IN VITRO EFFECT OF NAD ON DNA SYNTHESIS IN ISOLATED NUCLEI FROM REGENERATING RAT LIVER AND NOVIKOFF HEPATOMA

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1. Introduction

Incubation of rat liver nuclei or chromatin with NAD results in the transfer of the ADP-Rib† moiety to nuclear proteins [1–4]. The interaction takes place principally with the histone fractions [5, 6]. Burzio and Koide [7, 8] reported that DNA synthesis was inhibited when rat liver nuclei or chromatin were pre-incubated with NAD and that the block was related to ADP-ribosylation of nuclear proteins. These results suggest that ADP-ribosylation of nuclear proteins may play a role in DNA replication. The recent reports of Smulson et al. [9] and Clark et al. [10] support this proposition.

The present study was undertaken to determine whether a relationship exists between ADP-ribosylation of histones and DNA synthesis in other rapidly replicating tissues. Studies were performed with nuclei of normal and regenerating liver and Novikoff hepatoma. The poly (ADP-Rib) synthetase and DNA polymerase activities were measured under varying conditions.

2. Materials and methods

^{3}H-NAD (590 mCi/mmole), (15 Ci/mmole) and ^{3}H-ATP (18 Ci/mmole) were purchased from New England Nuclear Corp., Boston, USA; DNA polymerase from *M. lysodeikticus* (411 units/mg protein) from Miles Labs, Elkart, USA, and other chemicals from Sigma Chemical Co., St. Louis, USA. Female rats weighing about 200 g were used throughout the present study. Partial hepatectomy was performed as described by Higgins and Anderson [11]. About 60% of the liver was excised.

Novikoff hepatoma was a generous gift of Dr. Novikoff, Albert Einstein Medical College, Bronx, USA, and was transplanted into Holtzman rats each fifth day. The fourth day after the transplantation of the tumor the rats bearing the tumor were sacrificed and the tumor and the liver removed. The tissues were excised from the necrotic area. Nuclei from tumor and from liver (control nuclei) were prepared according to DeBellis et al. [12]. The same procedure was applied to prepared nuclei from normal and regenerating liver. It should be pointed out that the ratio of protein to DNA of isolated nuclei from normal, regenerating and control liver ranged between 4.5–5 and that of Novikoff hepatoma nuclei ranged from 6.5–7.0. The slightly higher content of protein in hepatoma nuclei was consistently observed. The assay systems for poly (ADP-Rib) synthetase activity, DNA synthetase activity and the template capacity of isolated nuclei for DNA polymerase were described in previous reports [7, 8].

Fractionation of nuclear proteins was performed as described in an earlier report [8]. Chromatin was prepared from isolated nuclei according to Marushige and Bonner [13]. DNA was measured as described by Schneider [14] and protein by Lowry et al. [15].

3. Results and discussion

To establish whether or not isolated nuclei from Novikoff hepatoma possess poly (ADP-Rib) synthetase

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† Abbreviation used: ADP-Rib, adenosine diphosphate ribose.

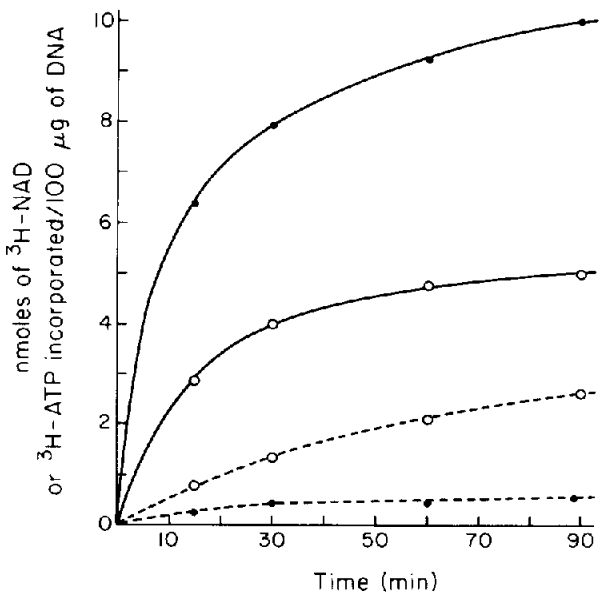

Fig. 1. Kinetics of incorporation of ^{3}H-NAD and ^{3}H-ATP plus NMN by Novikoff hepatoma nuclei and control liver nuclei. The assay systems are described in the text. The nuclei fractions used contained about 100–150 μg of DNA per 0.5 ml. ^{3}H-NAD (——); ^{3}H-ATP plus NMN (- - - -); Novikoff hepatoma nuclei (•); control liver nuclei (○).

activity, the kinetics of incorporation of ^{3}H-NAD or ^{3}H-ATP plus NMN by isolated nuclei of Novikoff hepatoma and control liver were determined (fig. 1).

The activity of the enzyme in hepatoma was about 2-fold greater than in control liver. In a series of 10 separate experiments the synthetase activity of nuclei from hepatoma was consistently higher than that of control liver although increase in activities varied from 50–300%. One noteworthy observation was that the incorporation of ^{3}H-ATP in the presence of NMN with nuclei from Novikoff hepatoma was low and was essentially equivalent to the rate of incorporation of ^{3}H-ATP alone. The basis for the low incorporation of ^{3}H-ATP plus NMN with nuclei from hepatoma may be related to the report that NAD pyrophosphorylase (EC 2.7.7.1) activity of hepatoma was lower than that of normal liver nuclei [16].

In a previous report, we showed that preincubation of rat liver nuclei with NAD inhibited the capacity to incorporate ^{3}H-TTP into DNA when the assay system contained all 4 deoxynucleotide triphosphates [7]. Furthermore, the inhibitory effect was on the template capacity of chromatin for DNA polymerase and was correlated with ADP-ribosylation of the histone fraction [8].

Poly (ADP-Rib) synthetase activities of various kinds of nuclei are presented in table 1. These results support the data that the enzymic activity in Novikoff hepatoma nuclei was higher than that in the nuclei of normal and regenerating (14 and 20 hr post-operative) livers and control livers from hepatoma-carrying rats as shown in fig. 1. However, the dramatic inhibition of DNA synthesis induced by preincubation of

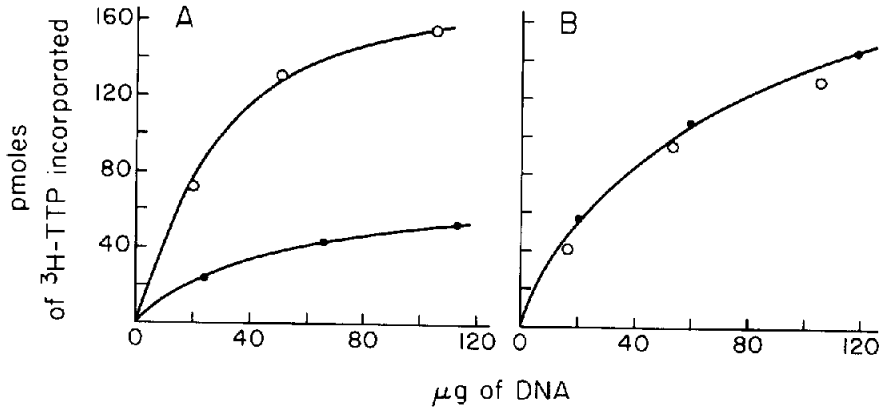

Fig. 2. Template capacity of nuclei from control liver (A) or Novikoff hepatoma (B) for DNA synthesis. DNA polymerase obtained from *M. lysodeikticus* was added to the assay system. Nuclei fractions were preincubated with (•) or without NAD (○), washed and assayed.

Table 1
Poly (ADP-Rib) synthetase activity and its effect on the capacity of DNA synthesis in various types of nuclei.

Source of nuclei	Poly (ADP-Rib) synthetase activity (nmoles of ^{3}H-NAD/100 μg DNA)	DNA synthesis (pmoles ^{3}H-TTP/100 μg DNA)	
		Control	Preincubated with NAD
Normal liver	4.2	38	4
Regenerating liver (14 hr)*	4.9	42	4
Regenerating liver (20 hr)*	5.0	40	3
Control liver	4.0	27	10
Novikoff hepatoma	7.7	11	12

Nuclei were preincubated without (control) or with NAD for 40 min at 25°, washed and DNA synthesis determined [5].

* The time in parenthesis indicates when rats were killed after the operation.

Table 2
Distribution of the radioactivity in different fractions of nuclear protein obtained from normal and control liver nuclei and Novikoff hepatoma nuclei preincubated with ^{3}H-NAD.

Nuclear protein fractions	Normal liver		Control liver		Novikoff hepatoma	
	cpm	mg protein	cpm	mg protein	cpm	mg protein
Whole nuclei	71,300	6.78	37,700	5.2	68,600	7.92
Globulin	10,000	2.97	8,000	1.64	11,700	2.65
Histones	43,200	2.57	20,000	1.52	14,200	2.68
Residue	25,000	2.16	10,000	1.54	48,100	2.05

In each experiment nuclei were incubated in a volume of 2 ml which contained 2 mM ^{3}H-NAD (5 μCi/μmole), 15 mM $MgCl_2$ and 80 mM Tris-HCl buffer, pH 7.5. The mixture was incubated for 60 min at 25°, and the nuclear protein was fractionated as described in the text. Aliquots of each fraction were precipitated with 20% trichloroacetic acid (TCA). The precipitate was collected on a millipore filter, and washed 4 times with 10 ml of 20% TCA. The amount of DNA was 1.62 mg, 1.18 mg and 1.12 mg for normal and control liver and Novikoff hepatoma, respectively.

nuclei from normal, regenerating or control liver nuclei with 4 mM NAD was not observed with Novikoff hepatoma nuclei (table 1). Since the rate of incorporation of ^{3}H-TTP by Novikoff hepatoma nuclei was very low, DNA polymerase from *M. lysodeikticus* was added to the assay system (fig. 2). When control liver nuclei were preincubated with NAD, the template capacity was inhibited by about 60% (fig. 2A) whereas with Novikoff hepatoma the template capacity remained unchanged (fig. 2B).

To elucidate the factors responsible for the divergent results obtained with liver nuclei versus hepatoma nuclei, factors influencing the assay for poly (ADP-Rib) synthetase activity were investigated. The optimal pH and concentrations of Mg^{2+} and ^{3}H-NAD in the assay system for synthetase activity were identical with nuclei of control liver or Novikoff hepatoma. Nicotinamide added to the assay system inhibited the enzymic activity of both types of nuclei. The recoveries of the poly (ADP-Rib) synthetase activity in chromatin prepared from control liver and Novikoff hepatoma were about 85% and 95% of the activity initially in the nuclei, respectively. The distribution of the radioactivity of ^{3}H-NAD into the various nuclear protein fractions were analyzed (table 2). With normal or control liver nuclei about 60% of ^{3}H-NAD was incorporated into the histone fraction. On the other hand, the amount of radioactivity in the histone and residual fractions of hepatoma nuclei were about 20% and 60%, respectively. This low incorporation into the histone fraction of hepatoma nuclei was observed consistently in 5 separate experiments.

In a recent report Smulson et al. [9] demonstrated that in synchronized HeLa cells the activity of poly (ADP-Rib) synthetase varied inversely with the rate of DNA synthesis, i.e., during the S phase of the cell cycle when DNA synthesis was greatest, the activity of the poly (ADP-Rib) synthetase was lowest. The results of Smulson et al. paralleled the findings of Haines et al. [17] although these investigators studied a different system. They separated from rat liver diploid and tetraploid nuclei and nuclei undergoing active DNA synthesis [17]. Their results indicated that nuclei undergoing active DNA synthesis possessed lower poly (ADP-Rib) synthetase activity, suggesting that poly (ADP-Rib) synthetase may be of importance in DNA replication. Our previous study [7, 8] revealed that the suppression of template capacity of chromatin for DNA polymerase was related to ADP-ribosylation of histones. The present results suggest that ADP-ribosylation may play a role in the regulation of DNA synthesis in normal and regenerating liver and that the regulatory control was ineffective in Novikoff hepatoma. One of the factors may be the very low ADP-ribosylation of the histone fraction in hepatoma nuclei. In a preliminary study we found that ADP-ribosylation of the histones with nuclei of cells from acute myelogenous, acute and chronic lymphocyte leukemia was very low. The basis for the inability of ADP-ribosylation of nuclear proteins in neoplastic cells to suppress DNA synthesis *in vitro* is presently under study.

Acknowledgements

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