INFLUENCE OF POLYNUCLEOTIDES ON POLY (ADENOSINE DIPHOSPHATE RIBOSE) SYNTHETASE ACTIVITY OF RAT LIVER

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Received 26 January 1973

1. Introduction

The formation of poly (ADP-Rib)[*] from NAD by an enzyme system in mammalian cell nuclei and the nature of the product have been reported [1–8]. Following an inquiry into the biological function of the polymer, it was suggested that poly (ADP-Rib) formation might play a regulatory role in the synthesis of DNA in mammalian cells [9–12]. The mechanism of the enzymic reaction has not been completely clarified in view of the tight binding of the enzyme to chromosomal DNA which has hampered its purification. In a previous study [13], the enzyme was dissociated from rat liver chromosomal DNA by CsCl density gradient centrifugation and that the dissociated enzyme possessed an absolute requirement for DNA. In support of this finding Yamada et al. [14] reported that the synthetase which was partially solubilized from rat liver chromatin upon treatment with DNAase was found to be dependent upon DNA for activity.

In the present study, various synthetic polynucleotides were substituted for rat liver DNA in a reconstituted poly (ADP-Rib) synthetase system to establish the nature of the polynucleotide dependency of the enzyme. Evidence will be presented demonstrating that the enzymic activity varied greatly with the polynucleotide used.

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[*] *Abbreviation:*
ADP-Rib, adenosine diphosphate ribose.

2. Materials and methods

Ammonium sulphate (special enzyme grade) was purchased from Schwarz/Mann, Orangeburg, USA; poly (dG), poly (dC), poly (dI–dT) and poly (dA)·poly (BrU) from General Biochemicals, Chagrin Falls, USA; other polyribonucleotides and polydeoxyribonucleotides from Miles Laboratories, Elkart, USA; and [^{14}C]NAD from Amersham/Searle Corp., Arlington Heights, USA. Rat liver DNA was isolated according to the method of Kay et al. [15]. Poly (dA)·poly (dT) and poly (rA)·poly (rU) were prepared by mixing poly (dA) and poly (dT) (1.2:1) and poly (rA) and poly (rU) (1:1). The mixtures were heated at 100° for 10 min and cooled gradually. The amount of polynucleotides was estimated by measuring their absorbance at 260 nm.

The assay system for synthetase activity was described in a previous report [13]. A mixture containing 2 μg of rat liver histones, various amounts of polynucleotides or rat liver DNA, and appropriate amounts of poly (ADP-Rib) synthetase preparation (2 to 5 μg of proteins), 5 μmoles of Tris-HCl buffer (pH 8.0), 440 μmoles of $(NH)_2SO_4$ in a final vol of 0.2 ml was incubated for 30 min at 0°. The reaction was started by the addition of 50 μl of a mixture containing 2.5 nmoles of [U-^{14}C]adenine NAD (16,000 cpm/nmole), 7.5 μmoles of $MgCl_2$, 1.25 μmoles of NaF, and 10 μmoles of Tris-HCl buffer (pH 8.0).

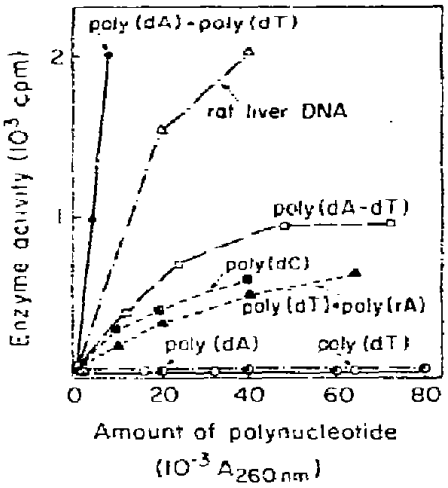

Fig. 1. Effect of polynucleotides on poly (ADP-Rib) synthetase activity. The polynucleotides were used in place of rat liver DNA in the assay system.

After 2 hr of incubation at 25°, the reaction was stopped by the addition of 5 ml of 10% trichloroacetic acid. The mixture was passed through a Millipore filter. The radioactivity of the acid insoluble materials on the filter was counted in a Packard liquid scintillation spectrophotometer.

Male adult rats weighing 150 g were purchased from Holtzman Co., Madison, USA. The procedure for the preparation of chromatin from liver nuclei and the dissociation of the enzyme from chromatin by CsCl density gradient centrifugation in the presence of 30% glycerol was reported [13]. The dissociated enzyme was partially purified by chromatography on a hydroxylapatite column. The details of the purification procedure will be described elsewhere. The partially purified enzyme preparation was used in all of the reported experiments.

3. Results

Poly (ADP-Rib) synthetase activity was examined by the addition of each polynucleotide to the standard reaction assay system in place of rat liver DNA (fig. 1). The enzymic activities increased with increasing concentrations of polydeoxyribonucleotides in the reaction mixture (fig. 1) and were linear at low concentrations. The rates measured in this region were

Table 1

Capacity of synthetic polynucleotides to stimulate reconstituted poly (ADP-Rib) synthetase activity.

Polynucleotides used	$s_{20,w}$ or mol. wt.	Relative capacity
Rat liver DNA	–	100
Poly (rA)	7.0–11.5 S	0
Poly (rU)	3.3– 6.4 S	0
Poly (rC)	4.8– 7.5 S	0
Poly (rA)·poly (rU)	–	0
Poly (dG)	10^6–5 × 10^6 daltons	19
Poly (dC)	10^6–5 × 10^6 daltons	38
Poly (dT)	2.1 S	0
Poly (dA)	2.9 S	0
Poly (dI–dT)	10^6–5 × 10^6 daltons	3
Poly (dG)·poly (dC)	17.0 S	22
Poly (dA)·poly (dT)	–	333
Poly (dA)·poly (BrU)	–	13
Poly (dA–dT)	12.7 S	43
Poly (dT)·poly (rA)	9.8 S	28

Each polynucleotide (8×10^{-3} absorbance unit measured at 260 nm) was added to the standard reaction mixture in place of DNA. The values are relative capacities based on the enzymic activity with rat liver DNA (600 cpm of ADP-Rib incorporated per assay system).

used to calculate the activation capacity based on the enzymic activity obtained with rat liver DNA which was arbitrarily set at 100% (table 1). The highest enzymic activity was observed with renatured poly (dA)·poly (dT) which exceeded by few folds the rate induced with rat liver DNA. Other single and double-stranded polydeoxyribonucleotides and DNA–RNA hybrid tested induced enzymic activities which are about 10 to 40% of that observed with rat liver DNA. On the other hand, the single-stranded polydeoxyribonucleotides, poly (dA) and poly (dT), had no activating influence on the enzymic activity. None of the polyribonucleotides tested were effective in stimulating the enzymic system.

Various synthetic polynucleotides were added to the reaction mixture containing rat liver DNA. The mixture was equilibrated at 0° for 30 min before the reaction was initiated. A marked inhibition of the enzymic reaction (greater than 90%) was observed following the addition of 8×10^{-3} absorbance units (measured at 260 nm) of poly (dT) to the reaction mixture (fig. 2). This amount of poly (dT) was

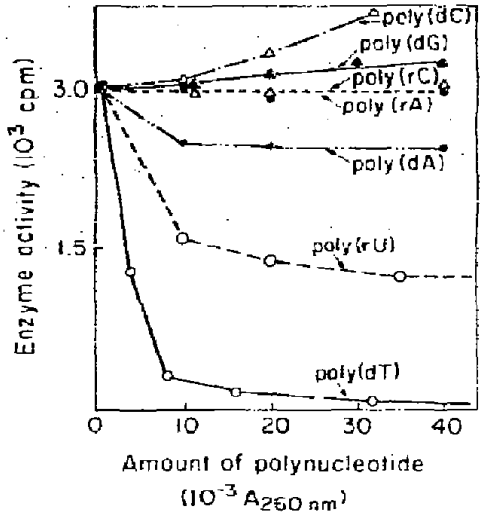

Fig. 2. Influence of single stranded polynucleotides on poly (ADP-Rib) synthetase activity. Each polynucleotide was added to the standard reaction mixture containing 2 μg of rat liver DNA and 2 μg of rat liver histones.

equivalent to about 20% of the content of rat liver DNA in the assay system. Other single-stranded polydeoxyribonucleotides, e.g., poly (dI–dT) exhibited an inhibition of about 70% with the addition of 20×10^{-3} absorbance units to the reaction mixture while poly (dA) affected a slight inhibition. In contrast, poly (dC) and poly (dG) stimulated slightly the synthetase activity. Poly (dT)·poly (rA) and double-stranded polydeoxyribonucleotides examined did not influence the reaction. The polyribonucleotide, poly (rU), inhibited the reaction by about 50–60% at a concentration of $10–40 \times 10^{-3}$ absorbance units (260 nm), while poly (rC) and poly (rA) had no influence. The inhibitory effect of poly (dT) was observed when poly (dA–dT), poly (dA)·poly (dT) or poly (dG)·poly (dC) were used in place of rat liver DNA in the assay system.

4. Discussion

In a previous report it was demonstrated that the poly (ADP-Rib) synthetase system required DNA for activity [13]. It is conceivable that in addition to its ability to stabilize the enzyme, DNA might play some other role in the reaction. It might act as an activator of the enzyme or combined with nuclear proteins and function as receptors of ADP-Rib. In support of this hypothesis is the observation that 70% of the original enzyme activity was recovered after incubation at 25° for 2 hr in a standard assay system from which DNA was omitted, demonstrating that the enzyme is stable in the absence of DNA (unpublished data). Furthermore, the finding that double-stranded polydeoxyribonucleotides, some single-stranded polynucleotides, and DNA–RNA hybrid can, more or less, replace rat liver DNA in stimulating the synthetase suggests that DNA might activate the enzyme in some unknown manner. The possibility that DNA interacts with the enzyme was suggested by the finding that some of the polynucleotides tested inhibited the synthetase activity which was activated with rat liver DNA. Poly (dT) was the most effective blocking agent. Its inhibitory potency was about 100 times greater than that of thymidine and its analogs [16]; i.e., poly (dT) at a concentration of 2 μg/ml (fig. 2) inhibited the synthetase activity by about 90% while thymidine at a concentration of 240 μg/ml was required to sustain a comparable inhibition.

The present finding that poly (dA)·poly (dT) and poly (dT) were the most potent activator and inhibitor, respectively, of the synthetase activity suggests that the enzyme activity might be regulated or be dependent upon the presence of areas rich in dA and dT in the DNA.

Acknowledgements

The investigation was supported in part by grant No. P01-HD05671-01 from the National Institutes of Health.

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