# RELEASE OF DNA POLYMERASE FROM RAT LIVER CHROMATIN ON INCUBATION WITH NAD

Koichiro YOSHIHARA * and S.S. KOIDE+
*Biomedical Division, The Population Council, The Rockefeller University, New York, N.Y. 10021, USA*

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## 1. Introduction

Rat liver nuclear chromatin possesses an enzymic activity which transfers the ADP-Rib ‡ moiety of NAD to nucleoproteins to form a polymer [1–3]. It was suggested that the formation of poly(ADP-Rib) might play a role in the regulation of DNA synthesis [4, 5] since its formation resulted in an inhibition of the template activity of isolated rat liver nuclei for DNA synthesis [4, 5]. This finding was confirmed by Nagao et al. [6]. They reported, however, that the endogenous DNA polymerase was inhibited rather than the template activity. In a previous communication we reported that the observed inhibition of DNA synthesis following incubation of isolated rat liver nuclei with NAD was associated with a block of the nuclear endonucleolytic activity [7].

In the present paper evidence will be presented to show that the observed inhibition of the endogenous DNA polymerase activity of rat liver chromatin following poly(ADP-Rib) formation was due to a liberation of the enzyme from chromatin into the incubation medium, paralleled with a loss of activity in the chromatin.

* On leave from Cancer Research Institute, Faculty of Medicine, Kyushu University, Fukuoka, Japan.
+ To whom correspondence should be sent.
‡ Abbreviations: ADP-Rib, adenosine diphosphate ribose; EDTA, disodium ethylene diamine tetraacetate.

## 2. Materials and methods

### 2.1. *Materials*

DNA polymerase obtained from *Micrococcus lysodeikticus* was purchased from Miles Laboratory, Indiana, USA. One unit of the enzyme was equivalent to an incorporation of 1.2 nmoles of [$^3$H]TMP into acid-insoluble material in 15 min which was assayed as described below. The assay system contained 50 $\mu$g of activated rat liver DNA. [Adenine $^{14}$C(u)]NAD and [thymidine-methyl $^3$H]TTP were obtained from Amersham/Searle, Ill., USA, and New England Nuclear, Massachusetts, USA, respectively. Activated rat liver DNA was prepared according to the method of Meyer and Simpson [8].

### 2.2. *Preparation of chromatin*

Adult male rats weighing 150–200 g were killed by decapitation. The livers were washed with medium A which contained 0.25 M sucrose, 5 mM Tris–HCl (pH 8.0), 3 mM $CaCl_2$, 1 mM EDTA, cut into small pieces in 4 vol of the same medium and homogenized in a Teflon homogenizer. All subsequent procedures were carried out at 0–4°C. The homogenate was centrifuged at 3000 *g* for 15 min. The pellet was resuspended in 5 vol of heavy sucrose solution containing 2.2 M sucrose, 5 mM Tris–HCl (pH 7.4), 3 mM $CaCl_2$, 1 mM EDTA, homogenized and centrifuged at 70 000 *g* for 1 hr. The nuclei sedimented at the bottom were washed with medium A. Chromatin was prepared by washing the nuclei with the following media: 1) 0.075 M NaCl, 25 mM EDTA (pH 8.0); 2) 50 mM Tris–HCl (pH 8.0), 1 mM EDTA; 3) 10 mM Tris–HCl (pH 8.0), 1 mM EDTA twice; 4) 1 mM Tris–HCl (pH 8.0), 1 mM EDTA. Chromatin was washed with 3–5 vol of medium.


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The chromatin suspensions were centrifuged at 10 000 *g* for 15 min. The crude chromatin fraction obtained by washing was resuspended in medium (4) and sheared by passing twice through a hypodermic needle. The suspension was placed on top of a medium containing 1.7 M sucrose, 10 mM Tris–HCl (pH 8.0), 1 mM EDTA and centrifuged at 27 000 rpm in a SW rotor for 1 hr. The gelatinous pellet was suspended in 30% glycerol, 50 mM Tris–HCl (pH 8.0), 2 mM EDTA and stored at −20°C. The ratio of protein to DNA in the chromatin preparation was 2.5.

2.3. *Preincubation with NAD*

The incubation medium contained 10 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 50 mM Tris–HCl buffer (pH 8.0), chromatin preparation (2.0 mg DNA) and indicated amount of NAD or NAD analogs in a total volume of 2.0 ml. The system was incubated at 25°C for 30 min and stopped by placing the tubes in ice. The tubes were centrifuged at 4000 rpm for 20 min. The supernatant was mixed with an equal volume of a solution containing 100 mM potassium phosphate (pH 6.8), 20 mM EDTA, 4 mM 2-mercaptoethanol and dialyzed overnight against a medium containing 30% glycerol, 2 mM potassium phosphate buffer (pH 6.8), 0.5 mM EDTA, 2 mM 2-mercaptoethanol. The sedimented chromatin was resuspended in a medium containing 30% glycerol, 2 mM 2-mercaptoethanol, 2 mM EDTA, 50 mM potassium phosphate buffer (pH 6.8) and 0.35 M NaCl. The DNA polymerase activity in the chromatin and the supernatant was measured in the presence of 50 μg of activated rat liver DNA.

2.4. *DNA polymerase activity*

The standard reaction for the assay of DNA polymerase activity contained 30 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 25 mM KCl, 2 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM ATP, and 20 μM each of dATP, dGTP, dCTP and [$^3$H]TTP (1.0 μCi/4 nmoles), appropriate amounts of chromatin (10–20 μg DNA) or supernatant and 50 μg of activated DNA. The mixture was incubated at 37°C for an indicated time and stopped by the addition of 2.5 ml 10% trichloroacetic acid. The acid-insoluble radioactive material was collected on a glass fiber filter and counted in a Packard Tri-Carb scintillation spectrometer.

Table 1
DNA polymerase activity of chromatin incubated with NAD.

| | [$^3$H]TMP incorporation (cpm) | | |
|---|---|---|---|
| | Chromatin | Incubation medium | Total |
| Control | 1 883 | 55 | 1 938 |
| +5 mM NAD | 599 | 1 050 | 1 647 |
| +5 mM NAD and 20 mM nicotinamide | 1 678 | 278 | 1 956 |

Chromatin containing 2.0 mg of DNA was incubated with or without NAD. The DNA polymerase activities of the reaction medium and chromatin were measured as described under Materials and methods. The reaction mixture was incubated for 15 min. The values were adjusted and expressed as cpm of activity per 20 μl of the incubation mixture which contained 20 μg of DNA of the original chromatin during the initial incubation.

2.5. *Other Assay*

Protein was determined as described by Lowry et al. [9] and by Burton [10].

## 3. Results and discussion

Nagao et al. [6] reported that the DNA polymerase activity of chromatin was inhibited following incubation with NAD and suggested that the DNA polymerase was inactivated. An alternative explanation is that NAD might affect a release or liberation of the polymerase from chromatin into the incubation medium. To verify this interpretation, chromatin was incubated with NAD and the mixture was centrifuged and the DNA polymerase activities of the supernatant and chromatin were assayed (table 1). The DNA polymerase activity in the supernatant fraction was markedly increased, paralleled with a corresponding loss of enzymic activity in the chromatin. This release or liberation of the polymerase from chromatin into the incubating medium in the presence of NAD was blocked by the addition of nicotinamide (table 1 and 2). Of the various related nucleotides tested for their ability to effect a release of DNA polymerase from chromatin (table 2), NAD was the most effective agent. The

Table 2
Effect of NAD analogs on the release of DNA polymerase from rat liver chromatin.

| Compounds | Released DNA polymerase activity ([$^3$H]TMP incorporation) (cpm) |
|---|---|
| Control | 133 |
| 1 mM NAD | 2287 |
| 1 mM NAD + 20 mM Nicotinamide | 334 |
| 1 mM ADP-Ribose | 304 |
| 1 mM NMN | 192 |
| 1 mM $NADH_2$ | 566 |
| 1 mM NADP | 424 |
| 1 mM $NADPH_2$ | 638 |
| 2 mM ATP | 645 |

Rat liver chromatin (2.0 mg DNA) was incubated with the various nucleotides and DNA polymerase activity of the incubation medium was assayed as described in the text. The polymerase assay system contained 50 $\mu$g of activated DNA and was incubated at 37°C for 30 min. DNA polymerase activity released into the incubation medium from chromatin was determined and values calculated as described in the legend of table 1.

fact that rat liver chromatin possessed an active poly(ADP-Rib) synthetase activity and that nicotinamide inhibited the enzymic activity [11] suggests that the observed effect was probably due to poly(ADP-Rib) formation rather than due to a direct influence of NAD on chromatin. The former contention is further supported by the finding that NAD was unable to affect a release of the polymerase in the presence of high concentrations of nicotinamide (table 2). It should be noted that since the combined total polymerase activities of the supernatant fraction and chromatin incubated with or without NAD were essentially the same (table 1) the active site of the enzyme was probably not affected on incubation of chromatin with NAD.

Following incubation of chromatin with NAD the protein and DNA content of the two fractions were determined. The DNA and protein content of chromatin after incubation with or without NAD in the medium were 1.7–1.9 mg, and 4.1–4.9 mg, respectively. The supernatant fraction contained 0.1 mg of DNA and 0.3–0.4 mg of protein. These results suggest that although NAD affected a release of DNA polymerase from chromatin, no gross liberation of nucleoproteins or DNA occurred.

The results of the present study suggest that the apparent block of the DNA polymerase activity observed by Nagao et al. [6] was due to a release or liberation of the enzyme from chromatin following incubation with NAD. The molecular mechanism accounting for the release of the polymerase from chromatin and the functional significance of this phenomenon are presently under study.

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