# DEPRESSION OF DNA POLYMERASE ACTIVITY IN CHROMATIN BY INCUBATION WITH NAD[1]

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## SUMMARY

DNA polymerase activity of a disrupted nuclear preparation of rat liver was depressed by preincubation with NAD. This depression was observed either using endogenous DNA or poly d( A-T ) as template. DNA polymerase activity in a solubilized fraction from the disrupted nuclear preparation, was also reduced when obtained from a preparation which had been preincubated with NAD. Exogenously added E. coli DNA polymerase could be used equally well by the disrupted nuclear preparation, preincubated either with or without NAD. Addition of purified poly( ADP-Rib ) did not inhibited the activity of DNA polymerase using poly d( A-T ) or endogenous DNA as template.

## INTRODUCTION

Chromatin can synthesize poly( ADP-Rib )[2] from NAD ( 1, 2, 3, 4 ). ADP-ribosylation of histone has also been described ( 5, 6 ). Burzio and Koide reported that previous incubation of chromatin with NAD under conditions leading to formation of poly( ADP-Rib ) resulted in depression of the incorporation of the radioactivity of [ $^{3}H$ ] dTTP into acid-insoluble material on incubation in the presence of other 3 dNTP with or without externally added DNA polymerases of rat liver and of Micrococcus luteus. This effect of

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2. The abbreviations used are: poly( ADP-Rib ), polymer of adenosine diphosphate ribose; NAD, nicotinamide adenine dinucleotide; Nam, nicotinamide; poly d( A-T ), copolymer of alternating deoxyadenylate and deoxythymidylate residues; dNTP, deoxyribonucleotide triphosphate; DTT, dithiothreitol.

incubation with NAD in depressing incorporation was prevented by the presence of Nam, which inhibited the formation of poly( ADP-Rib ). Thus, the decrease in template activity resulting from alteration in the functional structure of chromatin was suggested to be due to the formation of poly( ADP-Rib ) ( 7, 8 ). DNA isolated from chromatin on which poly-( ADP-Rib ) had been formed, showed the same template activity with micrococcal DNA polymerase as DNA from normal chromatin, indicating that DNA itself was not altered by poly( ADP-Rib ) formation and providing further support for the above suggestion ( 8 ).

This paper describes evidence that the activity of DNA polymerase is depressed by incubation of disrupted nuclei with NAD. Incubation of disrupted nuclei with NAD reduced their activity to form the acid-insoluble material from [ $^3$H ] dTTP and dATP in the presence of poly d( A-T ). DNA polymerase was solubilized from the disrupted nuclei with 0.2 M phosphate buffer ( pH 7.4 ). Like the preparation of disrupted nuclei, it had less activity than enzyme from a preparation which had been incubated without NAD.

## MATERIALS AND METHODS

Preparation of disrupted nuclei: Nuclei were prepared by the method of Chauveau et al. ( 9 ), and homogenized with 50 mM Tris-HCl buffer ( pH 8.0 ) in a Potter-Elvehjem-type glass homogenizer with a tightly fitting Teflon pestle. The preparation was then dialyzed against the same buffer for 2 hrs. The mixture was centrifuged at 105,000 x g for 60 min and the precipitate was used as the preparation of disrupted nuclei.

Incubation of disrupted nuclei with NAD: The disrupted nuclear preparation ( 1.2-1.9 mg protein/ml ) was incubated with 4 mM NAD, 100 mM Tris-HCl buffer ( pH 8.0 ), 8 mM $MgCl_2$ and 1 mM DTT at 25°C for 20 min. In some experiments, 20 mM Nam was added.

After incubation, the mixture was diluted with 6 volumes of ice-cold 50 mM Tris-HCl buffer ( pH 7.4 ) and centrifuged at 105,000 x g for 60 min. The precipitate was suspended in 10 mM Tris-HCl buffer ( pH 8.0 ) and its capacity to synthesize DNA was tested.

Incubation of the disrupted nuclear preparation for syntheses of DNA and poly d( A-T ): After various treatments preparations of disrupted nuclei were incubated under conditions leading to synthesis of DNA or poly d( A-T ). The reaction mixture contained [ $^3$H ] dTTP with 3 dNTP or [ $^3$H ] dTTP with dATP on the template of DNA in the preparation or added poly d( A-T ). Details of the components of the mixtures are given in the legends to figures and tables. Acid-insoluble radioactivity was collected on glass-filter paper ( GF/C, Whatman ) and counted with a liquid scintillation counter.

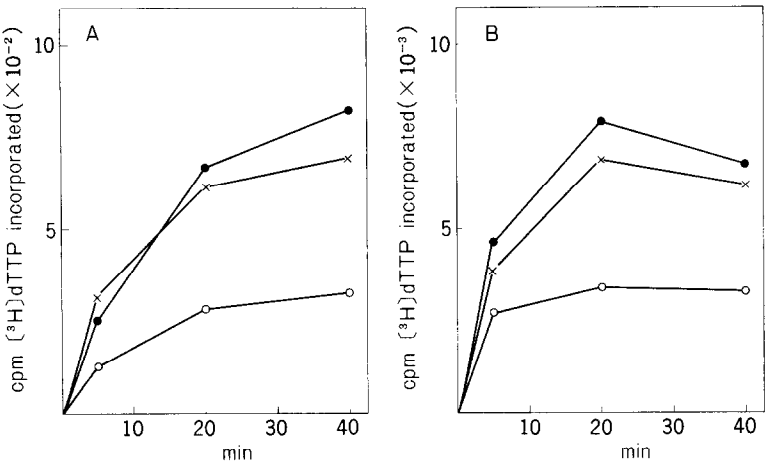


Fig. 1 DNA polymerase activity of disrupted nuclei A. Template, endogenous DNA. The reaction mixture consisted of 10 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.5 mM EDTA, 1 mM $\beta$-mercaptoethanol, 20 mM KCl, 80 μM dATP, dCTP, dGTP and 3.3 μM [ $^3$H ] dTTP ( 2.3 x $10^6$ cpm/nmole ) and preincubated disrupted nuclei, ( 100 μg protein ) 0.2 ml. Incubation for DNA synthesis was carried out at 37°C. A 40 μl aliquot was pipetted onto glass filter paper and washed successively with cold 5 % TCA-1 % sodium-pyrophosphate, 5 % TCA, ethanol and ether. Preincubation; - O -, with NAD, -X- with NAD and Nam, - ● - without NAD. B. Template, poly d( A-T ). The reaction mixture was same as in A. 80 μM dATP and 3.3 μM [ $^3$H ] dTTP were used as substrate and poly d( A-T ) was added to a final $OD_{260}$ of 0.8. Preincubation; - O -, with NAD, -X-, with NAD and Nam, - ● -, without NAD.

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Solubilization of DNA polymerase from the disrupted nuclear preparation: DNA polymerase was solubilized from disrupted nuclei after various treatments, by the method described by Chiu and Sung ( 10 ) and Chang and Bollum ( 11 ). The preparation was suspended in 0.2 M phosphate buffer ( pH 7.4 ) containing 2 mM $\beta$-mercaptoethanol and centrifuged at 105,000 x g for 60 min. Almost 90 % of the DNA polymerase activity was recovered in the supernatant fraction.

Chemicals: dATP, dGTP, dCTP, NAD and ATP were purchased from Sigma Chemical Co., St. Louis. [ $^3$H ] dTTP was obtained from Schwarz Bio Research, Orangeburg, N. Y. DNA polymerase I of Escherichia coli was prepared by the method of Jovin et al. ( 12 ). Pure poly( ADP-Rib ) was obtained by the method of Sugimura et al. ( 13 ).

## RESULTS AND DISCUSSION

Fig. 1A shows the decrease in DNA synthesis of the disrupted nuclear preparation due to preincubation with NAD. This effect was prevented by the presence of Nam, which inhibited the formation of poly( ADP-Rib ) from NAD. This result confirms that of Burzio and Koide ( 7 ).

Table 1

Effect of preincubation with NAD on solubilization of DNA polymerase

| | Incorporation of [ $^3$H ] dTTP | | |
|---|---|---|---|
| | Total ( cpm ) | Supernatant ( cpm ) | Residue ( cpm ) |
| Preincubation | | | |
| ( a ) -NAD | 2533 | 2455 | 1163 |
| ( b ) +NAD | 1300 | 1100 | 490 |
| Ratio of ( b )/( a ) | 0.51 | 0.45 | 0.42 |

DNA polymerase activity was determined with poly d( A-T ) after incubation at 37°C for 10 min, as described in the legend to Fig. 1B.

Fig. 1B shows a similar experiment on incorporation of [ $^3$H ] dTTP into acid-insoluble material in the presence of dATP and poly d( A-T ) indicating that the activity of DNA polymerase in the disrupted nuclear preparation to form poly d( A-T ) decreased greatly on preincubation of the preparation with NAD. As in the case of DNA synthesis, the depression of poly d( A-T ) synthesis was prevented by the presence of Nam during preincubation. Under the present experimental conditions, no radioactivity formed from [ $^3$H ] dTTP and dATP was found in the acid-insoluble fraction unless poly d( A-T ) was added. This suggests that decrease of template activity may not be the only reason for the depression of DNA synthesis of the disrupted nuclear preparation on preincubation with NAD. Another reason might be decrease in the activity of DNA polymerase.

To test the latter possibility the disrupted nuclear fraction was preincubated with NAD and then the DNA polymerase activity was recovered in the soluble form by treating the preparation with 0.2 M phosphate buffer ( pH 7.4 ). Table 1 shows that about 90 % of the DNA polymerase activities determined with poly d( A-T ) template were recovered in the supernatant fractions of preparations preincubated with and without NAD. However,

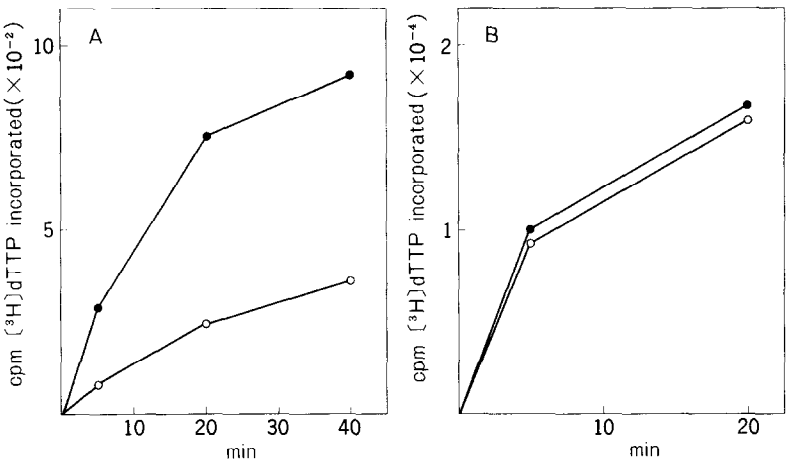


Fig. 2 Template activity of disrupted nuclei preincubated with NAD A. Endogeneous DNA polymerase. Enzyme was assayed as described for Fig. 1A. Preincubation; - O - with NAD, - ● - without NAD. B. E. coli DNA polymerase. 0.74 unit ( as defined by Jovin et al. ) of E. coli DNA polymerase was added in a total of 0.2 ml. Preincubation; - O - with NAD, - ● - without NAD.

the activity of the preparation which had been incubated with NAD was about 50 % of that which had been without NAD. This again suggests that DNA polymerase itself was affected by preincubation with NAD.

Purified DNA polymerase I of Escherichia coli was added to disrupted nuclear preparations which had been incubated with or without NAD. As shown in Fig. 2B, DNA synthesis occurred at the same rate with both these preparations. DNA synthesis in this experiment was completely dependent on the added amount of the disrupted nuclear preparation serving as DNA-template. Thus DNA polymerase I of Escherichia coli can utilize DNA template in disrupted nuclear preparations which have been incubated with or without NAD equally well.

The activity of endogeneous DNA polymerase in the disrupted nuclear preparation, measured after preincubation with NAD was depressed, as shown in Fig. 2A. This again confirms the results of Burzio and Koide ( 7 ). Burzio and Koide reported that the activity for DNA synthesis of the disrupted nuclei which had been preincubated with NAD was

Table 2

Effect of poly( ADP-Rib ) on DNA polymerase

| Template | Poly( ADP-Rib ) ( nmole ) | [ $^3$H ] dTTP incorporated/10 min ( cpm ) |
|---|---|---|
| Endogenous DNA | 0 | 364 |
| | 1.2 | 369 |
| | 2.4 | 578 |
| | 12.0 | 670 |
| | 23.9 | 400 |
| Poly d( A-T ) | 0 | 2643 |
| | 0.48 | 4021 |
| | 0.96 | 3529 |
| | 4.78 | 4259 |
| | 9.55 | 4061 |

The disrupted nuclear preparation was preincubated without NAD under the conditions described in the text. Endogenous DNA : DNA polymerase activity was assayed as described for Fig. 1A except that poly( ADP-Rib ) was added. The concentration of disrupted nuclear preparation was 67.8 µg protein/0.1 ml and a 40 µl aliquot was taken to determine acid insoluble radioactivity. Poly d( A-T ) : The assay conditions were as for Fig. 1B except that poly( ADP-Rib ) was added. The disrupted nuclear preparation contained 27.1 µg protein/0.1 ml, and a 30 µl aliquot was taken to determine acid insoluble radioactivity.

depressed, even when DNA polymerase of Micrococcus luteus was added. This discrepancy requires further investigation.[3]

Various amounts of a purified sample of poly( ADP-Rib ) were added to the disrupted nuclear preparation during synthesis of DNA or poly d( A-T ). As shown in Table 2, the range of concentrations of added poly( ADP-Rib ) was quite broad ranging to about ten times the amount of poly( ADP-Rib ) formed under this preincubation condition. Addition of poly( ADP-Rib ) had no effect on the capacity to synthesize DNA and slightly increased

3. Dr. S. S. Koide ( Rockefeller University ) incubated nuclei with NAD, washed them with 0.25 M sucrose containing 2 mM $CaCl_2$ and found that they showed depressed DNA synthesis in the presence of E. coli DNA polymerase. ( personal communication )

the capacity to synthesize poly d( A-T ). It is clear that the depression of poly d( A-T ) synthesis on the disrupted nuclear preparation observed after preincubation with NAD was not due to the effect of endogeneously formed poly( ADP-Rib ) as polyanionic material ( 14 ). The present data all indicate that DNA polymerase in the disrupted nuclear preparation is inactivated by preincubation with NAD. The mechanism of this inactivation requires further investigation.

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