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RELEASE OF SPECIFIC PROTEINS FROM NUCLEI OF HL-60 AND MOLT-4 CELLS BY ANTITUMOR DRUGS HAVING AFFINITY TO NUCLEIC ACIDS

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Abstract—The binding sites for mitoxantrone (MIT), Ametantrone (AMT), doxorubicin (DOX), actinomycin D (AMD) and ethidium bromide (EB) in nuclei from exponentially growing and differentiating human promyelocytic HL-60 and lymphocytic leukemic MOLT-4 cells were studied by gel electrophoresis of proteins selectively released during titration of these nuclei with the drugs. Each drug at different drug:DNA binding ratios resulted in a characteristic pattern of protein elution and/or retention. For example, in nuclei from exponentially growing HL-60 cells, MIT affected 44 nuclear proteins that were different from those affected by EB; of these 29 were progressively released at increasing MIT:DNA ratios, 11 were transiently released (i.e. only at a low MIT:DNA ratio) and 4 entrapped. Patterns of proteins displaced from nuclei of exponentially growing HL-60 cells differed from those of cells undergoing myeloid differentiation as well as from those of exponentially growing MOLT-4 cells. The first effects were seen at a binding density of approximately one drug molecule per 10–50 base pairs of DNA. The observed selective displacement of proteins may reflect drug-altered affinity of the binding sites for those proteins, for example due to a change of nucleic acid or protein conformation upon binding the ligand. The data show that the binding site(s) for each of the ligands studied is different and the differences correlate with variability in chemical structure between the ligands. The nature of the drug-affected proteins may provide clues regarding antitumor or cytotoxic mechanisms of drug action.

A new method for studying binding sites of antitumor drugs in nuclear chromatin was developed recently in our laboratory [1]. The method is applicable to drugs presumed to interact with nucleic acids, and is based on exposure of isolated cell nuclei to antitumor drugs followed by electrophoretic analysis of the proteins specifically eluted by these drugs from the nuclei. The rationale of this method is based on the assumption that binding of the ligand to nucleic acids in chromatin, at a site(s) occupied by particular proteins, may dissociate and replace these proteins, which then will be released from the nucleus. Furthermore, a change in the nucleic acid native conformation (e.g. topological stress, distortion of the double helix) induced by the drug may also lead to dissociation and release of the proteins. Hence, analysis of proteins released from nuclei following their interaction with the drugs could provide information on drug-binding sites and an insight into the mechanisms underlying antitumor activity. In our earlier study, we observed that nuclei isolated from rat liver cells release drug-specific sets of proteins when exposed to several antitumor drugs [1].

We have continued and expanded these studies and now report results of experiments in which: (i) human promyelocytic leukemic HL-60 cells were compared with human lymphocytic leukemic MOLT-4 cells, to observe whether there is a tissue (lineage) specificity in the extracted proteins; (ii) nondifferentiated HL-60 cells were compared with HL-60 cells induced to myeloid differentiation, to assess effects of differentiation; (iii) several intercalating drugs differing in potency and binding mechanisms, namely mitoxantrone (MIT§), Ametantrone (AMT), doxorubicin (DOX), actinomycin D (AMD) and an intercalating ligand with no significant antitumor activity, ethidium bromide (EB), were compared.

Furthermore, in the present study the nuclei were suspended in 0.15 N NaCl rather than in solutions of low ionic strength as previously used [1], and a modified gel electrophoresis methodology was used, employing longer, 5–20% gradient acrylamide gels which enabled higher resolution of proteins. We also studied mechanisms involved in the release and retention of proteins in nuclei treated with the drugs.

MATERIALS AND METHODS

Cells. The human promyelocytic cell line HL-60 and the human lymphocytic leukemic MOLT-4 line were maintained in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 0.1 μg/mL

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§ Abbreviations: AMD, actinomycin D; AMT, Ametantrone; DOX, doxorubicin; DTT, dithiothreitol; EGTA, ethyleneglycol-bis-(β-aminoethyl ether) *N,N,N'N'*-tetraacetic acid; EB, ethidium bromide; MIT, mitoxantrone (Novantrone); NPB, nuclear preparation buffer; DMSO, dimethyl sulfoxide; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; and SDS, sodium dodecyl sulfate.

streptomycin (all from Gibco, Grand Island, NY). To induce differentiation of HL-60 cells, dimethyl sulfoxide (DMSO; Sigma Chemical Co., St. Louis, MO) was added to cultures containing 3.5×10^5 cells, to a final concentration of 2×10^{-1} M (1.4%), and the cells were kept in culture for 5 days. Proliferation of cells in DMSO-treated cultures was suppressed, as tested by flow cytometry following differential staining of cellular DNA and RNA with acridine orange [2]. Cell viability was assayed by the trypan blue exclusion test.

Nuclei isolation. Nuclear preparation buffer (NPB) contained 20 mM HEPES/KOH, pH 7.6; 1.5 mM $MgCl_2$; 0.5 mM DTT; 2% Tween 40; 0.15 N NaCl; and protease inhibitors [aprotinin, 0.2 μg/mL; leupeptin, 0.2 μg/mL; antipain, 0.1 μg/mL; ethyleneglycol-bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), 50 μM; phenylmethylsulfonyl fluoride (PMSF), 0.2 mM]. Buffer A consisted of: 20 mM HEPES/KOH, pH 7.6; 0.5 mM DTT; 0.5 mM EDTA; 10% glycerol; and the above protease inhibitors (without PMSF). All chemicals were from Sigma.

Nuclei were isolated essentially as described recently by Bunce *et al.* [3]. Briefly, the cells obtained from cultures at a density of $0.8–1.0 \times 10^6$/1 mL were pelleted by centrifugation at 500 *g* for 5 min, and rinsed once with phosphate-buffered saline (PBS; without Ca^{2+} and Mg^{2+}). The cells were then suspended in the ice-cold NPB, at a density of 1×10^8 cells/1 mL. The cell suspension was frozen by immersing tubes in liquid nitrogen and stored frozen, at −70°, for up to 2 months. The cells were thawed by immersing tubes in a 37° water bath. After thawing, the cells were transferred to an ice-cold Dounce homogenizer and nuclei were released by twenty gentle strokes of a type B pestle. Then the nuclei were centrifuged through a 50% sucrose cushion [50% (w/v) sucrose in NPB] in Eppendorf microtubes (1 mL suspension/500 μL sucrose cushion) at 2°, 3000 *g*, for 15 min. After washing with buffer A (1×3 mL/1×10^9 nuclei) and counting with a cytometer, the nuclei were ready to use.

Incubation of nuclei with drugs and analysis of released proteins. The nuclei prepared as described above were resuspended in buffer A at a density of 2.5×10^8 nuclei/1 mL and divided into samples of 2.5×10^7 nuclei each, in 100-μL aliquots. Each sample was then preincubated with 1 mL of buffer A for 25 min on ice, the nuclei were separated by centrifugation (2 min, 3000 *g*), resuspended in 1.5 mL of buffer A, sedimented again, and resuspended in 200 μL of buffer A containing one of the studied drugs. Nuclear samples were incubated with drugs for 25 min on ice, the nuclei were then removed by a 2-min centrifugation at 3000 *g*, and supernatants were centrifuged at 12,000 *g* at 4° for 10 min. Proteins were precipitated from the supernatants with 10% trichloroacetic acid (TCA) (final concentration) for 1 hr on ice and pelleted by centrifugation at 4°, 12,000 *g*, for 10 min. The pellets were washed once with 1.5 mL acetone, dried in a Speedvac (Savant, Farmingdale, NY), dissolved in the loading buffer, and applied to the discontinued 5–20% gradient polyacrylamide gels containing 0.1% SDS [4]. Gels were stained with Coomassie Brilliant Blue R 250 (Bio-Rad Laboratories, Richmond, CA) [4]. Each experiment was repeated at least twice, and there was good reproducibility with respect to proteins specifically released or retained in the nuclei under identical treatments.

RNase A treatment. In some experiments nuclei were preincubated with RNase prior to titrations with the drugs. RNase A (type II A, Sigma) was dissolved in 5 mM HEPES/KOH, pH 7.6, buffer containing 0.15 M NaCl at a concentration of 0.95 Kunitz units/1 μL, boiled for 15 min, and allowed to cool slowly to room temperature. The nuclei (2.5×10^7) were treated with 0.75 units/1 μL RNase A (final concentration) in 200 μL total volume, for 45 min on ice, separated by centrifugation, and washed twice with 1.5 mL of buffer A. RNase treatment, even at that low temperature, was effective in removing RNA from the nuclei, as tested by subsequent control staining of nuclear RNA and DNA with acridine orange and flow cytometry [2].

Analysis of proteins precipitated from the solution by MIT. Aliquots (100 μL) of the supernatant obtained from preincubation of the nuclei of exponentially growing HL-60 cells with the buffer (see above) were incubated with 100 μL of MIT solution, at final MIT concentrations ranging from 0 to 1 mM, for 90 min on ice. The samples were then centrifuged at 14,000 *g* for 10 min at 4°, proteins from supernatants were precipitated with TCA, and proteins from both pellets and supernatants were analyzed on SDS polyacrylamide gels, as described above.

Estimation of bound and free MIT and AMT. Aliquots of 2.5×10^7 nuclei isolated from exponentially growing HL-60 cells were titrated with MIT or AMT as described above. At different points of titration the nuclei were sedimented (10 min, 12,000 *g*), and the concentrations of the drugs in the supernatants ("free drug") were estimated spectrophotometrically assuming $\varepsilon_{686} = 8.4$ mM cm^{-1} for MIT and $\varepsilon_{645} = 7.1$ mM cm^{-1} for AMT [5].

Drugs. MIT and AMT were from American Cyanamid (Pearl River, NY), and EB and DOX (with no additives) were from Sigma. Stock solutions of the drugs, at a 5 mM concentration, made in 5 mM Tris/HCl, pH 7.0, 0.15 M NaCl buffer were stored in aliquots at −70°. A fresh aliquot was used for each experiment. AMD (Sigma) was dissolved in ice-cold water at a 5 mM concentration, each time prior to use.

RESULTS

We have observed in pilot experiments that exposure of isolated cell nuclei to buffer alone, at 0.15 N NaCl, results in release of a number of proteins, which apparently are either present in soluble form in the nucleoplasm or are loosely bound to the nuclear structure. The release of proteins from isolated nuclei also has been observed at lower salt concentration, in the presence and absence of several DNA binding ligands [1, 6–8]. Preincubation of the nuclei for 25 min with buffer, in the absence of the drugs, removes most of these proteins, thus significantly reducing background ("noise") of the experiment. Such preincubation, prior to addition

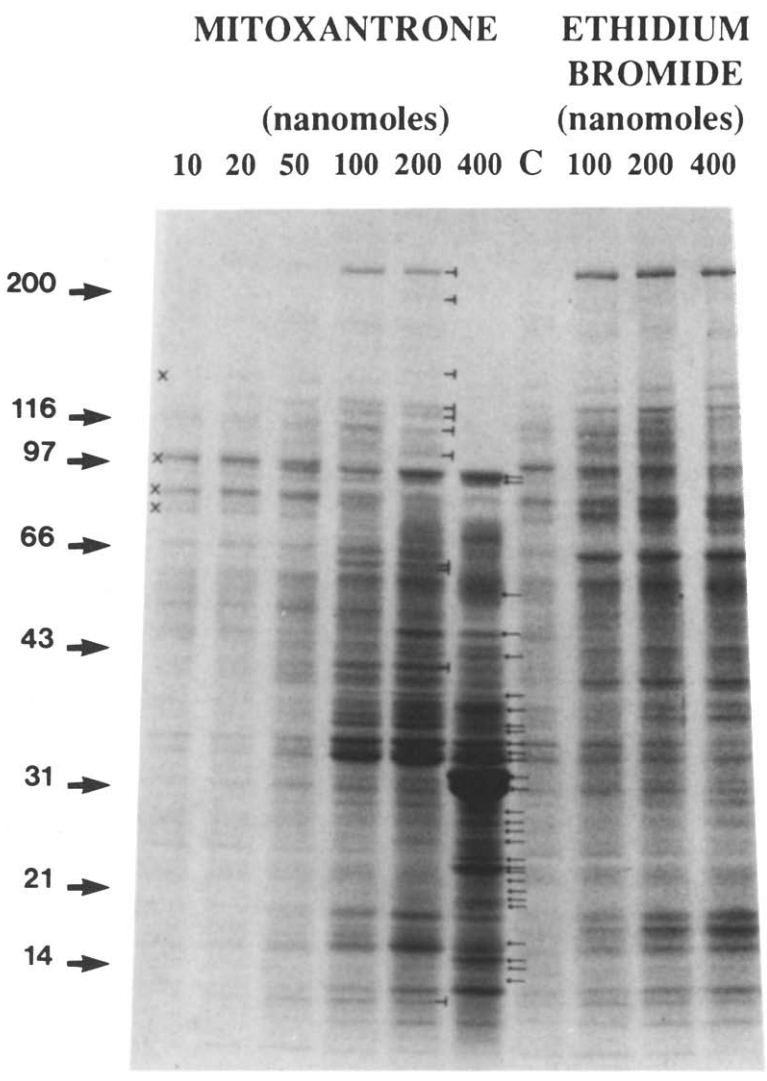

Fig. 1. Proteins released from the nuclei of exponentially growing HL-60 cells upon titration with MIT. Positions of the molecular weight markers are shown on the left side of the gel (in all figures). Key: (×) proteins entrapped; (-|) proteins transiently eluted (only at low MIT:DNA ratio); (←) proteins continuously eluted at the increasing MIT:DNA ratio. Effects of EB are shown for comparison (three right lanes). c (control), proteins eluted in the absence of the drugs. See text for further explanation.

of the studied drugs, was routinely carried out in the present studies.

Incubation of the nuclei in the presence of drugs resulted in a specific pattern of proteins released from the nuclei, depending on the amount of the drug needed (drug:DNA ratio). Three types of changes were observed: (i) appearance of new bands in gels, or enhancement of the existing (in the absence of drug) bands, representing release of specific proteins by the ligand; (ii) loss of bands compared to the control, indicating entrapment of proteins in the nuclei; and (iii) transient appearance of the bands, indicating that proteins eluted at a low drug:DNA ratio become entrapped at a higher ratio. These effects were drug- and cell-specific.

Figure 1 illustrates the changes in the pattern of proteins released from the nuclei of exponentially growing HL-60 cells upon titration with MIT. In this figure, MIT is compared with another intercalating ligand, EB, which has no significant antitumor activity. EB, especially at higher concentrations, affected release of several proteins. In comparison with EB, however, MIT specifically affected elution of at least 44 proteins: there were 29 new or enhanced protein bands in the gels of proteins released at increasing drug:DNA ratios, 11 proteins were transiently eluted (i.e. released at lower, and entrapped at higher, MIT concentration), and 4 proteins were specifically entrapped by MIT.

The pattern of proteins eluted by MIT from nuclei of HL-60 cells induced to myeloid differentiation by DMSO is shown in Fig. 2. This pattern is clearly different from the one obtained from nondifferentiated cells, with respect to proteins

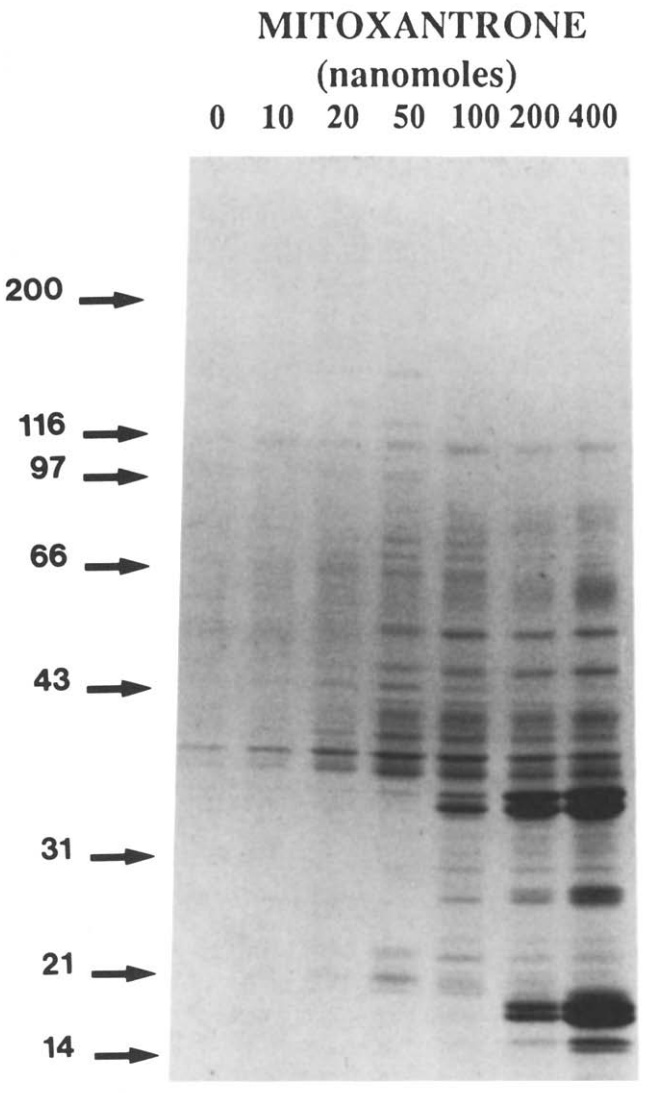

Fig. 2. Proteins released from the nuclei of HL-60 cells induced to differentiation by DMSO upon titration by MIT.

released in: (i) the absence of any drug; (ii) the presence of EB; and (iii) the presence of MIT. In the absence of EB or MIT, very few proteins were released from the differentiated cell nuclei compared with the nondifferentiated cells (Fig. 1). In comparison with EB, MIT affected at least 55 nuclear proteins in the differentiated cells, of which 42 were progressively released by the increasing MIT concentration, 11 transiently released, and 2 entrapped.

A comparison of patterns of proteins released from nuclei of HL-60 cells by MIT and its very close analog AMT is presented in Fig. 3. These drugs affected the release of similar, but not identical, sets of proteins. The arrows indicate proteins which were differentially eluted, at equivalent concentrations, by MIT versus AMT.

Patterns of proteins released by DOX and AMD differed markedly from those released by MIT or AMT (Fig. 4). In general, fewer proteins were affected by DOX or AMD compared to MIT or AMT. DOX, at increasing drug:DNA ratio specifically released only 3 proteins, entrapped 7, and transiently eluted 4. AMD, on the other hand, specifically entrapped 4 and did not release a single protein.

There was at least one large protein (mol wt >200 kD) which appeared to be transiently eluted by all three (MIT, AMT, DOX) drugs. Also one protein of an approximate molecular weight of 105 kD appeared to be entrapped by all three drugs.

There were significant differences in proteins extracted from nuclei of MOLT-4 cells in comparison with nuclei of HL-60 cells (compare Figs. 1 and 5). As in the comparison of differentiated and nondifferentiated HL-60 cells, the differences were evident in: (i) proteins released into the buffer in the absence of any drug; (ii) proteins released by

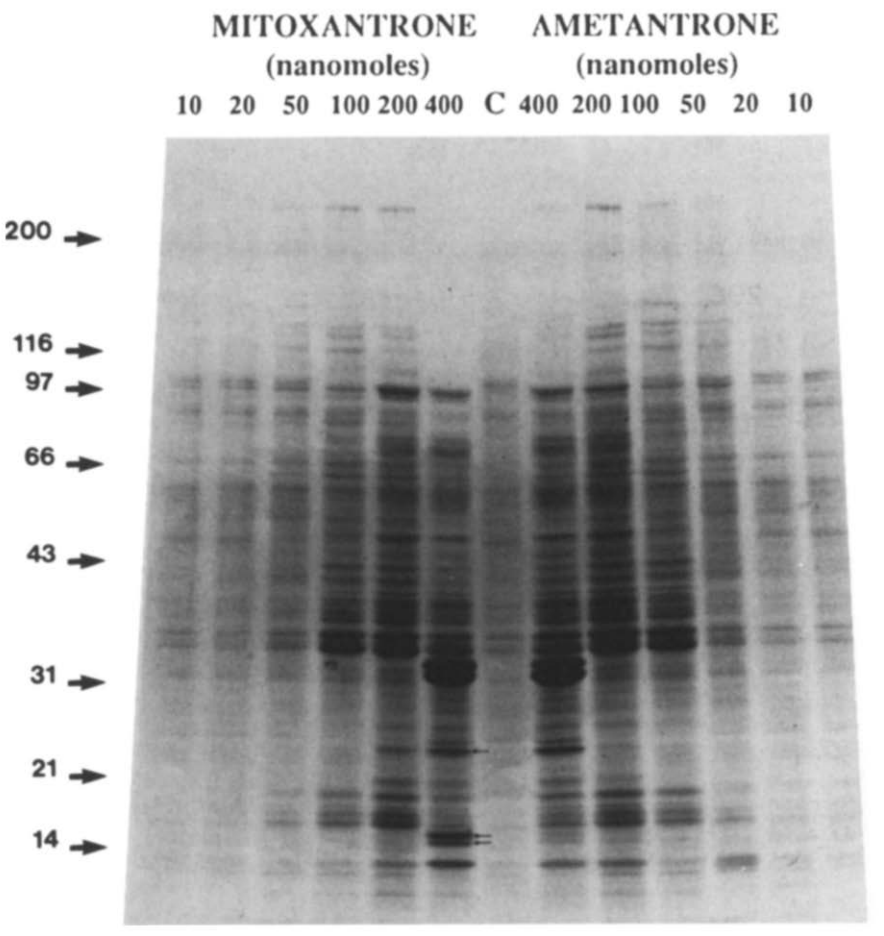

Fig. 3. Comparison of proteins released from the nuclei of exponentially growing HL-60 cells upon titration with MIT and AMT. Arrows indicate proteins released at lower MIT:DNA ratio compared to a similar AMT:DNA ratio.

EB; and (iii) proteins released by MIT. In comparison with EB, MIT specifically affected elution of at least 30 proteins from MOLT-4 cell nuclei: 20 proteins were released by increasing drug:DNA ratio, 6 were entrapped and 4 eluted at a lower and were retarded at a higher ratio. At least 12 of these proteins had apparent different molcular weights from any proteins of HL-60 nuclei affected by MIT.

Since one of the possible targets of MIT may be RNA [9], studies also were done on nuclei pretreated with RNase A (Fig. 6). Incubation of nuclei with RNase alone released a number of proteins. It was difficult, however, to obtain good electrophoretic patterns of these proteins because of the over-abundance of the RNase protein in gels. After removal of the incubation medium containing RNase and the released proteins, incubation of the RNase-pretreated nuclei with the drug resulted in many fewer proteins being subsequently released into the buffer, both in the absence and presence of MIT (Fig. 6). Thus, the RNase-pretreated nuclei when incubated with the buffer alone released only 15 proteins, compared to 55 proteins released from the control nuclei identically treated except for the presence of RNase. At the highest MIT:DNA ratio 76 proteins were eluted from the buffer-pretreated nuclei compared with 50 proteins from the RNase-pretreated nuclei. The RNase treatment thus either released from the nuclei some of the proteins which otherwise would be released by MIT or prevented their subsequent extraction by the drug.

MIT is capable of precipitating proteins out of solution already at concentrations as low as 25 μM (Fig. 7). Interaction between the drug and proteins leading to their precipitation appeared to be specific since, for example, some proteins were present exclusively in the pellet at a 125 μM MIT concentration while others remained in the supernatant even at a 1 mM concentration.

DISCUSSION

New drug design requires knowledge of the mechanism of interaction of the drug with cellular targets, which is responsible for its pharmacological activity. Because a large group of antitumor drugs are intercalators, new drugs are often designed under the assumption that their intercalative affinity to

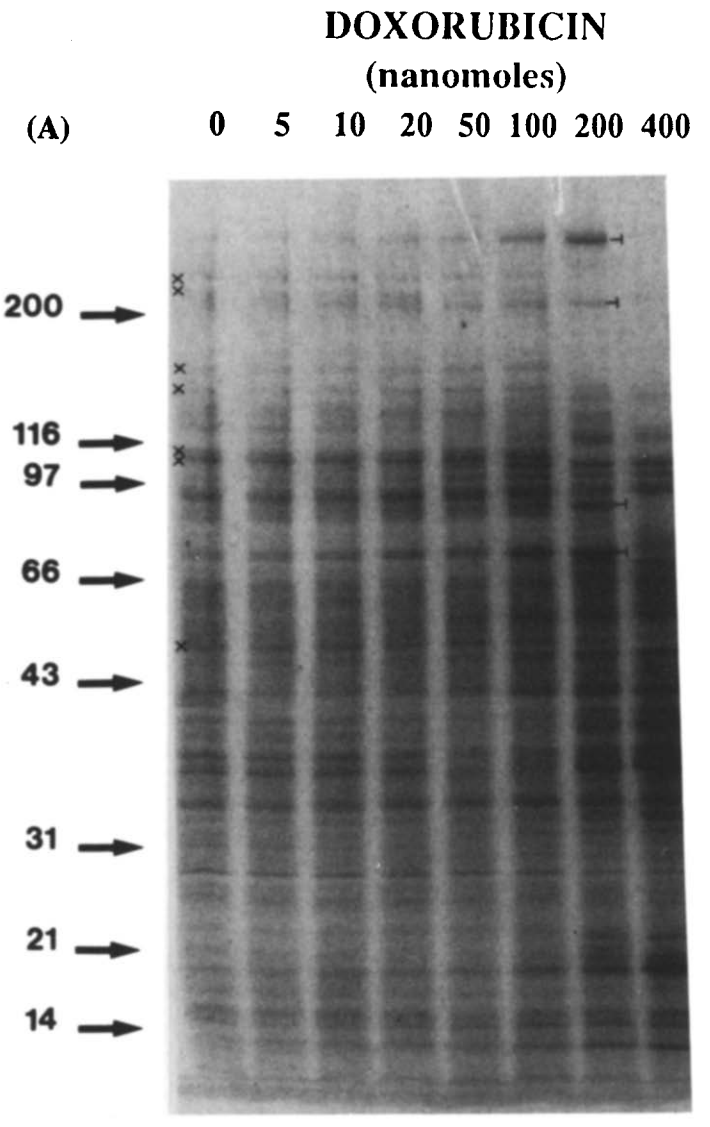

Fig. 4. (A) Proteins released from the nuclei of exponentially growing HL-60 cells upon titration with DOX. Key: (×) proteins entrapped; (⊣) proteins transiently eluted; and (←) proteins continuously eluted at the increasing DOX:DNA ratio. (B) Proteins released from the nuclei of exponentially growing HL-60 cells upon titration with AMD. Key: (×) proteins entrapped.

DNA correlates with antitumor potency [e.g. see Refs. 10 and 11]. Yet there is ample evidence that the intercalative binding to DNA cannot be the sole factor responsible for antitumor properties of many drugs. Thus, for instance, many strong intercalators lack pharmacological activity and generally no correlation is apparent between affinity of drugs to intercalate into DNA and their antitumor potency [e.g. Ref. 5]. Targets other than DNA, such as DNA topoisomerases [12] or nuclear (nucleolar) RNA [9, 13], were proposed to explain antitumor properties of several intercalating drugs.

It is likely, however, that the interactions are much more complex and involve a multiplicity of primary and secondary targets. This complexity explains, for instance, why some drugs are more potent with respect to certain tumor types (tissue specificity), or why histologically similar tumors have initially different sensitivity to a given drug.

The approach developed by us to study the drug-binding sites is based on an analysis of the proteins released from their original sites in nuclei incubated with the drugs. Clearly, there is great complexity with respect to nuclear proteins that are affected by the studied drugs, yet the effects are drug- and also cell- or tissue-specific. For example, MIT and AMT are very close analogs, differing only by the presence of two hydroxyl groups in positions 1 and 4 of MIT. Clinically, and with respect to their cytotoxic or cytostatic *in vitro* effects, MIT and AMT are also

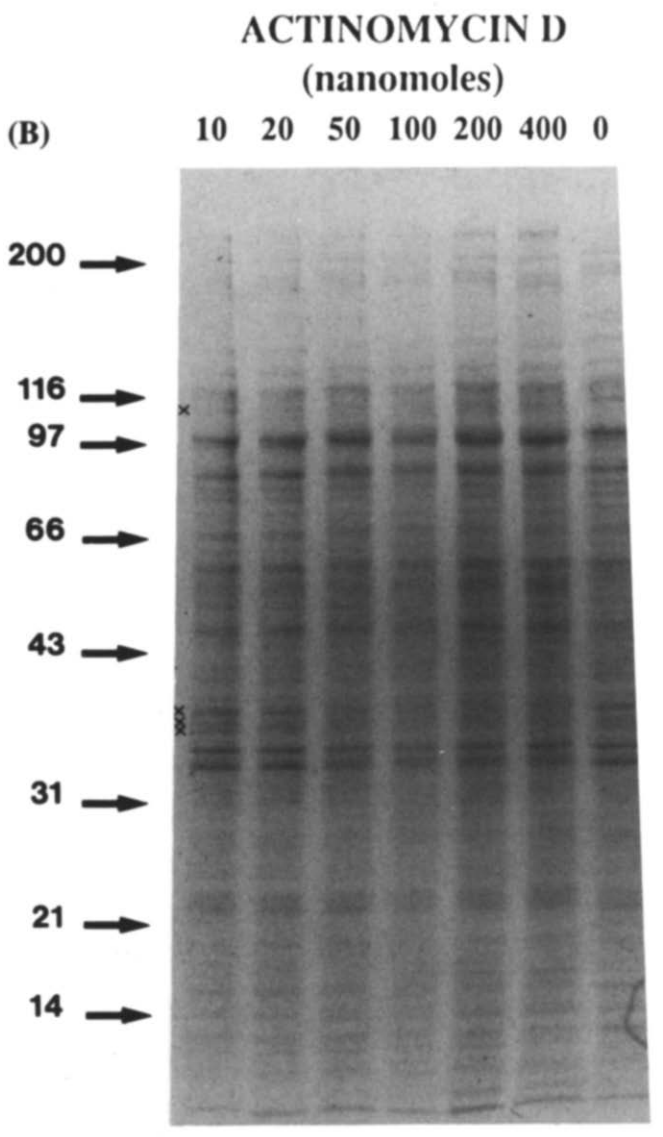

Fig. 4. Continued.

qualitatively similar, although MIT is significantly more potent, on a molar basis [6]. The observed patterns of proteins affected by MIT and AMT were also similar but differences in elution of 7 proteins were noted. Three of these proteins were eluted and the remaining four were entrapped at lower MIT:DNA than AMT:DNA ratios. Extraction of these proteins thus appears to correlate with pharmacological potency of these two drugs.

The differences in proteins extracted by MIT versus AMT are minor compared with proteins extracted by MIT or AMT versus DOX or AMD. These differences appear to correlate with differences in chemical structure of the drugs studied inasmuch as MIT and AMT are close analogs and they differ markedly from DOX or AMD. Thus, the binding sites for MIT and AMT, as revealed by the pattern of the extracted proteins, are similar, but not identical, whereas the sites for DOX or AMD are very different from those for MIT and AMT.

The observed differences are also cell-type specific. Namely, the pattern of proteins released by MIT from nuclei of the promyelocytic HL-60 cells was much different from that released from the lymphocytic MOLT-4 nuclei. The sets of proteins extracted by MIT from nuclei of the exponentially growing, nondifferentiated HL-60 cells were also dramatically different from those released from the same cells after myeloid differentiation. The latter differences may be due either to cell differentiation, withdrawal from the cell cycle (the differentiated cells are noncycling), or both.

Intercalation of the studied drugs into DNA, *per se*, cannot explain the specific release/retention of

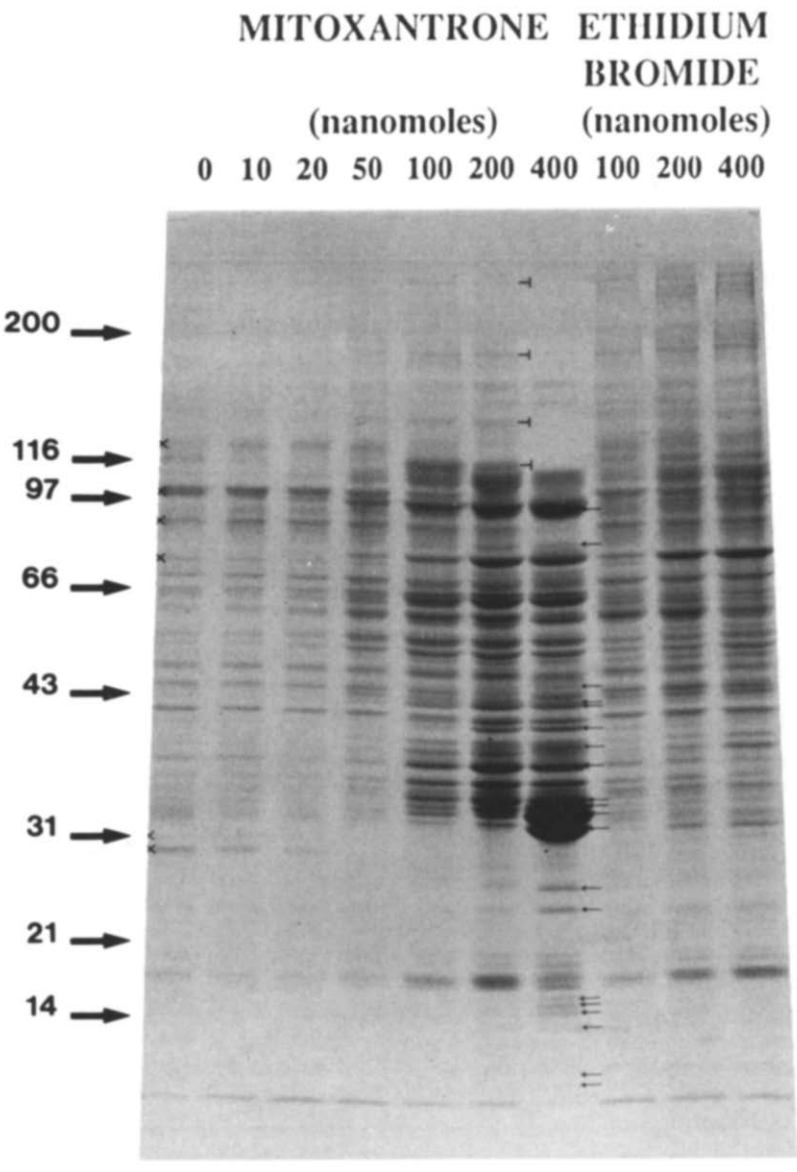

Fig. 5. Proteins eluted from the nuclei of exponentially growing MOLT-4 cells upon titration with MIT. Key: (×) proteins entrapped; (⊣) proteins transiently eluted; and (←) proteins continuously eluted at the increasing MIT:DNA ratio.

the proteins. The effects of EB, especially at the lower ligand concentration, were rather modest and the number of proteins specifically released or entrapped by the drugs, as listed in the Results, represents proteins other than those affected by EB; EB thus served as a secondary control representing an intercalating ligand with minimal antitumor activity.

To elucidate mechanisms involved in protein elution or retention by the drugs, control experiments were done to study the possibility of direct protein precipitation by the drugs (which could explain entrapment of proteins), and also the role of nuclear RNA. As is evident from Fig. 7, MIT directly precipitated numerous nuclear proteins. This phenomenon, and the relationship of the precipitated proteins to the proteins specifically entrapped by the drugs in the nuclei, are the subject of an ongoing study. These preliminary data indicate, however, that MIT can precipitate specific nuclear proteins, and this mechanism may explain the entrapment phenomenon. It is also possible that the drug modifies conformation of other molecules which then bind some proteins with higher efficiency, preventing their release.

Preincubation of nuclei with RNase precluded

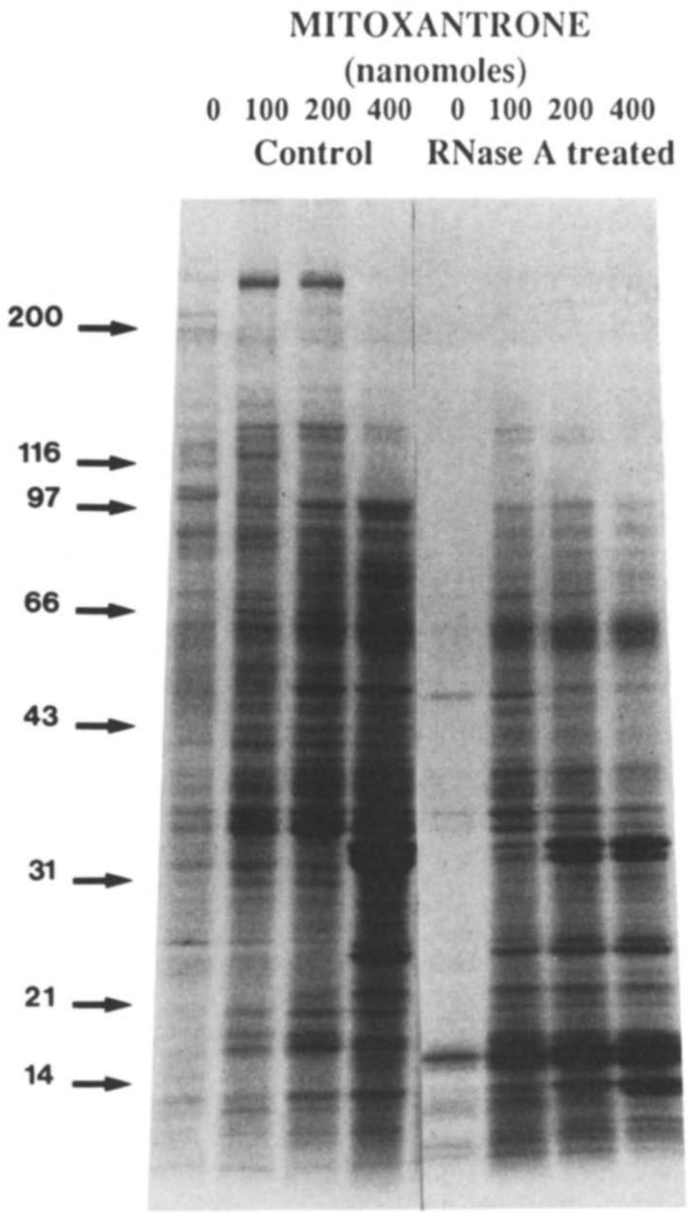

Fig. 6. Proteins released from the nuclei of exponentially growing HL-60 cells preincubated with RNase A upon titration with MIT. Control nuclei were preincubated with the buffer which did not contain RNase A.

release of several proteins by MIT (Fig. 6). It is likely that these proteins are released earlier as a result of enzymatic hydrolysis of RNA and thus may represent the RNA-binding proteins. These data are compatible with the assumption, supported by our earlier observations, that at least some pharmacological effects of MIT may be mediated via nuclear RNA [9]. The RNase effects, however, should be interpreted with caution, since this basic protein, *per se*, may extract some nuclear proteins rather than act via its enzymatic activity. This possibility is particularly difficult to exclude because to suppress activity of the endogenous nucleases and proteases RNase incubations must be performed in the cold; under these conditions its activity is low and a relatively high concentration of the enzyme is needed.

Identification of the drug released or entrapped proteins was not within the scope of this study. Casual observation of the gels, however, suggests that the heavy bands representing proteins released at the highest dose of MIT may be HMG and histone proteins (mol wt approx. 14–21 kD nucleosome core histones, 33 kD H1 histones and above H1, HMG1 and HMG2 proteins; see Fig. 2).

It is difficult to estimate how the drug concentrations presently used to release nuclear proteins compare to pharmacological doses. The first effects were generally observed at 10–20 nmol of the drug added to 2.5×10^7 nuclei and the effects were dose

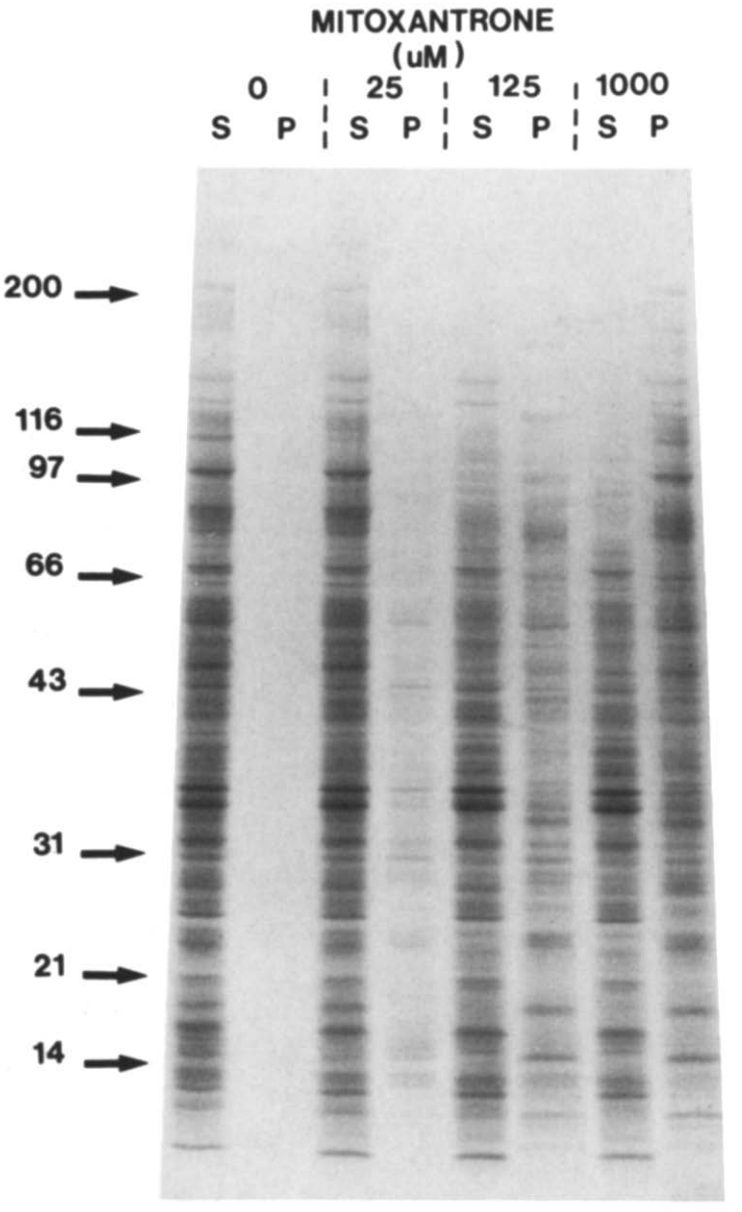

Fig. 7. Proteins precipitated from the solution by MIT. Key: (S) proteins from supernatant; and (P) proteins from pellet. Final MIT concentrations are indicated above the lanes (see Materials and Methods).

Table 1. Bound and free MIT and AMT during titration of nuclei isolated from exponentially growing HL-60 cells with these drugs

Amount of drug added (nmol)	Bound drug (% of total)		Free drug (μM)	
	MIT	AMT	MIT	AMT
20	99.3	98.3	0.7	1.7
50	99.4	97.6	1.4	6.0
100	99.4	96.6	2.8	17.0
200	99.2	93.0	7.9	69.8
400	98.4	87.5	28.0	250.0

See Materials and Methods for experimental details.

dependent. The drugs bound with high efficiency to the nuclei during titrations and free drug concentration in the buffer was minimal (Table 1). From the known number of nuclei in the sample (2.5×10^7), average DNA content per nucleus [14], corrected for cell cycle distribution (12 pg), and approximate molecular weight of DNA nucleotide, the number of drug molecules per DNA base pair was calculated under the assumption that the drugs bound only to DNA in the nuclei. First effects of the drugs on protein elution from the nuclei were observed at the drug doses equivalent to binding of one drug molecule per 10–50 base pairs. These are rather conservative estimates, given that the drugs

bind also to RNA [5] and proteins ([15], see Fig. 7). The actual binding densities were thus lower.

Comparison of these binding densities with *in vivo* drug effects is subject to many uncertainties. Little is known about the number and density of the drug-binding sites in cultured cells treated with pharmacological concentrations of the drugs or when drug is used in clinic, even under a simple assumption that the drugs bind only to DNA. The drug taken up from the medium or blood is accumulated in the cell, and perhaps even more in the nucleus, where its concentration is unknown. Also unknown is whether drug binding to DNA is uniform or clustered. Thus, because the actual density of drug binding per given length unit of nucleic acid cannot be established for living cells, the drug effects seen at the pharmacological concentrations cannot be directly compared with the drug:DNA ratios used in the present experiments.

The present and earlier [1] studies are still too preliminary to conclude that the observed patterns of elution/entrapment of nuclear proteins by the antitumor drugs can serve as a marker of drug activity. However, they open a new area of research which can lead towards the goal of evaluating drug pharmacological activity from knowledge of binding sites via analysis of released nuclear proteins. Further research can go in several directions: (i) from studies of large panels of active drugs and their inactive analogs a correlation may be sought between the pattern of protein elution and drug activity. If such correlation is found, the method can be used for rapid screening of newly synthesized drugs; (ii) from a panel of drug-sensitive and -resistant cells, a protein pattern can be sought which will be predictive of cell sensitivity to the drug; (iii) individual proteins specifically affected by the drugs may be analyzed with respect to their function (oncogenc products?) in the cell. Their presence and amount (e.g. assayed immunochemically) also may be correlated with cell sensitivity to the drug. This approach is applicable in pathology to cells collected by biopsy, and may help to assess sensitivity of specific tumors to particular drugs. The presently observed drug specificity of the phenomenon, as well as its specificity with regard to the tumor type (HL-60 vs MOLT-4) and to cell differentiation, suggests that information on the drug-binding sites in the nucleus obtained by this method can yield clues to the pharmacological activity of the drug.

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