A FUNCTIONAL ROLE OF POLYADPR IN DNA SYNTHESIS

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SUMMARY

Preincubation of rat liver nuclei or chromatin with NAD suppressed the incorporation of [^{3}H]TTP into DNA which was more pronounced at 25° than at 37°. Mg^{2+} or excessive amounts of DNA polymerase added to the assay system containing chromatin did not alter the incorporation of [^{3}H]TTP. On the other hand, when calf thymus DNA was added, the DNA polymerase activity was restored. Nicotinamide added to the preincubation medium containing chromatin blocked polyADPR synthesis and prevented the inhibition of DNA synthesis induced by NAD. The results of the present study demonstrated that preincubation of chromatin with NAD reduced its template capacity for DNA synthesis. Evidence is presented which suggests that the formation of polyADPR influences DNA synthesis.

In mammalian tissues, an enzymic system exists which transfers adenosine diphosphoribose (ADPR) moiety of NAD to nuclear proteins, adds successively ADPR to form a homopolymer (polyADPR) and liberates nicotinamide (1-5). This enzyme designated as polyADPR polymerase is located exclusively in the cell nuclei, is associated intimately with chromatin, and requires DNA for activity (6,7). PolyADPR has been isolated from chicken liver nuclei and identified as a product of NAD metabolism (8). To date the function of ADP-ribosylation of nuclear proteins and formation of the homopolymer has not been established. In the present paper, we will present evidence which suggests that polyADPR may regulate DNA synthesis.

MATERIALS AND METHODS

Non-radioactive NAD was added to [^{3}H]NAD (59.1 mCi/mmole) to a specific activity of 2.0 μCi/μmole. Rat liver nuclei were prepared according to Chauveau et al. (9), chromatin from nuclei suspension according to Ueda et al (7), and rat liver DNA polymerase (step two preparation) according to Montsavinos (10). The assay for the synthesis of polyADPR was carried out

according to Fujimura et al. (4) and DNA polymerase activity according to Wang and Patel (11) with slight modification.

Preincubated with NAD. To study the effect of the formation of polyADPR on DNA polymerase activity, isolated rat liver nuclei were incubated at 25° and 37° in 0.5 ml of a medium composed of 40 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 1.0 mM β-mercaptoethanol and varying amounts of NAD. After incubation for varying lengths of time, the reaction was terminated by placing the tubes in ice and by the addition of 3 ml of a cold solution of 0.25 M sucrose, 2.0 mM $MgCl_2$. The suspension was centrifuged at 900 x g for 10 min and the pellet was resuspended in a medium composed of 40 mM Tris-HCl buffer (pH 7.4), 10 mM $MgCl_2$, 1.0 mM β-mercaptoethanol, 150 mM sucrose and assayed for DNA polymerase activity.

When chromatin was used as template the incubation was carried out as described for the nuclei preparation. At the end of the incubation period, the reaction mixture was cooled, diluted with 3 ml of 0.05 mM Tris-HCl buffer (pH 7.4) and centrifuged at 105,000 x g for 4 hours in a Spinco Model L Ultracentrifuge. The pellet was resuspended in 0.01 M Tris-HCl buffer (pH 8.0) and sheared in a Polytron tissue disintegrator (Type 10, rheostat setting at 4.5) for 20 sec. The suspension was diluted to a concentration of 1 mg of DNA per ml. The recovery of radioactivity was greater than 90% when chromatin was incubated with [^{3}H]NAD and subjected to the washing procedure.

DNA was determined with the diphenylamine reaction as described by Schneider (12) and protein with the biuret reaction (13) or by the method of Lowry et al. (14).

RESULTS

Fig. 1 shows the effect of temperature on the incorporation of [^{3}H]NAD with isolated rat liver nuclei. A significant difference in the extent of incorporation was observed at 25° and 37°. The gradual decline in the in-

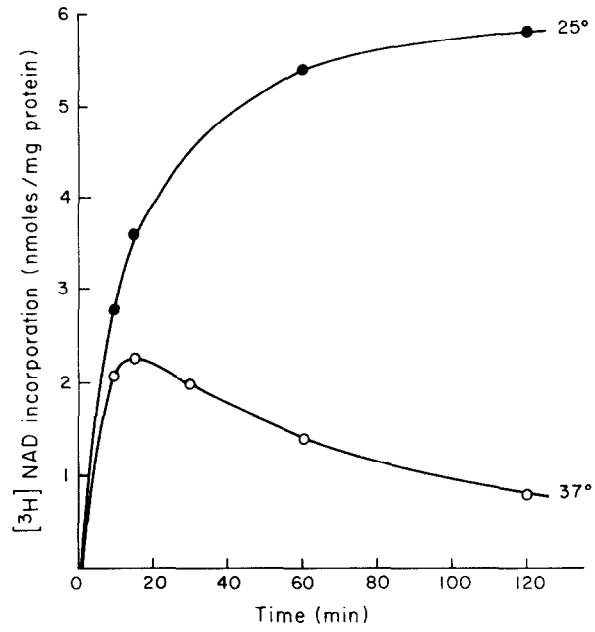

Fig. 1. Incorporation of [^{3}H]NAD into isolated rat liver nuclei at 37° (o—o) and 25° (●—●). The concentration of [^{3}H]NAD was 2 mM.

Table 1. Effect of Preincubation with NAD on [^{3}H]TTP Incorporation by Isolated Nuclei.

Preincubation Temperature (°)	Preincubation Time (min)	NAD (4 mM)	[^{3}H]TTP incorporation (cpm/mg protein)	Inhibition (%)
25	7	0	4350	—
		+	1440	67
"	15	0	5600	—
		+	1130	80
"	30	0	6400	—
		+	790	88
37	7	0	3980	—
		+	2250	57
"	15	0	4730	—
		+	1830	41
"	30	0	4850	—
		+	3070	36

The preparation of nuclei was preincubated for the length of time as indicated, washed and assayed for its capacity to incorporate [^{3}H]TTP.

corporation of [^{3}H]NAD at 37° with the duration of the incubation may be attributed to phosphodiesterase activity (2,4,7,15).

Fig. 2 shows that the incorporation of [^{3}H]TTP was depressed when preparations of isolated rat liver nuclei were incubated with various concentrations of NAD for 30 min. The inhibition increased with increasing concentrations of NAD and was maximal (about 85%) when the concentration of NAD ranged from 2 to 4 mM (Fig. 2). Table 1 shows that DNA polymerase activity of rat liver nuclei was inhibited to a greater extent upon preincubation with NAD at 25° than at 37°.

Since chromatin possessed polyADPR polymerase activity (16-18), it was incubated at 25° for 60 min with NAD (4 mM) and assayed for template capac-

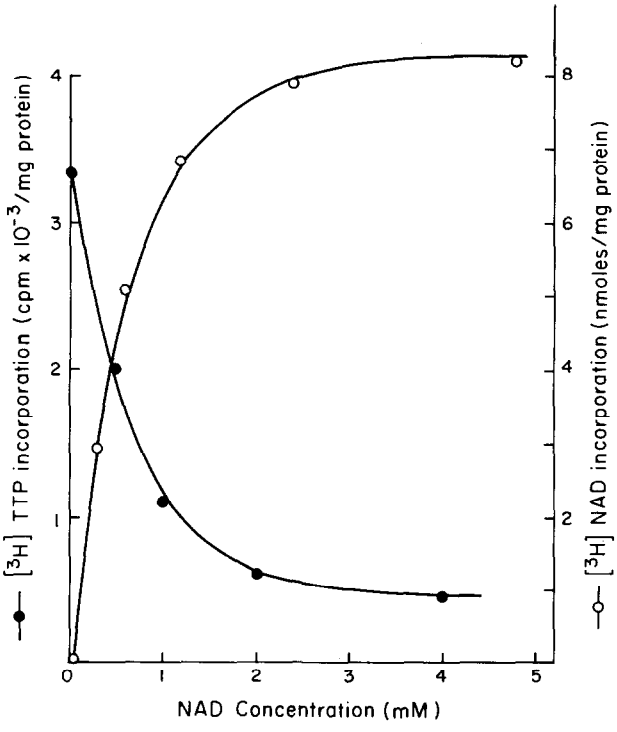

Fig. 2. Effect of varying amounts of NAD on the DNA polymerase activity of rat liver nuclei. The incorporation of [^{3}H]NAD was determined after preincubation of nuclei preparation at 25° for 30 min (O——O). The incorporation of [^{3}H]TTP was measured after incubation of the nuclei preparation in the DNA polymerase system at 37° for 30 min (●——●).

Table 2. Effect of Nicotinamide on the Inhibition of DNA Synthesis induced by PolyADPR.

Preincubation in the presence of:	[^{3}H]TTP incorporation (cpm/100 mg protein)	Inhibition (%)
——	2460	——
NAD (4 mM)	250	90
NAD (4 mM) + Nicotinamide (10 mM)	1500	39
NAD (4 mM) + Nicotinamide (20 mM)	2060	16

The chromatin preparation was preincubated at 25° for 60 min and washed with saline. Rat liver DNA polymerase preparation containing 148 μg of protein was added to the chromatin preparation for the assay of DNA polymerase activity. The reaction mixture was incubated at 37° for 60 min.

ity by the addition of DNA polymerase prepared from rat liver. When chromatin was preincubated with NAD its template capacity for DNA synthesis was depressed (Fig. 3). Nicotinamide at 10 and 20 mM concentrations suppressed the incorporation of [^{3}H]NAD by chromatin by 75 and 91%, respectively. These results confirm the report of Hayaishi et al. (16), that nicotinamide inhibited the formation of polyADPR by rat liver nuclei and chromatin. At those concentrations nicotinamide prevented the inhibition of [^{3}H]TTP incorporation into DNA induced by preincubating chromatin with NAD (Table 2).

To evaluate the factors influencing the inhibition, varying amounts of Mg^{2+}, DNA polymerase and DNA were added to the assay system containing chromatin. When the concentrations of Mg^{2+} were 4, 10 and 20 mM, the inhibition of [^{3}H]TTP incorporation resulting from preincubating chromatin with NAD was 31, 33 and 36% of the control values, respectively, suggesting that the inhibition was independent of Mg^{2+}. The addition of excess amounts of DNA polymerase to the assay systems did not alter the inhibition induced by preincubation with NAD. In contrast, when exogenous calf thymus DNA were added to the assay system, DNA polymerase activity was restored (Table 3). The incorporation was 19, 53 and 67% of the control values when 0, 6 and 12 g of DNA were added, respectively. It should be noted that the incorporation

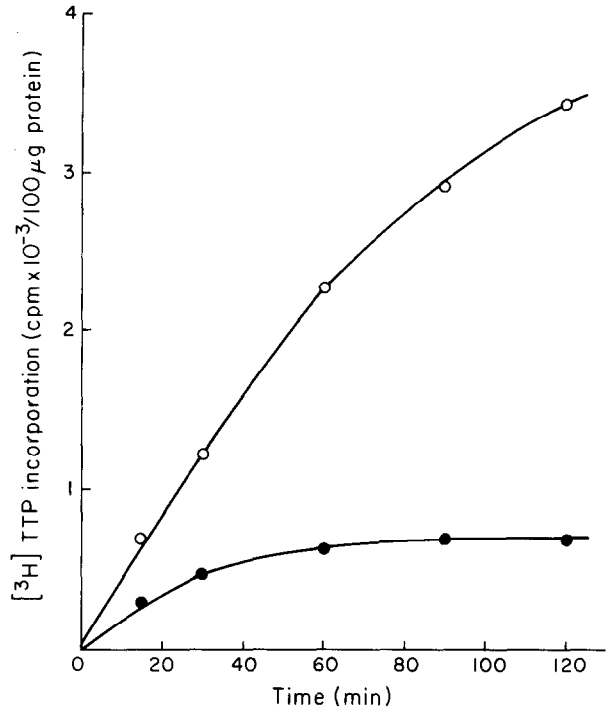

Fig. 3. Time course of the incorporation of [^{3}H]TTP into DNA of chromatin preincubated with NAD (●——●) and without NAD (○——○). Chromatin preparation was preincubated with 4 mM NAD for 30 min, washed and assayed for template activity with rat liver DNA polymerase preparation (85 µg of protein).

Table 3. Effect of exogenous DNA on the Template Capacity of Chromatin.

	Calf thymus DNA (µg)	NAD (4 mM)	[^{3}H]TTP incorporation (cpm/100 µg protein)
Chromatin	——	0	3670
		+	710
	6	0	5580
	6	+	2960
	12	0	6520
	12	+	4410
Without chromatin	6	——	2240
	12	——	3520

of [^{3}H]TTP with chromatin and exogenous DNA was equivalent to the sum obtained with chromatin and DNA alone. The present results demonstrate that the inhibition is due to a suppression of the template capacity of chromatin for DNA synthesis and not due to an inhibition of the enzyme, DNA polymerase.

DISCUSSION

The results of the present study suggest a possible biological function of polyADPR i.e., regulation of DNA synthesis. This thesis is supported by the findings that the degree of inhibition of DNA synthesis varies directly with the amount of incorporation of [^{3}H]NAD (Fig. 1) and with the concentration of NAD (Fig. 2), and inversely with the amount of nicotinamide (Table 1) which blocks the formation of polyADPR (2-5). Since ADP-ribosylation of nuclear proteins takes place concurrently with the formation of polyADPR on incubation of isolated rat liver nuclei with NAD (1-5), we are presently investigating the relative significance of ADP-ribosylation of nuclear protein versus the formation of polyADPR in causing the inhibition of DNA synthesis.

The results of the present study have a direct bearing on the control of DNA replication. There are several reports demonstrating that nicotinamide inhibits mitotic activity in neoplastic cells, regenerating liver and fertilized sea urchin eggs (19,20). On the other hand, nicotinamide stimulates NAD synthesis (21). The inhibition of mitosis induced by nicotinamide might be explained in the following manner. Nicotinamide is converted to NAD, resulting in an elevated level of NAD. Such an increase in availability of NAD would be expected to stimulate the formation of polyADPR and thereby suppress DNA synthesis and mitosis.

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