Influence of Agents that act on DNA and RNA Synthesis on the Activity of Poly(ADP-Rib) Polymerase

Poly(adenosine diphosphate-ribose) [poly(ADP-Rib)] is synthesized from NAD by the poly(ADP-Rib) polymerase, an enzyme which is located in chromatin of eukaryotic organisms[1-3].

The function of this enzyme is not yet fully understood, although several reports on in vitro (review[2,3]) and in vivo[4] systems indicate its importance. The enzyme reaction seems to be DNA-dependent[1-3]. It is suggested that poly(ADP-Rib) synthesis is not involved in regulation of DNA synthesis[5] but rather in gene expression[4] and/or in organization of chromatin[5] and acidic proteins[6]. In the present paper, the influence of agents which act on DNA and RNA synthesis in isolated enzyme systems or in in vitro systems, on the activity of the poly(ADP-Rib) polymerase is determined, in order to shed further light on the function of this enzyme.

The poly(ADP-Rib) polymerase is isolated from nuclei of oviducts of egg-laying japanese quails (*Coturnix japonica*). The incorporation is linear for 10 min; during this period an incorporation rate of 4.5 nmoles/min is observed.

The different agents described here can be classified into 4 groups: 1. Polyamines; 2. template inactivators; 3. substrate analogues and 4. enzyme poisons. Unless otherwise noted, the substrate concentration in the poly (ADP-Rib) polymerase assays amounts to 70 μM (= 46 µg/ml) NAD, the concentration of the different compounds was 220 µg/ml. The reaction mixture was composed as described in legend to the Figure and incubated for 10 min.

Two polyamines have been tested, spermine (Serva) and spermidine (Serva). Both stimulate the activity of poly(ADP-Rib) polymerase. Under optimal concentrations of the polyamines (5.3 mM spermine and 8.1 mM spermidine), the activity of the enzyme increases to 156% in the case of spermine and to 147% in the case of spermidine. Under optimal stimulation conditions, a ratio between the molar concentration of the polyamine (mole/ml) and the molar concentration of DNA (mole nucleotide/ml) in the enzyme assays amounts to 1.9 for spermine and to 2.8 for spermidine.

Two groups of template inactivators have been tested for their potency in the poly(ADP-Rib) polymerase assay; first, alkylating agents: Bleomycin (Mack), camptothecin (National Cancer Institute, Bethesda) and mitomycin C (Serva), and second, intercalating agents: Daunomycin (Mann), distamycin (Farmitalia), acridine orange (Merck), olivomycin (Mann), chromomycin (A_3; Mann), actinomycin D (Serva) and ethidium bromide (Serva). All these agents do not affect the activity of the enzyme, with the exception of ethidium bromide. This agent inhibits poly(ADP-Rib) polymerase competitively to NAD (Table). The relative affinity of an inhibitor can be expressed in the case of a competitive inhibition by the ratio K_i/K_m[7]. The higher the value of the ratio, the lower the inhibitory potency of a compound. Due to the high value of the ratio, the activity of ethidium bromide to inhibit poly(ADP-Rib) polymerase is relatively low.

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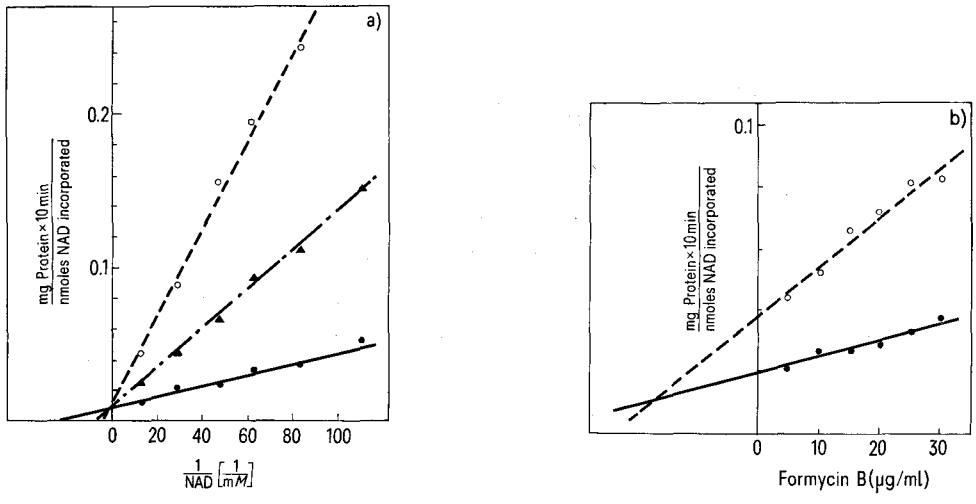

Inhibition of poly(ADP-Rib) polymerase by formycin B. Preparation of the poly(ADP-Rib) polymerase: Quail oviduct nuclei were isolated according to McGuire et al.[12] and washed with 0.7 M KCl by centrifugation (12,000 × g, 2 min, 2 °C). The nuclear preparation is characterized by a DNA: RNA ratio of 1.28 and a DNA: protein ratio of 0.15. The poly(ADP-Rib) polymerase was assayed in a reaction mixture containing in a total volume of 70 µl: 100 mM *Tris*-HCl, pH 8.5, 6 mM $MgCl_2$, 60 mM KCl, 4 mM dithiothreitol and different concentrations of [^{14}C] NAD$^+$ (0.4 mCi/mmole; Radiochemical Centre Amersham) and 20 µl of the nuclear preparation. The mixture was incubated at 25 °C for 10 min. The reaction was stopped by the addition of 1 ml of 5% trichloroacetic acid. After 30 min at 0 °C the mixture was transfered onto GF/C filters and counted[4]. a) Plot according to LINEWEAVER et al.[8]. ●—●, control; ▲— · —▲, inhibitor concentration 35 µg/ml; ○ – – ○, 75 µg/ml. K_i = 68.9 ± 6.5 μM; K_m = 43.7 ± 4.1 μM. b) Plot according to DIXON et al.[9]. Two different substrate concentrations have been used: ●—●, 30 µg NAD/ml; ○ – – ○, 10 µg/ml. K_i = 63.7 ± 6.9 μM; K_m = 48.2 ± 4.1 μM.

Inhibition of poly(ADP-Rib) polymerase by different agents

Inhibitor	V_{max} (nmoles/mg protein)	K_m (μM)	K_i (μM)	K_i/K_m
Ethidium bromide	98.4	42.2	143.8	3.4
Formycin B	96.0	43.7	68.9	1.6
Showdomycin	96.3	43.0	107.8	2.5
1-Methyl adenine	95.9	41.9	226.6	5.4

The standard enzyme mixture (see in legend to the Figure) with different NAD concentrations (in the range between 9 and 83 μM) has been used; the mixture was incubated for 10 min. K_m and K_i were determined according to LINEWEAVER et al.[8].

The substrate analogues related to the adenosine moiety of NAD exert a different inhibitory activity in the poly (ADP-Rib) polymerase assay. The natural analogues Ade, Ado, dAdo, AMP, ADP, ATP, dAMP, dADP and dATP are without influence on the reaction. The effects of some unusual analogues have been tested; without influence were: 9-β-D-arabinofuranosyladenine (Mack), 9-β-D-arabinofuranosyladenine-5′-monophosphate, -diphosphate and -triphosphate (Terra Marine), α-β-ATP-methylene diphosphonate (Terra Marine), β-γ-ATP-methylene diphosphonate (Terra Marine), adenosine-5′-*O*-(3-thiotriphosphate) (Boehringer, Mannheim), cordycepin (Sigma), coformycin and tubercidin (Sigma). Three analogues have been found to affect the activity of poly(ADP-Rib) polymerase in different strengths (Table): Formycin B (Calbiochem), showdomycin (Calbiochem) and 1-methyl-adenine (Sigma). These 3 compounds inhibit the enzyme competitively to the NAD substrate. The inhibition kinetics obtained are perfectly linear, both in LINEWEAVER and BURK plot[8] and in DIXON plot[9]. One example is shown for formycin B in the Figure. The highest affinity among the three inhibitors to the enzyme is found in the case of formycin B; showdomycin and 1-methyl adenine are less effective.

The three different enzyme poisons tested are without influence on the activity of poly(ADP-Rib) polymerase: Rifamycin (Calbiochem; final concentration in the assay 0.2 mg/ml), α-amanitin (Boehringer Ingelheim; final concentration 0.1 mg/ml) and 2-phenylethanol (Mack; final concentration 1 mg/ml). To rule out the possibility that these compounds which are capable of inhibiting poly(ADP-Rib) polymerase are contaminated with NMN adenylyltransferase, the compound solutions were assayed for this enzyme; no enzyme activity could be detected[10]. NMN adenylyltransferase could convert NAD into NMN, a potent inhibitor of poly(ADP-Rib) polymerase[11].

Summary. The activity of poly(ADP-Rib) polymerase is enhanced in the presence of spermine and spermidine. Among the adenosine-like antibiotics tested, only formycin B and showdomycin cause an inhibition of the enzyme, which is competitive to NAD. The activity of poly(ADP-Rib) polymerase is not reduced by rifamycin, α-amanitin and 2-phenylethanol.

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Chlorodimeform and its Effect on Monoamine Oxidase Activity in the Cattle Tick, *Boophilus microplus*

Galecron (active ingredient chlorodimeform) is a relatively new acaricide which is particularly effective against cattle ticks, including resistant strains[1]. Its mode of action is uncertain but it is known not to involve inhibition of acetylcholinesterase[2], the mode of action of organophosphorus and carbamate acaricides. Two different modes of action for chlorodimeform have been proposed. ABO-KHATWA and HOLLINGWORTH[3,4] using mitochondria from rat liver and from cockroach thoracic muscle found that chlorodimeform uncoupled respiratory-chain phosphorylation and stimulated ATP-ase. However, as pointed out by BEEMAN and MATSUMURA[5] this uncoupling action would not give rise to chlorodimeform's known action on the central nervous system. AZIZ and KNOWLES[6] and BEEMAN and MATSUMURA[5,7] have shown chlorodimeform to be a potent in vitro inhibitor of monoamine oxidase (MAO) from both rat liver and cockroach thoracic muscle and this led them to suggest that inhibition of MAO may be the mode of acaricidal action.

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