts have profound effects on the activity. These observations seem to suggest that DNA or some polyanionic structure is necessary for the manifestation of enzyme activity and that agents which change such a structure affect the activity. The exact mechanism of the reaction will be investigated with a more purified system.

REFERENCES

Chambon, P., Ramuz, M., and Doly, J., Biochem. Biophys. Res. Commun., 21, 156 (1965).

Chambon, P., Weill, J. D., Doly, J., Strosser, M. T., and Mandel, P., Biochem. Biophys. Res. Commun., 25, 638 (1966).

Chauveau, J., Moulé, Y., and Rouiller, Ch., Exp. Cell Res., 11, 317 (1956).

Doly, J., and Mandel, P., Compt. Rend. Acad. Sci. Paris, 264, 2687 (1967).

Fujimura, S., Hasegawa, S., Shimizu, Y., and Sugimura, T., Biochim. Biophys. Acta, 145, 247 (1967).

Futai, M., Mizuno, D., and Sugimura, T., Biochem. Biophys. Res. Commun., 28, 395 (1967).

Georgiev, G. P., Ananieva, L. N., and Kozlov, J. V., J. Mol. Biol., 22, 365 (1966).

Goldberg, I. H., Biochim. Biophys. Acta, 51, 201 (1961).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).

Marushige, K., and Bonner, J., J. Mol. Biol., 15, 160 (1966).

Nishizuka, Y., Ueda, K., Nakazawa, K., and Hayaishi, O., J. Biol. Chem., 242, 3164 (1967).

Oth, A., and Desreux, V., J. Polymer Sci., 23, 713 (1957).

Sugimura, T., Fujimura, S., Hasegawa, S., and Kawamura, Y., Biochim. Biophys. Acta, 138, 438 (1967).

Widnell, C. C., and Tata, J. R., Biochim. Biophys. Acta, 123, 478 (1966).