MODE OF INHIBITION OF DNA SYNTHESIS INDUCED BY ADENOSINE DIPHOSPHORIBOSYLATION OF NUCLEAR PROTEIN.

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Summary

DNA obtained after complete removal of the proteins from NAD-treated rat liver chromatin possessed template capacity equivalent to that obtained from untreated chromatin. The stepwise extraction of proteins from chromatin affected a gradual removal of labeled ADPR and a parallel decrease of the inhibition. The results of the present study suggest that the inhibition of the template capacity for DNA synthesis following preincubation of chromatin with NAD is dependent upon ADP-ribosylation of the associated proteins. The template capacity of chromatin and nucleoprotein complex (Weiss preparation) for DNA synthesis was inhibited, whereas the capacity for RNA synthesis was apparently not affected.

An enzymic system which is associated with chromatin transfers the adenosine diphosphoribose (ADPR) moiety of NAD to nuclear proteins with the liberation of nicotinamide (1-3). ADP-ribosylation occurs with histones and other proteins associated with chromatin and not with DNA (4-6). In a previous report, we demonstrated that the template capacity for DNA synthesis was inhibited when chromatin was incubated with NAD (7). In the present study, the basis of this inhibitory effect was further investigated.

Materials and Methods

[^{3}H]NAD (59.1 mCi/mmole, [^{3}H]TTP (12.5 Ci/mmole) and [^{3}H]UTP (17 Ci/mmole) were purchased from New England Nuclear Corp., Boston. DNA polymerase from Micrococcus lysodeikticus and RNA polymerase from E. coli were purchased from Miles Laboratories, Inc., Kankakee.

Rat liver nuclei were isolated according to Chauveau et al. (8), chro-

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matin as described by Huang and Huang (9), and rat liver DNA polymerase according to Montsavinos (10). The incubation system for the incorporation of [^{3}H] NAD (15 μCi/μmole) was described in a previous report (7). The template capacity of chromatin for DNA synthesis was assayed with rat liver DNA polymerase according to Montsavinos (10). When bacterial DNA polymerase was used, the procedure of Zimmerman (11) was utilized. The assay for RNA synthesis with E. coli polymerase was performed according to Marushige and Bonner (12).

DNA was isolated from chromatin by suspending chromatin in 4 M CsCl and centrifuging the suspension at 35,000 rmp for 18 h: (SW 40 Spinco rotor). The pellet containing DNA was dissolved in 0.01 M Tris-HCl buffer (pH 8) and the suspension dialyzed against the same buffer overnight (12).

Chromatin (2 mg of protein) was preincubated at 25°C for 30 min. in 10 ml of a medium containing 40 mM Tris-HCl buffer (pH 7.4), 4 mM $MgCl_2$, and 4 mM NAD and designated as inhibited chromatin. The control was prepared by incubating chromatin in a medium containing all ingredients except NAD. Chromatin was washed and the proteins extracted with NaCl solution of various concentrations according to Ohlenbusch et al. (13). The measurement of the radioactivity of material insoluble in 10% TCA, DNA and protein were carried out as described in a previous report (7).

Results and Discussion

Table 1 shows the incorporation of [^{3}H]NAD into the various fractions of rat liver nuclei and chromatin. The largest incorporation was found in the histone fraction (Table 1) which is in accord with previous reports (4-6). Table 2 shows the effect of ADP-ribosylation on RNA and DNA synthesis by isolated rat nuclei and Weiss preparation (14) and on the template capacity of chromatin. When these preparations were preincubated with NAD, DNA synthesis were inhibited. On the other hand, the template capacity of chromatin or Weiss preparation for RNA synthesis was not altered significantly. With isolated rat liver nuclei, however,

Table 1. Incorporation of [^{3}H]NAD into Various Fractions of Rat Liver Nuclei and Chromatin.

Fractions	Radioactivity cpm	%
Whole Nuclei *	108,000	100
Globulins	12,500	11
Histones	71,160	67
Residue	10,700	10
Chromatin +	62,300	100
Histones	37,500	61
Residue	8,300	13

* Nuclear proteins were fractionated by the method of Steele and Busch (15).

\+ Chromatin was incubated with [^{3}H]NAD and extracted 4 times with 0.25 N HCl. The remaining insoluble pellet after centrifugation was designated as residue.

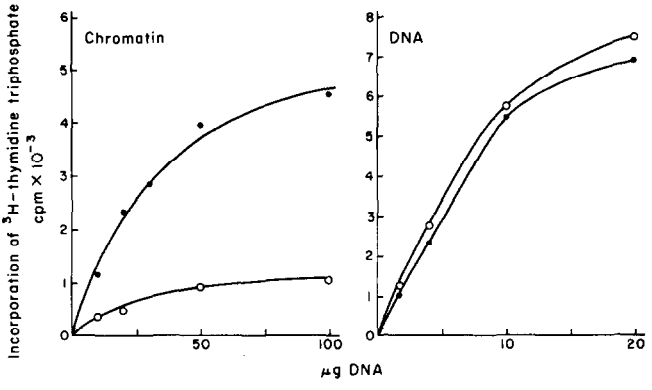

Fig. 1. Template capacity of control (●——●) and inhibited (○——○) chromatin and of the DNA obtained following centrifugation of the respective chromatin in CsCl. The template capacity was assayed with rat liver DNA polymerase in the presence of 1.5 µCi of [^{3}H]TTP (12 Ci/mmole) following the procedure of Montsavinos (10).

a slight stimulation in the incorporation of [^{3}H]UTP into RNA was observed which was inconstant and ranged between 10-60% of the control value.

Fig. 1 shows the template capacity of control and inhibited chromatin

Table 2. Effect of Preincubation with NAD on the Incorporation of [^{3}H]UTP and [^{3}H]TTP by Rat Liver Nuclei, Weiss Preparation and Chromatin.

Fraction	NAD	Radioactivity [^{3}H]UTP (cpm/mg protein)	[^{3}H]TTP (cpm/mg protein)
Nuclei+	—	6960	4740
	+	7350	504
Weiss Preparation+	—	3364	5830
	+	3220	1300
Chromatin++	—	2840	4980
	+	2670	1040

The values are means of triplicate runs of a representative experiment. The three preparations were washed after incubating with and without NAD and assayed.

+ The RNA synthesis was determined by measuring the incorporation of [^{3}H]UTP as described by Weiss (14) and DNA synthesis with [^{3}H]TTP (7).

++ The assay for RNA and DNA synthesis was carried out in the presence of RNA polymerase of E. coli (12) and rat liver DNA polymerase (10), respectively.

for DNA synthesis with increasing amounts of chromatin in the assay system. The degree of inhibition remained fairly constant (about 75%) over the concentrations of chromatin used (Fig. 1). The template capacity of DNA which was prepared from control and inhibited chromatin by the CsCl method and assayed with DNA polymerase from rat liver was identical (Fig. 1). These findings suggest that the inhibition of DNA synthesis induced by incubating chromatin with NAD is mediated by changes in the associated proteins and not on the DNA. This conclusion was further substantiated by the observation that when chromatin preincubated with labeled NAD was extracted successively with 0.6, 1.2 and 2 M NaCl, the radioactivity progressively diminished and a parallel increase in the ability of chromatin to incorporate [^{3}H]TTP into DNA was observed (Table 3). The template capacity was determined with the addition of DNA polymerase obtained

Table 3. Effect on the Template Capacity of Chromatin for DNA Synthesis Following the Extraction of Associated Proteins.

Concentration of NaCl (M)	Chromatin*	[^{3}H]TTP incorporated (μμmoles)	Inhibition (%)
None	Control	165	—
	Inhibited	37	78
0.6	Control	820	—
	Inhibited	540	34
1.2	Control	1510	—
	Inhibited	1240	18
2.0	Control	1890	—
	Inhibited	1780	5

*Chromatin preincubated with and without the addition of NAD in the suspending media was designated as inhibited and control, respectively.
The assay medium for DNA synthesis contained: 6 mM MgCl; 40 mM Tris-HCl buffer (pH 8); 0.5 mM β-mercaptoethanol; 0.1 mM each of dATP, dGTP and dCTP; 0.05 mM (100 μCi/μmole) of [^{3}H]TTP; 4 units (0.06 mg protein) of DNA polymerase (M. lysodeikticus); and chromatin and nucleohistones (10 μg of DNA) in a total of 0.5 ml. The tubes were incubated at 37° for 60 min.

from M. lysodeikticus to the assay system. When chromatin was incubated with [^{3}H]NAD and extracted with 2 M NaCl, the radioactivity remaining with the DNA complex was less than 10% of the initial value. The residual nucleoprotein complex obtained after extracting control and inhibited chromatin with 2 M NaCl possessed equivalent template capacity. Similarly, when the associated proteins were removed from these chromatins by the CsCl method (Fig. 1), the template capacity of the DNA was the same. These results suggest that ADP-ribosylation of nuclear proteins influences the template capacity of chromatin for DNA synthesis.

The results of the present study supports the finding that ADP-ribosylation occurs principally with the histone fraction of chromatin as reported by others (4-6). It is suggested that ADP-ribosylation induced

alterations in the conformation of histones which affected their ability to interact with DNA and thus influenced the template capacity for DNA synthesis.

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