INFLUENCE OF SPERMINE AND HISTONES ON A NUCLEAR NUCLEOSIDASE WITH ADP-RIBOSE TRANSFERASE ACTIVITY IN EHRLICH ASCITES CELLS

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1. Introduction

Two different NAD nucleosidases (NADases) (EC 3.2.2.5) could be localized in Ehrlich ascites cells, one in microsomes presumably associated with the plasma membrane and the other in the nuclear fraction associated with chromatin [1, 2]. The nuclear NADase is very probably identical with the enzyme forming an acid-insoluble polymer of ADP-ribose first described in liver nuclei [3–5]. With Ehrlich ascites cell nuclei similar results have been published [6]. An ADP-ribosylation of histones by the nuclear enzyme has recently been observed [7].

NADase and ADP-ribose transferase activities in nuclei are sensitive to DNase but not to RNase [1,3,5]. The detailed mechanism of this DNase sensitivity is still obscure. The following experiments were undertaken to elucidate whether this inhibition is caused by removal of polyanionic DNA or by a release of polycations which had been bound to DNA. It will be shown that the inhibition by DNase pretreatment can be overcome by ammonium sulfate. Polycations like spermine and histones inhibit the enzyme activities at high concentrations. It is interesting to notice, however, that at low concentrations of the polycations used, an activation of both enzyme activities can be observed.

2. Materials and methods

Nuclei of ascites cells were prepared as described earlier [1]. Assay of NADase and ADP-ribose transferase: 1.5 μmole NAD-(adenine-8)-^{14}C (45 000 cpm), 50 μmole Tris HCl pH 7.5 and nuclear fraction (6 mg protein) were incubated at 37° in a total volume of 1.0 ml. The reaction was started with NAD and stopped at the time indicated with 1.5 ml 5% trichloroacetic acid (TCA). After centrifugation, the remaining NAD was measured in the supernatant as the CN-addition product of NAD [8] and with alcohol dehydrogenase [8]. The pellet was washed 3 times with 5% TCA, dried with ethanol and diethylether and dissolved in formic acid (98%). Radioactivity was determined in a tri-Carb liquid scintillation system [9]. Blancs were run in which the enzyme was inhibited by 0.2 M nicotinamide.

NAD-(adenine-8)-^{14}C was prepared with ATP-(adenine-8)-^{14}C, NMN and NAD pyrophosphorylase from ascites cells [10]. ATP-(adenine-8)-^{14}C was obtained from The Radiochemical Center, Amersham, Calf thymus histones (type II) from Sigma Chem. Co., St. Louis, Spermine tetrahydrochloride from Calbiochem, Los Angeles, bovine pancreatic DNase I from Worthington Biochem. Corp. Freehold and alcohol dehydrogenase from Boehringer, Mannheim.

3. Results and discussion

As shown in table 1, 0.4 M ammonium sulfate (AS) inhibits both NADase and ADP-ribose transferase activity in nuclei of Ehrlich ascites cells to more than 90%. Increasing the AS concentration to 1.6 M, however, causes a restoration of both enzyme activities up to at least 50%. This is in agreement with experiments on the rat liver enzyme [11, 12]. In contrast to the nuclear NADase, RNA synthesis is stimu-

Table 1

Influence of ammonium sulfate on DNase treated nuclear NADase and ADP-ribose transferase.

Treatment	Addition of ammonium sulfate	NADase (μmole/5 min)	ADP-ribose-transferase (cpm/5 min)
no preincubation	–	0.22	1920
–	+ 0.4 M	< 0.03	110
–	+ 1.6 M	0.17	960
preincubation			
– DNase	–	0.17	1400
– DNase	+ 1.6 M	0.08	760
+ DNase (0.8 mg/ml)	–	< 0.03	10
+ DNase (0.8 mg/ml)	+ 1.6 M	0.14	720

Enzyme activities were measured as described in methods. Nuclei were preincubated for 30 min at 37° with bovine pancreatic DNase (0.8 mg/ml) and 30 mM $MgCl_2$.

lated by low AS concentrations [11, 12]. This stimulation has been tentatively ascribed to removal of histones from DNA.

Treatment of nuclei with DNase produces a complete disappearance of NADase and ADP-ribose transferase. After addition of AS, however, the enzyme activities can be restored (table 1). This phenomenon may be explained by the assumption that DNA hydrolysis leads to a release of histones. The high concentration of free cationic groups may interact with the enzyme protein producing an inhibition. Addition of AS is probably causing a dissociation of the enzyme inhibitor complex.

Fig. 1 shows that indeed spermine as well as histones cause an inhibition of NADase associated ADP-ribose transferase activity. The inhibition by spermine is easily reversible by dialysis. Relatively high concentrations of histones are necessary to produce a 50% inhibition. Because of the impeded diffusion into nuclei the actual histone concentration at the site of the enzyme may be considerably lower. Furthermore, fig. 1 demonstrates that both activities are activated at low concentrations of the polycations used. The activation may be in connection with the recent findings that histones are acceptors of ADP-ribose moieties [7].

A maximum of activation was observed with 3 mM

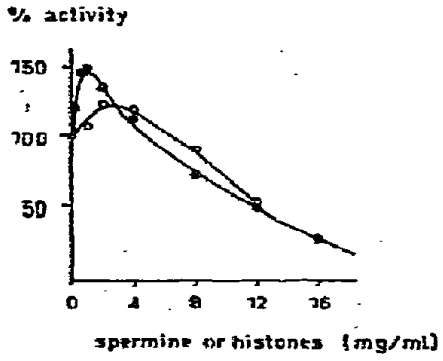

Fig. 1. Influence of spermine and histones on ADP-ribose transferase. • spermine, ○ histones. Enzyme activity after 5 min incubation was measured as described in methods. % activity is based on mean values of two experiments.

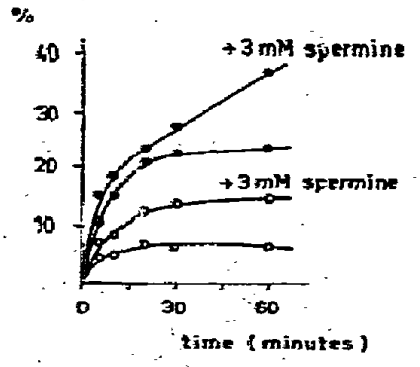

Fig. 2. Influence of 3 mM spermine on NADase and ADP-ribose transferase. • % NAD hydrolyzed, ○ % radioactivity of NAD-(adenine-8)-^{14}C incorporated into acid-insoluble material. Enzyme assay was performed as described in methods.

spermine. Fig. 2 shows that both reactions are leveling off after 15 min of incubation. Part of this inhibition is due to the produced nicotinamide [1]. In the presence of 3 mM spermine, however, both enzyme reactions are increased. The discrepancy between % NAD hydrolysis and % radioactivity incorporated into acid-insoluble material was also observed with the liver enzyme [5]. It is interesting to notice that activation by spermine is observed at concentrations which are in the range of the concentration of polyamines in various tissues [13].

The experiments demonstrate that polycations markedly influence the nuclear NADase and ADP-ribose transferase activity. Doly et al. have shown that polyanions also affect the enzyme [14]. The mechanism(s) of interaction of polycations, polyanions and the enzyme have now to be elucidated with a purified system.

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