EFFECT OF MUSTINE (HN2) AND ITS MONOFUNCTIONAL ANALOGUE (HN1) ON TEMPLATE ACTIVITY OF CHROMATIN AND DNA IN AN RNA—POLYMERASE SYSTEM IN VITRO

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Mustine inhibits the template activity of chromatin and DNA in an RNA—polymerase system in vitro much more strongly than its monofunctional analogue. Chromatin is more sensitive to the action of mustine than is deproteinized DNA. However, the template activity of DNA is reduced to a greater degree by the action of the monofunctional analogue of mustine than is that of chromatin. The mechanism of inhibition of the template activity of chromatin by mustine is probably connected with the ability of the compound to form DNA—protein cross-linkages in the chromatin structure.

KEY WORDS: *alkylating mutagens; template activity of chromatin; template activity of DNA.*

Bifunctional alkylating compounds have a very broad spectrum of action on the genetic apparatus of eukaryote cells. They not only injure the structure of DNA but they can also disturb the native state of DNA—protein and protein—protein bonds in the structure of chromatin. Investigations [3, 4, 6] have shown that these disturbances can be reduced mainly to the formation of stable DNA—protein complexes. It can be expected that disturbance of the structural organization of chromatin ought to modify its function significantly. There is evidence in the literature that alkylating compounds inhibit the incorporation of labeled precursors into RNA of tumor cells [10]. However, it is not clear on what this effect is based. The hypothesis that it is connected with direct interaction between these agents and DNA and with various changes arising under these circumstances in the structure of DNA [7] is not supported by other workers who consider that the decisive factor in this case is either breakdown of the deoxyribonucleoprotein complex of chromatin [5] or a change in metabolic processes in the cell nucleus [9]. In order to shed light on this problem it is important to rule out as far as possible any possible effect of the enzyme systems of the nucleus and also to compare the action of alkylating compounds of similar structure but differing in their ability to create stable DNA—protein complexes [4].

For this purpose, in the investigation described below, the effect of the bifunctional nitrogen mustard, mustine, and its monofunctional analogue in vitro on the template activity of isolated preparations of chromatin and DNA in an RNA—polymerase system is studied.

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EXPERIMENTAL METHOD

Methyl-bis(2-chloroethyl)amine (mustine, HN2) and its monofunctional analogue methylchloroethylamine (HN1), in the hydrochloride form, contained not less than 95% of the active principle.

DNA and chromatin were obtained from freshly frozen calf thymus. DNA was isolated by Kay's method [6a] with additional chloroform deproteinization, and chromatin preparations were obtained by Shaw's method [9a]. The incubation mixture contained DNA or chromatin (as DNA) in a concentration of 1 mg/ml, 10^{-2} M Tris-HCl buffer, pH 7.5, and the corresponding concentration of HN2 or HN1. Incubation was carried out at 37°C and the unreacted agent was removed by dialysis at 4°C for 14-16 h.

Template activity of the chromatin and DNA was determined by the use of RNA–polymerase from Escherichia coli in samples containing, in a volume of 0.25 ml, the following components in the concentration specified: 0.05 M Tris-HCl, pH 7.8; 0.15 M KCl; 10^{-2} M $MgCl_2$; 5 X 10^{-4} M β-mercaptoethanol; 4 X 10^{-4} M each of ATP, GTP, and CTP; 4 X 10^{-5} M H^3-UTP (specific activity 100 Ci/mole); 25 μg DNA in the form of chromatin or free DNA, and 2-15 μg RNA–polymerase. The reaction was carried out at 37°C for 10 min and stopped by the addition of 0.1 ml 1% RNA–coprecipitator + 2.5 ml 5% TCA and the samples were placed in an ice bath. The residues were washed with 5% TCA in 10^{-2}M sodium pyrophosphate Radioactivity was determined in a toluene scintillator on the Mark II counter.

The percentage of DNA going into the interphase during chloroform–salt deproteinization of the chromatin preparations treated with HN2 was determined as described previously [3]. DNA was determined by Spirin's method [2] and the protein concentration by Lowry's method [8].

EXPERIMENTAL RESULTS AND DISCUSSION

Preparations of chromatin and DNA were treated with increasing concentrations of HN2 and HN1 (Fig. 1). With an increase in the HN2 concentration the template activity of the chromatin fell gradually, and in a concentration of 10^{-2}-10^{-1} M it reached 10% of its initial level. It must be emphasized that with concentrations as low as 10^{-7} M definite changes were found in the template activity of the chromatin. The sensitivity of chromatin is shown by the fact that the HN2 concentrations producing an effect (10^{-6} M and 10^{-7} M), with the working concentrations of chromatin that were used are equivalent to ratios of one molecule HN2 to at least 3 X 10^3 and 3 X 10^4 molecules of the nucleotides, respectively. This ratio was in fact greater still, for some HN2 molecules did not take part in the reaction or they reacted with the chromatin proteins.

As Fig. 1 shows, HN1 inhibited the template activity of chromatin only to a very slight degree, thus demonstrating differences in the reactivity of HN2 and HN1 with respect to chromatin.

By contrast with chromatin, the decrease in template activity of DNA treated with HN1 was roughly similar in character to that of DNA treated with HN2. Presumably the harmful action of these compounds on DNA when freed from the protein component is largely comparable. It also follows from Fig. 1 that whereas HN2 inhibited the template activity of chromatin more strongly than that of DNA, the opposite relationship was found with its monofunctional analogue. The same pattern also was observed when the kinetics of inhibition of template activity of deproteinized DNA and chromatin by HN1 and HN2 was investigated (Fig. 2). It is important to note that the template activity of DNA fell to a certain level practically immediately after the beginning of the reaction with the alkylating agents and more prolonged treatment had no significant further effect. The template activity of chromatin, however, changed gradually on interaction with HN2 and flattened out to a plateau after about 1 h; this result points to fundamental differences in the mechanisms of interaction of deproteinized DNA and chromatin with HN2.

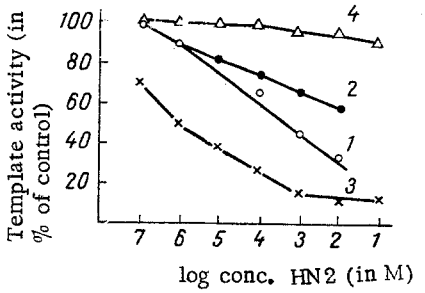

Fig. 1

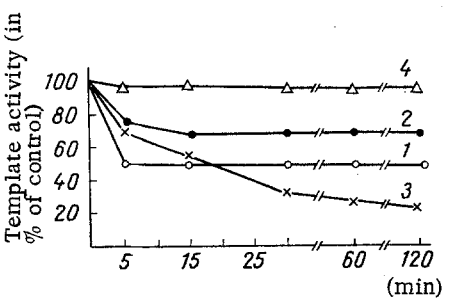

Fig. 2

Fig. 1. Effect of HN2 and HN1 on template activity of chromatin and DNA in an RNA–polymerase system. Incubation time 30 min. 1) DNA + HN2; 2) DNA + HN1; 3) chromatin + HN2; 4) chromatin + HN1.

Fig. 2. Template activity of chromatin and DNA as a function of duration of reaction with HN2 and HN1 in an RNA–polymerase system. Concentration of HN2 and HN1 in an incubation mixture 10^{-4} M. Legend as in Fig. 1.

The results indicate that the presence of a protein component bound with DNA in animal cells has a significant and, at the same time, a different effect on the injurious action of monofunctional and bifunctional alkylating compounds during their interaction with the genetic apparatus of the cell. Whereas in the case of HN1, the protein evidently plays the role of a special type of protector for DNA, it intensifies the harmful action of HN2. In the latter case the effect is presumably additive, and due on the one hand to damage to the DNA molecules themselves, and on the other hand to the formation of stable DNA–protein complexes. This hypothesis is in good agreement with the results of previous experiments [4] in which HN1, unlike HN2, did not induce the formation of such complexes.

One test which proves the formation of stable bonds between DNA and protein as a result of various forms of treatment is the passage of DNA into the interphase during the chloroform–salt deproteinization of deoxyribonucleoproteins damaged by cross-linking agents [3]. It was therefore decided to investigate the degree of correlation between the passage of DNA into the interphase during deproteinization of chromatin treated with various concentrations of HN2 and the decrease, in %, in the template activity of the same chromatin. As Fig. 3 shows, curves showing the effect of HN2 on the decrease in template activity of chromatin and strengthening of the DNA–protein bonds are very close to one another. It can be concluded that the sensitivity of chromatin as an integral functional system to HN2 is largely determined by the formation of strong DNA–protein cross-linkages, which evidently play the role of transcription blockers.

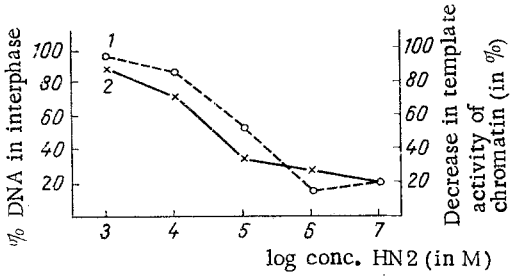

Fig. 3. Effect of HN2 on template activity of chromatin and passage of DNA into interphase during chloroform–salt deproteinization of corresponding chromatin preparations. Incubation time 30 min. 1) Passage of DNA into interphase (in % of initial content); 2) decrease in template activity of chromatin (in %).

However, other possible factors that could inhibit the template activity of chromatin under the influence of HN2 cannot be ruled out. These include the formation of a three-dimensional chromatin lattice through the formation of bonds of the protein–protein type. Such a supramolecular structure could evidently create obstacles to contact between RNA–polymerase and the template. Another possibility is that the DNA in the structure of chromatin is in a conformation more accessible to attack by nitrogen mustard, as shown by the results of a series of experiments [1]. Another cause of the greater sensitivity of chromatin than DNA could be the fact that protein molecules, when bound with HN2 molecules, increase its local concentration and thus create more optimal conditions for subsequent reaction with DNA molecules. All these suggestions require experimental verification.

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