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INTERACTION OF AN INTERCALATING ANTITUMORAL AGENT: 9-HYDROXY-2-METHYL ELLIPTICINIUM (NMHE) WITH CHROMATIN

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Abstract—In this work we study the effects of an intercalating antitumoral agent: 9-hydroxy-2-methyl ellipticinium (NMHE) on the structure of chromatin, using micrococcal nuclease and DNase 1 as structural probes.

The binding of the drug to chromatin, either *in vitro* or in the nuclei, induces two structural changes of chromatin: (a) an unfolding of the overall structure which results in an activation of the rate of degradation of chromatin by micrococcal nuclease and (b) a disorganisation of the core particle structure leading to the unwrapping of the DNA from the histone core.

Moreover, by studying the interaction of MMHE with nuclei labeled in the active regions of the genome through a nick-translation reaction, it appears that the drug is overconcentrated in these regions and does not induce any new structural changes. The interaction of NMHE with DNase 1-sensitive regions of chromatin indicates that these regions are already "open" or relaxed and represent a preferential target for the drug.

9-Hydroxy-2-methyl ellipticinium: NMHE (NSC 264-137) is a derivative of ellipticine [1], which binds to DNA mainly by intercalation, giving an unwinding angle of 21°6 [2].

NMHE is used in the treatment of metastatic breast cancer [3, 4] and has been reported to be active on experimental tumor cell lines: L1210, P388, B16 Melanoma and Lewis Carcinoma. It inhibits their cellular growth and lowers their cloning potential in agar together with their tumorigenicity once transplanted to female DBA/2 mice [1, 2, 5]. This antitumoral activity is not a common feature of all the ellipticine derivatives, and though the binding affinity for DNA *in vitro* has been recognized as a necessary requirement for antitumoral activity [6–8], intercalation *per se* cannot explain the observed differences in biological activity among the ellipticines [9]. Correlations have often been done by comparing biological effects *in vivo* to properties of the complexes formed *in vitro* between the drugs and isolated DNA. However, in the cell a huge amount of DNA is packed in a small nucleus, and this DNA is associated with both histones and acidic proteins under a highly organized complex termed chromatin (for a review see reference [10]). Because of this arrangement the drug, once it reaches the nucleus, is faced with different types of structurally-defined binding sites: linker DNA and DNA of the core particle. Moreover these sites can be under different conformations corresponding to active or inactive genes (see references [11] and [12]) and identified through their reactivity toward DNase 1 [13–20].

In this report we describe the interaction of NMHE with chromatin, whether isolated *in vitro* or in the nucleus. In both cases we have followed the drug-induced changes in chromatin structure using the nuclease digestion of the drug–chromatin complexes. The results thus obtained provide further insights into the action of this antitumoral agent on the overall genomic structure and into its interaction with particular regions of the cellular genome.

MATERIALS AND METHODS

Nuclei isolation. Erythrocytes were obtained from the circulating blood of chickens by cardiac puncture. All following operations were performed at 4°. Blood was homogenized in Tris–HCl† 10 mM (pH 7.6), NaCl 75 mM, EDTA 24 mM and PMSF 0.1 mM. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant discarded. The pellet was then resuspended in the same solution and Nonidet P40 added to the suspension to yield a final concentration of 0.25% (v/v). This suspension was incubated for 40 min with gentle agitation and centrifuged at 1000 rpm for 5 min. This step was repeated 4 times and the nuclei thus obtained were used immediately.

Chromatin extraction. Chromatin extraction was performed using the process described by Muyldermans [21]. The nuclei were resuspended in Tris–HCl 10 mM (pH 7.6), NaCl 40 mM, $MgCl_2$ 3 mM and $CaCl_2$ 1 mM, at a concentration of 2×10^9

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† Abbreviations: Tris, tri-(hydroxymethyl)aminomethane; EDTA, ethylene diamine tetraacetic acid; PMSF, phenyl methyl sulfonyl fluoride; BSA, bovine serum albumin; PCA, perchloric acid; *r*, represents the ratio of bound drug per nucleotide.

nuclei/ml in the presence of 150 U/ml of micrococcal nuclease. The nuclear suspension was then incubated for 6–8 min at 37°. The reaction was then stopped at 4° with 10 mM EDTA (final concentration) and the digested nuclei were dialysed 12 hr against a buffer containing 80 mM of NaCl, 1 mM Tris–HCl (pH 7.6) and 0.2 mM EDTA. After homogenisation, the suspension was centrifuged 10 min at 3000 rpm and the supernatant represents the soluble chromatin.

Nick-translation of isolated nuclei. Isolated nuclei, after a centrifugation at 3000 rpm for 10 min, were resuspended in Tris–HCl 10 mM (pH 7.6), 2β-mercapto-ethanol 10 mM, SAB 50 μg/ml, $MgCl_2$ 5 mM (final DNA concentration = 1 mg/ml). According to the method of Levitt *et al.* [22] and Gazit and Cedar [23], this suspension was then incubated in the presence of DNase 1 (0.09 μg of enzyme for 1 mg of DNA was the amount of DNase 1 necessary for optimum nick-translation, in our conditions), for 7 min at 37°. The reaction was stopped by cooling to 4° and the nuclei were incubated for 20 min at 15° in the presence of Kornberg DNA polymerase (Boehringer, Lot 1472108) with 10 U of polymerase per ml and 4 μM each of dATP, dGTP, dCTP and dTTP mixed with ^3H dTTP (CEA Saclay, France: lot TMM 234 with a specific activity of 65–75 Ci/mMol). The reaction was then stopped by cooling to 4° and the nuclear suspension centrifuged 5 min at 3000 rpm. The pellet was then washed 3–4 times in the intial buffer to avoid any further polymerization and remove labeled nucleotides.

Drug-binding to chromatin. After an overnight dialysis at 4° against 10 mM Tris–HCl, pH 7.6, 0.2 mM EDTA, 10 mM NaCl and 0.5 mM $CaCl_2$, the chromatin was diluted to a concentration of 3.24×10^{-4} M, expressed relative to the DNA, and the solution made 0.5 mM in $CaCl_2$. Then NMHE was added at the desired final concentration comprised between 0 and 3.24×10^{-5} M. Under these conditions, i.e. high concentration of chromatin, all the drug should be bound to chromatin DNA. The value of r = bound drug/DNA is thus equal to the ratio: total drug/DNA. This point has been checked by measuring the amount of bound drug under these conditions. The spectrophotometric measurements have been made on a Perkin–Elmer λ5 spectrophotometer.

The spectrum of free NMHE in solution shows a maximum at 311 nm ($\varepsilon_m = 32{,}000$), and in the presence of a large excess of DNA or chromatin this spectrum is moved toward the higher wavelengths with a maximum at 328 nm. If ε_f represents the molar absorbance of free NMHE at a given wavelength and ε_b the molar absorbance of the bound drug at the same wavelength, the difference between the optical density of a given amount of drug and the optical density of the same amount in the presence of DNA or chromatin allows us to derive the concentration of bound drug (C_b) from the equation: $C_b = \Delta OD/(\varepsilon_b - \varepsilon_f)$. In order to measure the amount of bound drug, the wavelength was fixed at 330 nm. In these conditions, $\varepsilon_b - \varepsilon_f$ was found equal to 16,615 and we were able to accurately measure concentrations of bound drug as low as 3×10^{-7} M, which corresponds to a difference of 0.005 between the optical density of the bound drug and the optical density of the free drug. Under our conditions, i.e. 3.24×10^{-4} M of chromatin or DNA, by adding a concentration of NMHE equal to 3.24×10^{-5} M we found that the concentration of bound drug is equal to 3.16×10^{-5} M indicating that 97.5% of the total drug is bound as predicted from the affinity constant of the reaction. Taking as r value the ratio of the concentration of the total added drug over the concentration of DNA or chromatin, implies an error which is lower or equal to 2.5%.

Nuclei digestion. The nuclei were resuspended in Tris–HCl 10 mM pH 7.6, $MgCl_2$ 5 mM, $CaCl_2$ 0.5 mM and KCl 25 mM to a final concentration of 100 μg/ml in DNA (3.22×10^{-4} M). The DNA concentration was measured by diluting an aliquot of a nuclear suspension in NaCl 4 M and by measuring the optical density at 260 nm. In these conditions 0.3 OD represents 10 μg/ml of DNA. The appropriate amount of drug was then added to the nuclear suspension and the nuclei were incubated at 37° for 10 min and then the nucleases were added either in the presence of micrococcal nuclease (Worthington) at 100 U per mg of DNA or 15 U per mg of DNA or in the presence of DNase 1 at 20 μg per mg of DNA, as indicated in the text. At various times during the digestion, an aliquot (200 μl) of the nuclear suspension was diluted with 800 μl of stop solution: BSA 2.5 mg/ml, EDTA 5 mM and NaCl 3 M and 100 μl of PCA (11 × N) added. The final volume (1.1 ml) was then incubated for 30 min at 4° and after centrifugation of the pellet, the optical density of the supernatant was measured at 260 nm to determine the percent of digested DNA. The 100% digestion was measured by hydrolysing the DNA at zero time for 50 min at 80° in the presence of PCA (1 × N) and the mixture was precipitated in the same conditions as for the sample. After a 20 min centrifugation at 7000 rpm, the optical density of the supernatant represents the 100% digestion.

For labeled nuclei, the procedure was the same except for the amount of acid-soluble DNA which was determined by measuring the radioactivity in the supernatant.

Chromatin digestion. The chromatin–dye complexes in 10 mM Tris pH 7.6; 0.2 mM EDTA; 10 mM NaCl and 0.5 mM $CaCl_2$, were incubated at 37° for 10 min then micrococcal nuclease was added at a final concentration of 15 U/ml. In these conditions the DNA concentration was 100 μg/ml. At different times the reaction was stopped as described for nuclei digestion.

DNA digestion. The digestion of DNA in the presence or in the absence of drugs was followed at 37° by measuring the optical density at 260 nm in 0.4 cm path length cuvettes, using a thermostated spectrophotometer (Kontron, Uvikon 810, Switzerland). One hundred μg/ml of calf thymus DNA (Boehringer, 104167) were mixed with various amounts of NMHE (ratio of drug over DNA varying from 0 to 0.1) in a buffer containing 10 mM Tris pH 7.6; 25 mM KCl; 0.5 mM $CaCl_2$ and 5 mM $MgCl_2$. Once equilibrated at 37°, micrococcal nuclease (Worthington) was added at a final concentration of 100 U/mg of DNA. The hyperchromicity was recorded as function of time and the slope at the

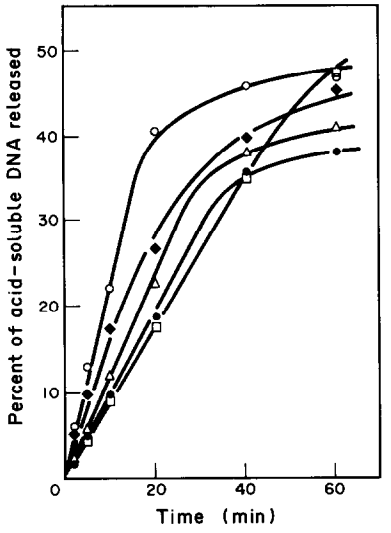

Fig. 1. Degradation of isolated chromatin–NMHE complexes in the presence of micrococcal nuclease as determined through acid-soluble release. Digestion was performed at 37° with 15 U of enzyme per mg of DNA: --●--, no NMHE; --△--, $r = 0.01$; --◆--, $r = 0.025$; --○--, $r = 0.05$; --□--, $r = 0.1$.

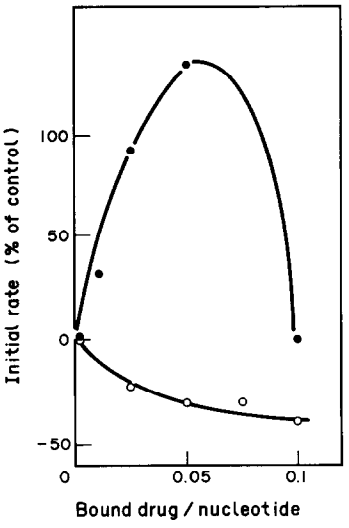

Fig. 2. Percentage changes in the initial rates of degradation of isolated chromatin or DNA by micrococcal nuclease as a function of r. The initial rates are determined from Fig. 1 and are expressed as percent of the control without drug: --●--, chromatin–NMHE complex; --○--, DNA–NMHE complexes. The value 0% on the ordinate axis corresponds to the initial rate of degradation of the control (chromatin or DNA) without drug.

origin determined to measure the initial rate of the reaction.

Gel electrophoresis. After digestion of the nuclei or chromatin, the sample was adjusted to 1 M in NaCl and sodium dodecylsulfate added to a final concentration of 1%. The DNA was then purified through two isoamylalcohol/chloroform (1:24 v/v) extractions and, after precipitation with two volumes of ethanol at −20°, resuspended in 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA pH 7.8. Five μg of DNA were then loaded on 3.6% polyacrylamide gels and run as already described [24]. The gels were stained with ethidium bromide (0.5 μg/ml) for 30 min and after a washing in water, were photographed under u.v. light. The negatives were scanned with a Joyce-Loebel microdensitometer.

Reagents. NMHE has been synthetized by SANOFI (Toulouse, France). All other chemicals were reagent grade.

RESULTS

Digestion of NMHE–chromatin complexes

The kinetics of the degradation of isolated chromatin with micrococcal nuclease in the presence of various amounts of NMHE is shown on Fig. 1. From these data we can derive the initial rates of degradation of the drug–chromatin complexes as shown in Fig. 2. It can be seen that the initial rate of degradation is enhanced as a function of increasing amounts of drug as compared to the control without drugs. This implies that NMHE activates the degradation of chromatin. The maximum of the activation takes place at a drug concentration corresponding to an r value of about 0.05. Since NMHE progressively inhibits the action of micrococcal nuclease on naked DNA, on chromatin the observed activation suggests that the drug induces new sites for the enzyme as previously shown for an other intercalating agents: ethidium bromide [25, 26]. When NMHE is bound to chromatin, the linker DNA is exposed and made fully accessible to the enzyme. This exposure of new DNA sites takes place for values of r comprised between 0 and 0.05. For higher values of r, the drug fully saturates the new available sites and therefore we observe a decrease in the rate of the enzymatic degradation. Furthermore, in the presence of drugs, the amount of acid-soluble material released by the degradation is higher than for the control without drugs. This increase is not solely an effect of rate but corresponds to a more efficient degradation of the DNA associated with the core particle, as evidenced by studying the migration pattern, on gel electrophoresis, of the limit-digest obtained in the presence of drugs (Fig. 3). The electrophoretic patterns thus obtained show that the limit-digest of the chromatin–drug complexes corresponds to DNA whose length is centered around 100 base pairs. This indicates that in the presence of drugs, the digestion of the chromatin can go further on in the DNA associated with the core particle, suggesting a partial disruption of this particle. Along this line NMHE acts like other intercalating agents: ethidium bromide [25–27] or adriamycin and daunorubycin [28].

However, the extraction of chromatin from nuclei could change the chromatin structure by releasing acidic proteins or simply by changing the constraints applied in the nucleus to the DNA. It is then necessary to check in isolated nuclei if we observe the same drug-induced structural changes as those observed for isolated chromatin. Therefore we have followed the degradation of chromatin by digesting

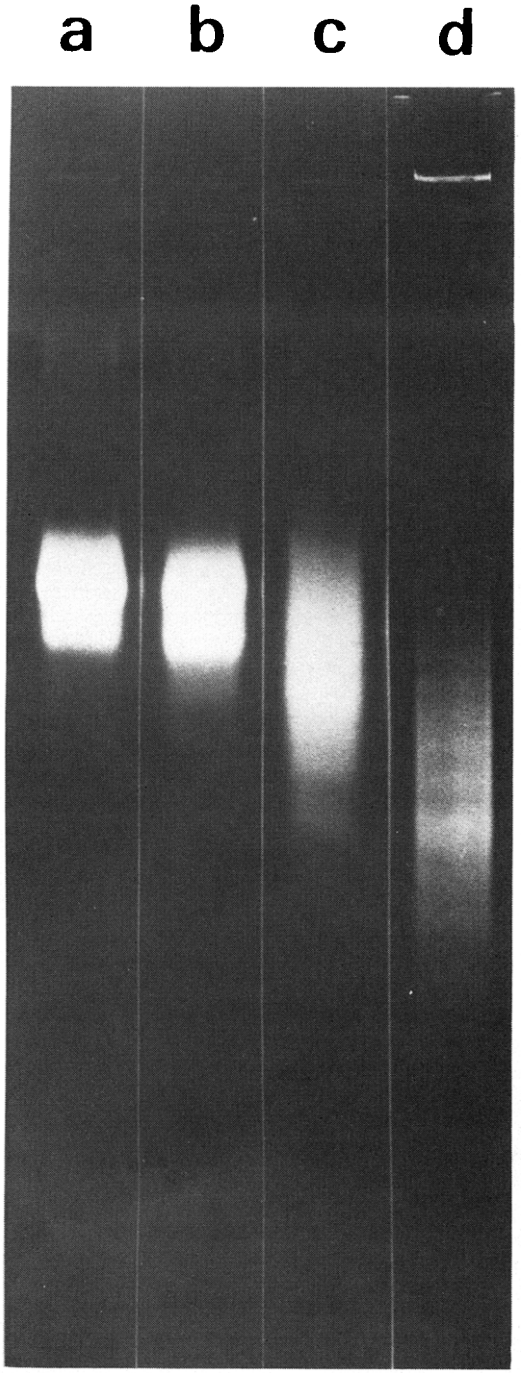

Fig. 3. 6% polyacrylamide gel electrophoresis of chromatin–NMHE complexes. The samples have been digested 30 min at 37° in the presence of 150 U of micrococcal nuclease per mg of DNA. Slot a: control without drug; slot b: $r = 0.01$; slot c: $r = 0.05$; slot d: $r = 0.1$.

isolated nuclei in the presence of different amounts of NMHE.

Digestion of NMHE–nuclei complexes

The rates of degradation corresponding to the digestion time-course of isolated nuclei in the presence of various amounts of NMHE are shown in Fig.

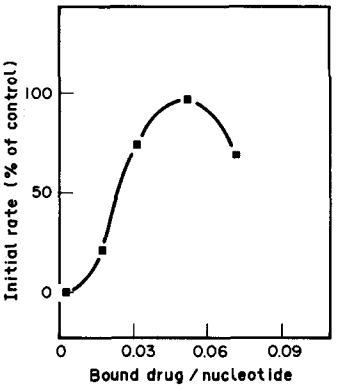

Fig. 4. Percent changes of degradation of isolated nuclei by micrococcal nuclease as a function of the amount of added NMHE. The digestion was performed in the presence of 100 U of enzyme per mg of DNA and determined through acid-soluble release. The value 0% of the ordinate axis corresponds to the initial rate of degradation of the control without drug.

4. As observed for isolated chromatin, the drug induces two different effects according to its concentration. For values of r smaller than 0.05 and by comparison with the control without drugs, we observe an increase in the micrococcal nuclease digestion rate. For r values greater than 0.05, the digestion rate decreases. By studying the migration on 3.6% acrylamide gel electrophoresis of the DNA fragments isolated from the digested NMHE–nuclei complexes, we confirm the action of NMHE on the chromatin structure (Fig. 5). Up to an r value of 0.05 no drastic changes in the gel pattern are observed. Only an activation of the digestion toward monomers is shown through a partial erasing of the bands which correspond to the multimeric DNA whose size is greater than three times the repeat length. Such a feature is in accordance with the observation made through the study of the released acid-soluble material. When the ratio of bound drug over DNA is higher than 0.05 there are changes in the shape and the position of the bands and particularly of the monomeric band which is more diffuse than the one observed for the control without drugs and which migrates to a position corresponding to about 100 base pairs. This indicates that not only the digestion of the chromatin inside the nucleus is activated by the drug binding, as already shown from the measurement of the rate of digestion of the nuclei in the presence of various amounts of the drug, but it also indicates a partial disruption of the core particle structure induced by this binding. The gels show that NMHE is able to partially unfold the DNA from the histone core thus allowing the enzyme to work further on in the core particle. Such a structural change corresponds to what has already been described for EthBr [27] interacting with isolated nuclei.

In nuclei it has been proposed that the regions of the genome which correspond to active genes or potentially active genes are under an open structure [29–32]. Therefore we have looked at the effects of NMHE on these particular regions.

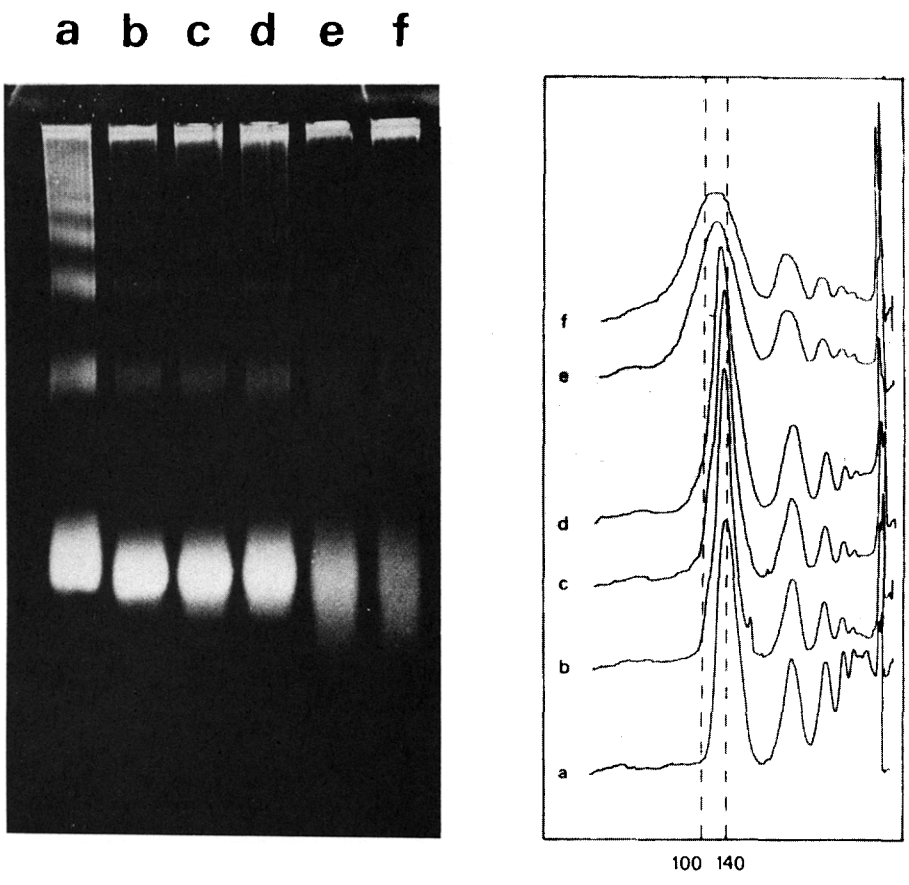

Fig. 5. 3.6% gel electrophoresis pattern of the DNA extracted from nuclei-NMHE complexes after a micrococcal nuclease digestion (left panel). Densitometer tracings of the preceding gel (right panel). Slot a: no drug; slot b: NMHE–DNA = 0.01; slot c: NMHE–DNA = 0.02; slot d: NMHE–DNA = 0.04; slot e: NMHE–DNA = 0.06; slot f: NMHE–DNA = 0.1. Same experimental conditions as Fig. 4.

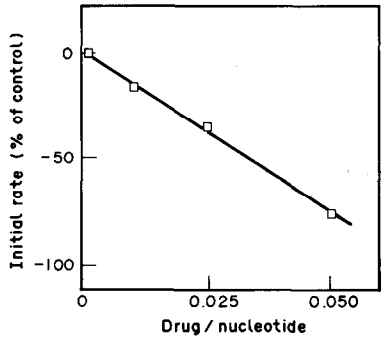

Fig. 6. Percentage changes of the initial rates of degradation of nick-translated nuclear DNA in the presence of DNase 1 as a function of added NMHE and determined through labelled acid-soluble release. The value 0% of the ordinate axis corresponds to the initial rate of degradation of the control without drug. The degradation was performed in the presence of 20 μg of DNase 1 per mg of DNA at 37°.

Digestion of NMHE-nick translated nuclei complexes

Nuclei, nick-translated as described in Material and Methods are labeled in the active part of the genome [22, 23]. They are incubated with increasing amounts of NMHE and these NMHE–nuclei complexes are digested in the presence of DNase 1 or micrococcal nuclease. In these conditions, we can follow separately the digestion of bulk chromatin, through UV determination of the acid-soluble material, and the digestion of active, DNase 1-sensitive chromatin through the appearance of titrated acid-soluble material.

In the presence of DNase 1, in conditions corresponding to almost no degradation of the bulk chromatin (20 μg of enzyme per mg of DNA and 2% of the total chromatin digested), we observe a strong inhibition in the degradation of the labeled DNA whatever the drug concentration used (Fig. 6), while no significant effect is detected on the degradation of bulk chromatin. The same result is found if we measure the rate of degradation of the labeled DNA by micrococcal nuclease for low enzyme concentrations (Fig. 7), conditions such as the enzyme shows a preference for the active regions of the

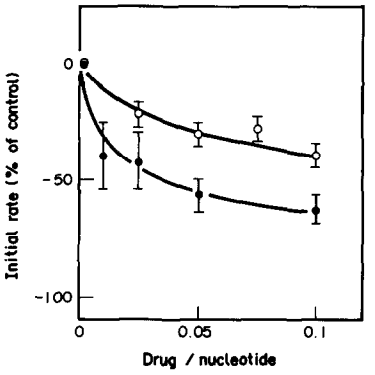

Fig. 7. Percentage changes of the initial rates of degradation of nick-translated nuclear DNA (--●--) or DNA (--○--) in the presence of micrococcal nuclease as a function of added NMHE and determined through labelled acid-soluble release. The value 0% of the ordinate axis corresponds to the initial rate of degradation of the control (nick-translated nuclear DNA or DNA) without drug. The degradation was performed in the presence of 15 U of enzyme per mg of DNA at 37°. Each point represents the average of four determinations and the error bars correspond to a 95% confidence interval.

genome [30]. NMHE strongly inhibits the action of the micrococcal nuclease on labeled chromatin, without altering the digestion of bulk chromatin. By comparing the extent of digestion of the labeled DNA to what was observed for naked DNA, it appears that NMHE is significantly more efficient to inhibit micrococcal nuclease action on the labeled DNA than on isolated naked DNA *in vitro*, especially for low concentrations of the drug.

DISCUSSION

The present work investigates the effects of the antitumor compound NMHE on chromatin structure at three levels: isolated chromatin, isolated nuclei and DNase 1-sensitive regions of the genome. The results show that NMHE is able to induce marked structural changes on isolated chromatin and on chromatin in isolated nuclei but does not act the same way on the DNase 1-sensitive regions of the genome.

The interaction of NMHE with chromatin, whether isolated or in nuclei, induces an activation of the rate of degradation of chromatin in the presence of micrococcal nuclease. This activation is maximum for a ratio of drug over DNA base pairs equal to 0.05 and is specific of the chromatin structure since, in the same conditions, NMHE inhibits the enzymatic degradation of naked DNA (Fig. 2). The intercalation causes a steric inhibition of the enzyme on naked DNA and the activation, which appears when chromatin–drug complexes are digested, has to be the result of the unmasking of new sites on the DNA which are normally protected in the regular nucleosomal structure. More sites on DNA are now rendered fully exposed to the action of the enzyme which overweights the inhibition due to the interaction of NMHE with DNA. Similar findings have been described for ethidium bromide [27] and for antracycline antibiotics [28]. Up to an r value of 0.05, the linker DNA is entirely exposed to the enzyme, inducing an augmentation in the rate of digestion. Furthermore, more drug binding disrupts the core particle structure leading to a partial degradation of core DNA.

The same pattern of events is observed when isolated nuclei are digested in the presence of NMHE. We observe an increase in the rate of digestion of the DNA, corresponding to an unfolding of the overall chromatin structure. At the same time we also observe a disruption of the core particle as shown by a kinetic analysis of the DNA products formed by digestion of nuclei–NMHE complexes. For r values higher than 0.05, we observe a change in the shape of the monomeric band corresponding to a disorganisation of the core particle. The new monomeric band, instead of migrating as a sharp band located to a position which corresponds to 145 base pairs, migrates as a diffuse band whose size is centered around 100 base pairs. Thus, in isolated nuclei, NMHE is able to induce a change in the overall chromatin structure through its binding to the linker DNA. At the same time, the drug can unfold the DNA from the histone core, allowing the enzyme to go further on in the degradation of core DNA. These structural changes observed for chromatin–NMHE complexes correspond to what has already been described for other intercalating agents deprived of antitumoral activity, leading to the conclusion that they are not responsible alone for the antitumoral activity of NMHE. Moreover, the structural changes induced on chromatin, either isolated or into the nucleus, appear on the overall genome at concentrations which are out of the range compatible with a pharmacological effect. However, toward DNase 1-sensitive regions of the genome, the action of NMHE is different from what is observed for the overall genome. In these regions, which should correspond to active genes [22, 23], the degradation of DNA is inhibited whatever the concentration of the drug. The inhibition of the nucleases action induced by NMHE indicates that the drug binds to these DNase 1-sensitive regions. However, the structure of these regions prevents the interaction of NMHE with DNA from inducing the structural changes observed for bulk chromatin. Since the nick-translated regions of the genome do not represent naked DNA as evidenced by the size of the limit-digest of these regions [23], our observation can be interpreted by assuming that the DNase 1-sensitive regions are already unfolded. Therefore, a drug which binds to the linker DNA cannot expose or generate new sites for the enzyme, and acts the same way as with naked DNA.

This result should be related to the existence of an altered nucleosomal structure corresponding to regions of the genome actively or potentially transcribed by RNA polymerase B [11]. In these regions it is proposed [19] that the structure of the nucleosomal array is altered by the absence of H1 and H5 histones and by a modification of the nucleosomal core structure. When the nuclei are labeled through

a nick-translation reaction, the DNase 1-sensitive regions of chromatin are preferentially labeled. From the numerous studies on the sensitivity of the actively or potentially transcribed regions of the genome [11–20], the DNase 1-sensitive regions of the genome correspond to "active" regions. The absence of exposure of new sites for the nucleases, when the drug is bound to the labeled chromatin, is then in accordance with the fact that this chromatin is under an altered nucleosomal conformation lacking H1 and H5 histones, and that is linker DNA is 40 bp longer and more accessible than in the inactive chromatin. As a consequence of this altered structure, this chromatin would already be "opened" and no more sensitive to structural changes induced by the intercalating drug.

Furthermore, the binding of NMHE to these labeled regions induces an inhibition of the nucleases which is significantly greater, as a function of drug concentration, than the one observed in isolated naked DNA. This later point is specially true for low drug concentrations. As the ratio of total drug vs DNA is the same in both experiments and the DNA concentration is high enough for neglecting the free drug, it is reasonable to assume that the r corresponding to NMHE bound to nuclear DNA is the same or even lower than the r measured for NMHE–naked DNA complexes. Thus the extensive inhibition which is observed for the DNase 1-sensitive regions is probably a consequence of an over-concentration of the drug in these regions. This over-concentration can result from a very high affinity of the drug for the DNase 1-sensitive chromatin or, more likely, can be a consequence of the organisation of this chromatin on the outside of the bulk chromatin [33].

In conclusion our data indicate that NMHE unwinds the chromatin structure leading to a greater accessibility of the linker DNA to nucleases and to an unwrapping of the core DNA from the histone core. Furthermore, NMHE binds preferentially to regions of the chromatin which correspond to DNase 1-sensitive sites of the genome. As already shown for other chemicals [34, 35], the active or potentially active genes in isolated nuclei are a preferential target for the interaction of NMHE. The inhibition of the degradation of these regions, in the presence of NMHE, can then reflect an already "open" and exposed structure of the DNA in accordance with the structure proposed for active genes as determined from previous studies [11, 20].

A precise description of a mechanism for the action of NMHE is not possible, but it is reasonable to assume that the opening by the drug of sites normally protected in the chromatin structure can play a biological role especially if this opening is modulated by the activity of the genome.

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