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DRUG-SENSITIVITY AND DNA-BINDING OF A SUBFORM OF TOPOISOMERASE IIα IN RESISTANT HUMAN HL-60 CELLS

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Topoisomerase IIα (170 kDa) expressed in human HL-60 cells is heterogeneous in charge. By two-dimensional electrophoresis and chromatofocussing two major subforms with pI of 6.5 and 6.7 can be resolved. By preparative anion-exchange chromatography we separated the known topoisomerase II isoenzymes (170/180 kDa) and in addition a late-eluting 170 kDa form, which has not been described before. The catalytic optimum of this late-eluting form is shifted to pH 9.4. It is more than 100-fold resistant to orthovanadate, amsacrine or etoposide, and has an increased salt stability. SDS-treatment induces covalent attachment of this enzyme fraction to calf thymus DNA in the absence of drug. The latter observations indicate an increase in DNA-binding. In the tightly DNA-bound state the late-eluting enzyme is not targeted by cleavable complex forming drugs. Accordingly, cells may become drug-resistant by expressing this form predominantly.

Type II DNA topoisomerases (Topo II) regulate the topological state of the DNA by passing DNA strands through transient enzyme-bridged double-stranded DNA breaks. They are part of the chromosomal structure and play a crucial role in DNA-synthesis, transcription and recombination (1–7). Two isoforms of Topo II (α and β) encoded by separate genes have been found in human cells (8, 9). Topo IIα and β differ in molecular mass (170 kDa vs. 180 kDa), functional properties (10), and are differently expressed during cell cycle and differentiation (3, 6, 11). Type II topoisomerases have recently been identified as the cellular targets for a number of important antineoplastic agents. These agents induce topoisomerase II mediated DNA strand breaks which are lethal to the cells (12–16). A special type of cellular resistance to Topo II inhibitors involves alterations of the drug target topoisomerase II itself (17). It can be caused by point mutations in the topoisomerase IIα gene (18–20), or changes in the expression levels of the two isoenzyme (21, 22).

We have previously reported on a sub-clone of the human leukemic cell line HL-60 (HL-60/R), which is 1000-fold resistant to the Topo II inhibitors mAMSA and etoposide, although it has not been selected for resistance to any drugs (23, 24). The multiple drug resistance in these cells is not associated with overexpression of P-glycoprotein. Here we report on the identification of two functionally different subforms of topoisomerase IIα by virtue of charge heterogeneity. The two subforms differ in sensitivity to clinically relevant topoisomerase II inhibitors by a factor of 1000. Our data demonstrate that these functionally distinct subforms coexist in drug-sensitive and resistant HL-60 cells and changes in their relative cellular levels affect the sensitivity of the cells to topoisomerase II targeting antineoplastic agents.

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Material and Methods

Material. Etoposide was from Bristol Myers, Troisdorf, FRG. Amsacrine was from Goedecke AG, Berlin, FRG. A fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) and columns from the same manufacturer were used in all chromatographic steps. pBR

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322 DNA, α_2-macroglobulin, and other protease inhibitors were obtained from Boehringer, Mannheim, FRG. Catenated Crithidia fasciculata kinetoplast DNA (kDNA) was obtained from TopoGen, Columbus, Ohio, USA. Rabbit-anti human topoisomerase II antibodies, cross-reacting with the α- and β-isoenzyme, were a kind gift of Prof. L.F. Liu, Baltimore, USA. Rabbit-anti-peptide antibodies, specific for the 18 carboxyterminal aminoacid residues (1513–1530) of human topoisomerase IIα (25) were obtained from Cambridge Research Biochemicals Ltd, UK. Detergent resistant endonuclease Benzonase was obtained from Merck, Darmstadt, FRG. A Hoefer Mighty Small II system for two-dimensional electrophoresis was obtained from Serva, Heidelberg, FRG. All other chemicals were of the highest degree of purity commercially available.

Methods. 10 mM dithiothreitol, 1 mM PheMeSO$_2$F, 1 μg/ml diisopropyl-fluorophosphate, and 10% glycerol were included in all buffers used for purification or assays. Purification and fractionation of topoisomerase II followed published procedures (26, 27). Briefly, nuclei were isolated and extracted with Tris-buffer, pH 7.4, containing 750 mM NaCl. Nuclear extracts were diluted with 50 mM potassium phosphate, pH 7.5 to a final NaCl concentration of 50 mM and applied to a heparin Sepharose column (1 ml bed volume) at a flow rate of 1 ml/min. Bound proteins were eluted by a gradient (50–500 mM potassium phosphate, pH 7.5, 20 ml). Fractions containing ATP-dependent catalytic DNA-decatenation avtivity were pooled, diluted 20-fold with 10 mM Tris buffer, pH 8.0, and adsorbed to a Mono Q HR 5/5 anion-exchange column. Bound proteins were eluted by a 50–500 mM NaCl gradient (20 ml), collecting 250 µl fractions. Alternatively, pooled and diluted fractions were absorbed to a Mono P HR 5/5 column and chromatofocussing was performed by elution with a gradient from pH 9-6 as previously described (26). Non-equilibrium pH gradient gel electrophoresis of whole cell lysates followed published procedures (28). Briefly, 10^7 HL-60 cells were lysed by 5 M urea, 2% Nonidet P40, 0.1% SDS. Lysates were digested with 50 units of detergent-resistant endonuclease benzonase for 10 min at room temperature and subjected to two-dimensional polyacrylamide gel-electrophoresis, followed by Western blot analysis (26, 27). Measurement of kDNA-decatenation and pBR 322 plasmid DNA cleavage was measured, as in (29, 30). Briefly, 4 μl of enzyme sample were incubated for 30 min at 37°C with 500 ng of pBR 322 or kDNA in a final volume of 40 μl buffer (150 mM glutamate mono-potassium salt, 50 mM bis-tris-propane, pH adjusted as stated with KOH or HCl). Analysis of drug-induced immuno-band-depletion was carried out as in (30). Filterbinding assays of DNA-protein complex formation induced in intact cells by topoisomerase II targeting drugs followed the procedure of Meyer et al. (31). The wild type of human HL-60 cells (HL-60/WT, American tissue culture collection No. CCL240) and the resistant strain HL-60/R were grown as described in (23). Briefly, cells were grown in liquid culture (RPMI 1640 + fetal calf serum 5% (vol/vol), 10 g/l penicillin/streptomycin) in a humidified atmosphere containing 5% (vol/vol) CO_2. Cells were routinely checked to be free of mycoplasms by immunoassays and cultural analysis.

Results and Discussion

Structural microheterogeneity of HL-60 topoisomerase IIα. It has recently been demonstrated (28) by two-dimensional gel-electrophoresis that topoisomerase IIα (170 kDa) of HeLa cells can be resolved into several subforms with isoelectric points (pI) between 6.4 and 6.8. Here, we obtained similar results with topoisomerase IIα of human HL-60 cells. By non-equilibrium pH-gradient-, followed by SDS-polyacrylamide gel-electrophoresis we resolved the enzyme into two major fractions with apparent pI values between 6.5 and 6.7 and a minor fraction focussing around pH 6.4 (Fig. 1a). To control for artifacts arising from the use of SDS and urea during cell lysis we also performed chromatofocussing of HL-60 topoisomerase II, which can be carried out in the absence of detergents and chaotropic agents (26). For this purpose a partially purified (by heparin Sepharose chromatography (27)) enzyme preparation was adsorbed to the weak anion exchange column Mono P, followed by a pH-gradient elution. As demonstrated in Fig. 1b, three distinct peaks of catalytically active topoisomerase II were resolved between pH 6.4 and 6.8 by this procedure, confirming the apparent pI values obtained by isoelectric focussing (Fig. 1a). Thus we can exclude, that in vitro modifications by SDS or urea are the cause of the multiple pI. Based on these unequivocal results of electrophoretic and chromatographic pI determination we assumed that the α-isoenzyme of human topoisomerase II consists of at least two structurally heterogenous subforms. In order to obtain sufficient material for further functional studies of these subforms we fractionated partially pure HL-60 topoisomerase by anion-exchange chromatography carried out at pH 8.0. When measuring topoisomerase II-specific ATP-dependent kDNA-decatenation activity in the chromatographic fractions eluted from the Mono Q column (Fig. 2a), we obtained different results with different pH in the assay buffer: at pH 7.5 the peak of decatenation activity was eluted by 230–250 mM NaCl (Fig. 2a, fractions 5, 6). This coincided with the elution profile of an immuno-reactive protein band of 180 kDa (Fig. 2b, left). When measuring at pH 8.5, we observed a single maximum of activity at an elutional strength of 150–200 mM NaCl (Fig. 2a, fractions 3, 4). This profile of activity at pH 8.5 was congruent with the elution profile of an immunoreactive band of 170 kDa (Fig. 2b, left and right). When the fractions were assayed at pH 9.4 we found a peak of activity in fractions 8–10, corresponding to an elutional strength of 300–

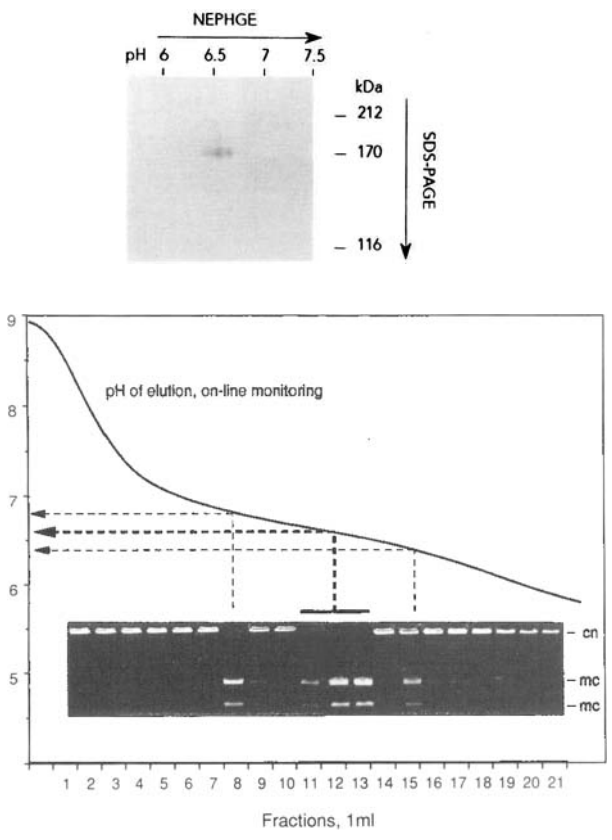

Fig. 1. Multiple isoelectric points of HL-60 topoisomerase II. a) 10^7 HL-60 cells were lysed, subjected to non-equilibrium pH-gradient electrophoresis followed by SDS-polyacrylamide gel electrophoresis. After electrphoretic transfer to nitrocellulose, topoisomerase II was stained with rabbit-anti-peptide antibodies, specific for the α-form of human topoisomerase II. The electrophoretic migration distance of the following marker proteins is indicated on the right margin: rabbit muscle myosin (212 kDa), α_2-macroglobulin from bovine plasma (170 kDa), β-galactosidase from E. coli (116 kDa). The pH values indicated on the top margin were derived from pH-measurements performed in empty pH-gradient gels run in parallel. b) HL-60 topoisomerase II was partially purified by heparin Sepharose chromatography, adsorbed to Mono P, HR 5/5 column and eluted with a pH gradient as indicated by the solid line. 1 ml fractions were collected and assayed for DNA-catenation activity, detected by agarose gel-electrophoresis from disappearance of the catenated network DNA in the sample application slot and simultaneous appearance of released minicircles (mc) in the gel. Activity peaks are related by dashed lines to the respective elutional pH value given on the x-axis.

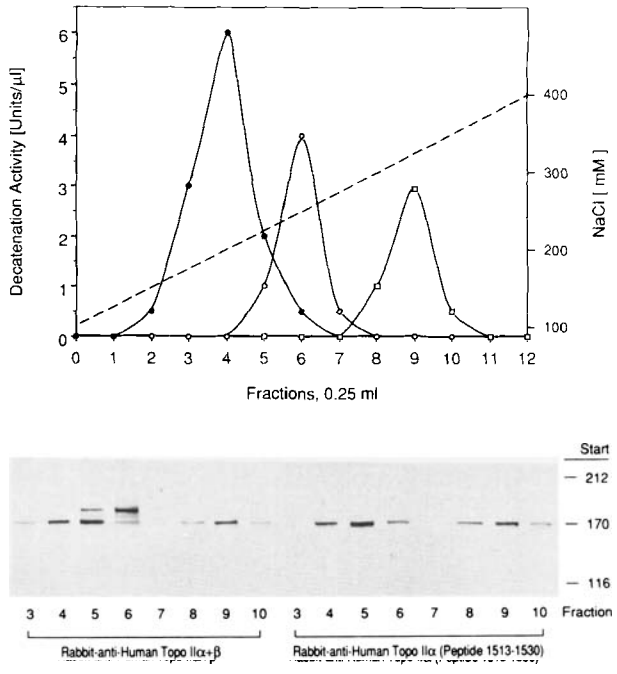

Fig. 2. Fractionation of subforms of HL-60 topoisomerase II by anion-exchange chromatography. Topoisomerase II partially purified from HL-60 cell nuclei by heparin-Sepharose chromatography was adsorbed to a Mono Q HR 5/5 column and eluted by a NaCl gradient (100–400 mM). a) Aliquots of each fraction were analyzed for DNA-decatenation activity at pH 7.5 (open circles), 8.5 (closed circles) and 9.4 (open squares) in various dilutions. One unit was defined as the minimal amount of enzyme that will completely decatenate 500 ng of kDNA within 30 min at 37 C. b) Immunoblot analysis of chromatographic fractions 3–10 employed rabbit-anti-human topoisomerase II antibodies crossreacting with α- and β-isoenzymes (left) and rabbit-antibodies directed against peptide 1513–1530 of the human β-isoenzyme (right). The electrophoretic migration distance of the following marker proteins is indicated on the right margin: rabbit muscle myosin (212 kDa), α_2-macroglobulin from bovine plasma (170 kDa), β-galactosidase from E. coli (116 kDa).

400 mM NaCl. These late-eluting fractions also contained an immuno-reactive band of 170 kDa (Fig. 2b, left and right). From these data we concluded in agreement with previous observations (32) that the α- and β-isoforms of topoisomerase II were eluted from the Mono Q column in close sequence (by 150 and 230 mM NaCl respectively). In addition, here we observed a late-eluting variant of topoisomerase II with altered charge properties, corresponding to the subform with the more acidic pI shown in Fig. 1. This late-eluting topoisomerase II, which has not been described before, bound more tightly and accordingly was eluted by higher ion concentrations (> 300 mM NaCl). It is clearly identified as a type II topoisomerase by its capability to release free double stranded DNA-circles from a catenated network kinetoplast DNA in an ATP-dependent fashion (Fig. 3). As can be seen in Fig. 2b, it has the same molecular mass as the early-eluting α-form of human topoisomerase II (170 kDa) and exhibits a similar pattern of immunoreactivity, cross-reacting with the rabbit-anti-human topoisomerase IIα + β antibody (Fig. 2b, left) and also with an α-form-specific rabbit-anti-peptide antibody raised against the carboxyterminal 18 amino acid residues of human topoisomerase IIα (Fig. 2b, right).

Catalytical properties of HL-60 topoisomerase II subform. A more detailed determination of the catalytic pH

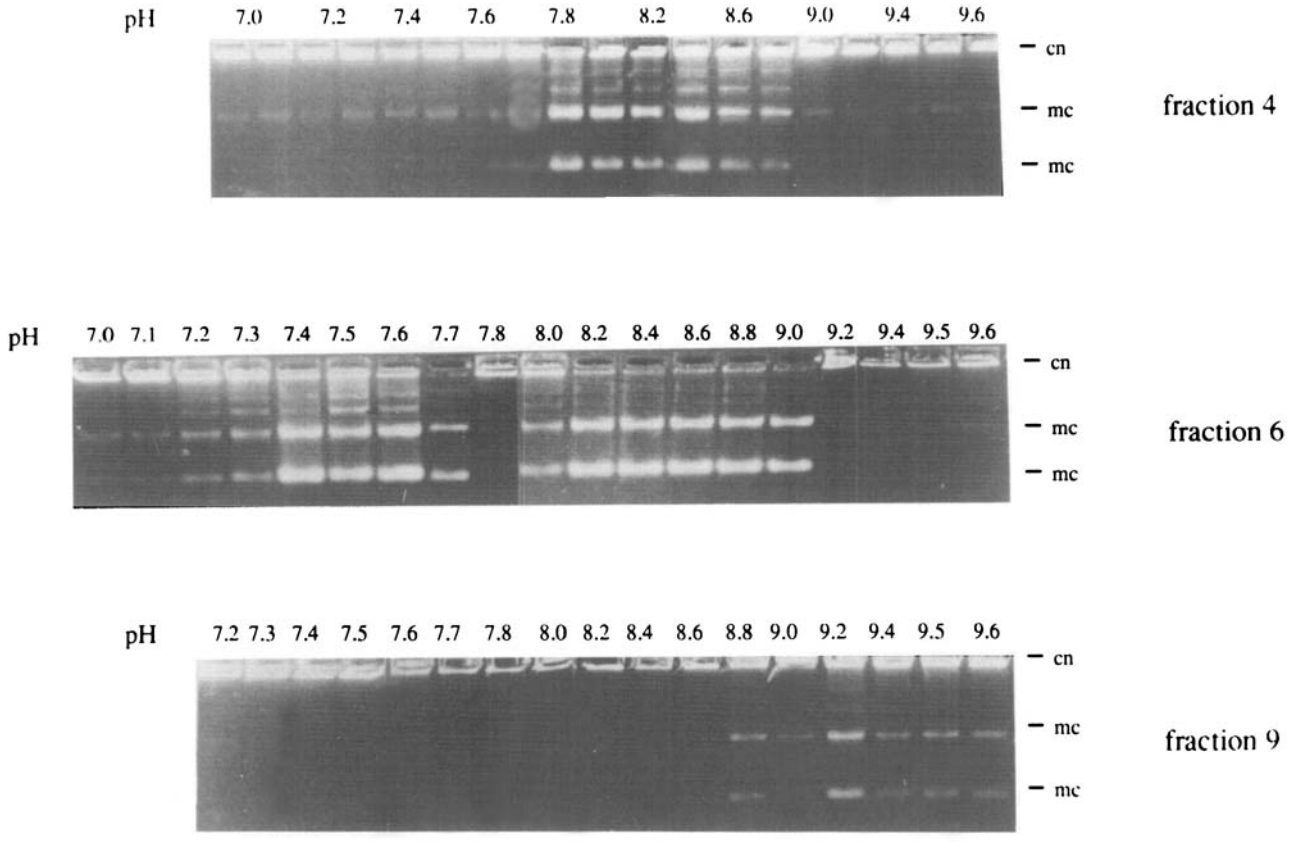

Fig. 3. Catalytic pH-optima of topoisomerase II subforms. DNA-decatenation of chromatographic fractions 4, 6 and 9 (refer to Fig. 2) was assessed at the pH values indicated.

optima of the enzymes in fractions 4 (early-eluting topoisomerase IIα), 6 (topoisomerase IIβ + traces of early-eluting topoisomerase IIα), and 9 (late-eluting topoisomerase IIα) is summarized in Fig. 3: the early-eluting form of topoisomerase IIα (fraction 4) is active between pH 7.8 and 8.6. Topoisomerase IIβ (fraction 6) is most active at pH 7.4–7.6. In contrast, the late-eluting variant of topoisomerase IIα (fraction 9) has its optimum at pH > 9.2. It should be noted that the pH-intervals of maximal activity of the three enzyme forms do not overlap. This can be most clearly seen in fraction 6 which contains both early-eluting α-form and β-form. Thus, in mixtures the various forms of topoisomerase II can be preferentially assessed by using the appropriate pH in the assay. On the assumption that proteins which bind more tightly to a synthetic anion-exchange resin should also exhibit a tighter interaction with the polyanion backbone of the DNA, we hypothesized that the Mono Q elution sequence of the topoisomerase II subforms would be reflected by their respective DNA-affinity, which in turn determines the salt stability of the catalytic activity. Accordingly, we assessed the catalytic activity of the three fractions at the appropriate pH in the presence of various concentrations of the physiological intracellular salt KGlu. These experiments are summarized in Fig. 4. Early-eluting topoisomerase IIα and topoisomerase IIβ exhibited similar properties. They were maximally active at KGlu concentrations of 150 mM. DNA-decatenation activity decreased rapidly, when salt concentrations were increased above 150 and 200 mM respectively. These figures agree well with the respective Mono Q elution concentrations (150 and 230 respectively). In contrast, the catalytic activity of the late-eluting subform of topoisomerase IIα (Mono Q elution at > 300 mM) was maximal at salt concentrations of 300–400 mM. Inactivation of the enzyme needed as much as 500 mM salt. We concluded that the DNA-affinity of the late-eluting form of topoisomerase IIα is significantly increased. This notion was further supported by the observation that SDS treatment induces covalent attachment of the late eluting enzyme fraction to calf thymus DNA. This could be monitored by the disappearance of the immunoreactive band from immuno-blots after preincubation with 1 μg of calf thymus DNA (data not shown).

Different inhibitor sensitivity of early and late-eluting topoisomerase IIα. Several studies, carried out with topoisomerases from different species, indicate that there may be a positive correlation between the DNA-affinity, the salt stability of the catalytic activity, and the susceptibility of the enzyme to cytostatic drugs that stabilize the cleavable enzyme-DNA complex (17, 33–35). The late-eluting vari-

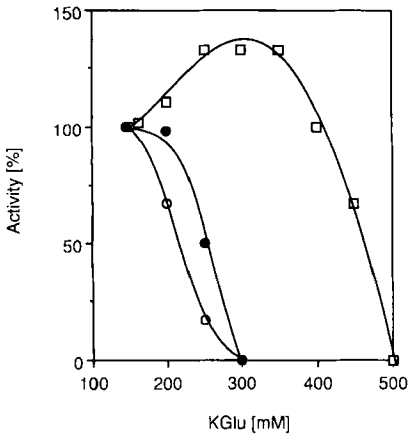

Fig. 4. Salt stability of topoisomerase II subforms: DNA-decatenation activity of fraction 4 (○), fraction 6 (●) and fraction 9 (□) was quantified at the salt concentration indicated by serial dilution and normalized to the activity measured with 150 mM salt. Controls in the absence of enzyme or ATP were negative. The fraction numbers refer to Fig. 2: fraction 4 contains early eluting topoisomerase IIα (assayed at pH 8.5); fraction 6 contains mainly topoisomerase IIβ (assayed at pH 7.5); fraction 9 contains late eluting topoisomerase IIα (assayed at pH 9.4).

ant of topoisomerase IIα of HL-60 described here has an increased DNA-binding and a salt stability similar to that observed in resistant enzyme mutants. Accordingly, we speculated that it might also be resistant to inhibitors which act by stabilizing the catalytic DNA-enzyme intermediate. To test this hypothesis, we measured the linearization of pBR322 DNA that could be induced by the two fractions of HL-60 topoisomerase IIα in the presence of various concentrations of an intercalating (amsacrine) and a non-intercalating (etoposide) inhibitor. As depicted in Fig. 5, pBR322 DNA-linearization, which is indicative of cleavable complex formation could be induced by nanomolar concentrations of both inhibitors when the early-eluting enzyme variant was present in the assay. In contrast, micromolar concentrations of the same inhibitors were needed to stabilize similar amounts of the catalytic intermediate of the late-eluting enzyme. From these experiments we conclude that the late-eluting enzyme is resistant in vitro and probably also not targeted by cytostatic topoisomerase II inhibitors in living cells. Another feature frequently observed in resistant forms of topoisomerases is an altered ATPase activity. Changes in ATP-turnover, substrate-specificity and sensitivity to ATPase inhibitors have been described (17, 36–39). To search for such alterations of the ATPase domain we made use of orthovanadate, which is a known inhibitor of the ATPase activity of human topoisomerase II (10). Measuring the inhibition of catalytic DNA-decatenation in the presence of various concentrations of orthovanadate, we found a marked difference between early-eluting topoisomerase IIα, which was normally sensitive to vanadate (IC_{50}, 30 nM) and the late-eluting form, which was highly resistant (IC_{50}, 3 μM) (data not shown). All in all, our investigation of topoisomerase IIα expressed in the wild type of human HL-60 cells demonstrates the simultaneous presence of two enzyme variants, which can be chromatographically separated by virtue of their different charges. The early-eluting enzyme exhibits catalytic properties which are in good agreement with published data (10) and is normally sensitive to specific topoisomerase II inhibitors. In contrast, a late-eluting variant, apparently, is not only structurally altered but has also undergone a complex functional change, including a shifted catalytic pH-optimum, an increased DNA-affinity, and alterations of the ATPase domain. In consequence of these changes the late-eluting

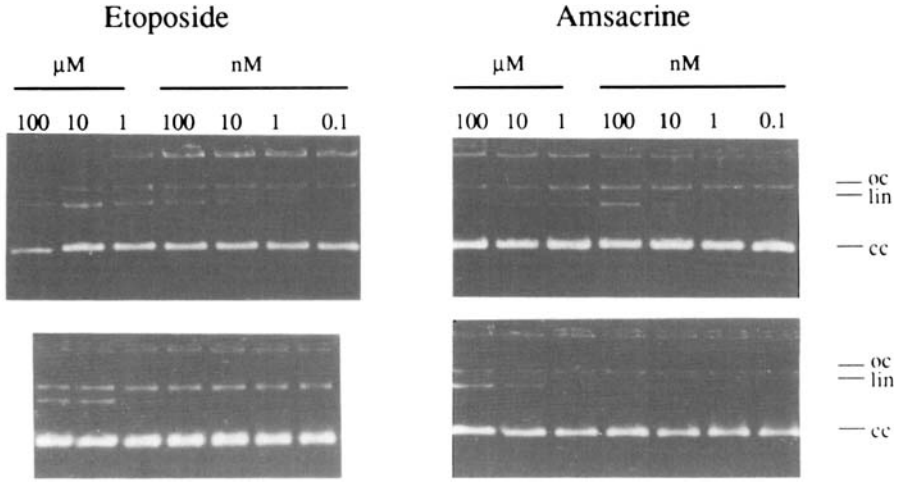

Fig. 5. In vitro sensitivity of early and late-eluting topoisomerase IIα to amsacrine and etoposide. Linearization of pBR 322 DNA in the presence of early (fraction 4, 10 μl, assayed at pH 8.5) or late-eluting (fraction 9, 10 μl, assayed at pH 9.4) topoisomerase IIα and various concentrations of etoposide or amsacrine. The various forms of pBR 322 DNA were electrophoretically separated in 1% agarose gels in the presence of ethidium bromide: oc, open circular; lin, linearized; cc, closed circular.

enzyme is highly resistant to two topoisomerase-targeting cytostatic drugs of different chemical classes.

A resistant clone of HL-60 cells has increased levels of late-eluting topoisomerase IIα. Previously, we reported on a subclone of the human leukemic HL-60 cell line, HL-60/R, which is 1 000-fold resistant to several topoisomerase II inhibitors, including amsacrine and etoposide, although it has not been selected for resistance to any drug (23). The multiple drug resistance in these cells is not associated with P-glycoprotein expression as determined by specific antibody-staining of the cells and quantification of cellular m-RNA levels. The resistance of HL-60/R to topoisomerase II inhibitors could not be reverted even by very high concentrations of verapamil, which are known to inhibit the P-glycoprotein mediated drug elimination (23). This phenotype of multidrug resistance could be due to altered expression levels of topoisomerase IIα and/or topoisomerase IIβ. However, Western blot analysis of whole cell lysates from these cells show that both the HL-60/WT and the HL-60/R cells have equal amounts of the α- and β-isoforms of topo II and that both isoforms are present in similar proportions (not shown). To examine whether the HL-60/R and the drug-sensitive HL-60/WT cells contain similar amounts of the late-eluting topoisomerase IIα variant, we performed two types of experiments which are summarized in the Table. First, the decatenation activity was measured in crude nuclear extracts from HL-60/WT and HL-60/R at the three different pH, favouring the catalytic activity of the respective subform. Nuclear extracts from both cell lines show similar decatenation activity at pH 7.5, indicating that the two cell lines have similar levels of topo IIβ. This is in agreement with the Western blot analysis of whole cell lysates from these cells (not shown). The resistant HL-60/R have a much higher decatenation activity at pH 9.4 than at pH 8.5. In contrast, the sensitive HL-60/WT have a higher decatenation activity at pH 8.5 than at pH 9.4. It should be noted that the total amount of decatenation activity (the sum of activities assayed at the three pH conditions) extractable per nucleus was equivalent between the HL-60/WT and HL-60/R cells. To confirm these results obtained in crude nuclear extracts and to exclude the possibility of unknown proteins in the nuclear extracts interfering with the assay, we adsorbed extracts prepared from 10^8 nuclei of HL-60/WT or HL-60/R cells to a Mono Q HR 5/5 column and separated early- and late-eluting topoisomerase IIα by stepwise elution. For the first elution step we chose 150 mM NaCl as a threshold concentration sufficient for isocratic elution of the early-eluting topoisomerase IIα. This was followed by a second elution with 400 mM NaCl for complete dissociation of the late-eluting form from the column (compare Fig. 2). We then compared the amount of enzyme activity that could be eluted by 150 or 400 mM NaCl respectively. In HL-60/WT cells only ≈ 20% of the enzymatic activity were recovered in the high salt fraction. In contrast, when the resistant HL-60/R cells were analyzed more than 80% of the topoisomerase II activity were in the high salt fraction (Table, lower part). Taken together, the data of both experiments unambiguously demonstrate that in HL-60/R

Table

pH-profile of ATP-dependent decatenation activity in crude nuclear extracts and relative distribution of early- and late-eluting topoisomerase IIα in HL-60/WT and HL-60/R cells

	DNA decatenation activity[1]	
pH in the assay	HL-60 WT	HL-60 R
	u/10^8 nuclei[2]	
7.5	98 ± 29	142 ± 54
8.5	465 ± 85	68 ± 36
9.4	121 ± 34	532 ± 90
NaCl elution mM	u/fraction[3]	
150	439 ± 120	42 ± 32
400	86 ± 26	475 ± 98

[1] DNA decatenation activity was quantified by serial dilution of the samples. One decatenation unit was defined as the minimal amount of enzyme that will completely decatenate 500 ng of kDNA within 30 min at 37°C. Controls in the absence of enzyme or ATP were negative. The results (mean ± standard error) of three independent experiments are shown. [2] Decatenation activity of freshly prepared nuclear extracts from HL-60 WT or HL-60 R cells was measured at the pH indicated. [3] Extracts of 10^8 nuclei were adsorbed to a Mono Q column (0.5 × 2 cm) and stepwise eluted with the NaCl concentrations indicated. For each cell line two fractions of 5 ml were obtained and DNA-decatenation activity was measured at pH 8.5 in the 150 mM fraction or at pH 9.4 in the 400 mM fraction.

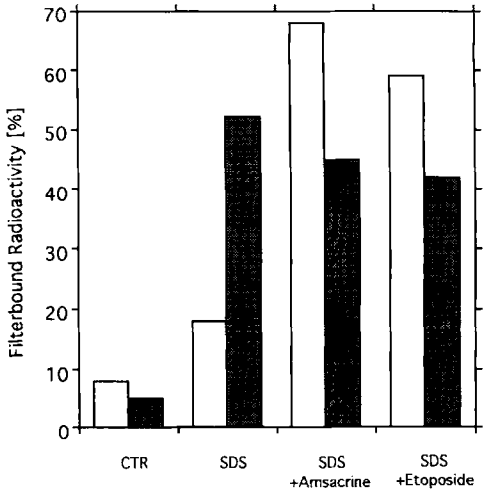

Fig. 6. Detection of DNA-protein complexes formed in HL-60/WT and HL-60/R cells in vivo. DNA of HL-60/WT (white bars) and HL-60/R (black bars) cells was labelled by ^{3}H-thymidin. Cells were then mechanically homogenized in the absence (CTR) or presence of SDS and protein-DNA complexes were captured on nitrocellulose filters. Filterbound radioactivity was determined by liquid scintillation counting and normalized to total DNA-incorporated radioactivity. The result pairs obtained after exposing the cells for 1 h to 10 μM concentrations of topoisomerase II targeting drugs are indicated by the respective names of the drugs.

cells the late-eluting topoisomerase IIα variant is increased at the expense of the early-eluting form. As demonstrated and discussed in Fig. 4, the late eluting variant of topoisomerase IIα has an increased DNA-binding affinity and as a consequence a tendency to form complexes with DNA upon SDS lysis. In order to compare this property between sensitive HL-60/WT and resistant HL-60/R cells we employed a whole cell filter binding assay which allows one to measure DNA-protein complex formation (31). After metabolic labelling of the cellular DNA by [^{3}H]-thymidine, we mechanically disrupted the cells and detected protein-DNA complexes by specific filter binding. As shown in Fig. 6, only about 10% of the cellular DNA become filterbound after mechanical disruption of both sensitive HL-60/WT and resistant HL-60/R cells. Upon treatment with SDS the amount of DNA-protein complexes increases only slightly in the wild type cells ($\approx$by 10%) but pronouncedly ($\approx$50%) in the resistant strain. This observation is in good agreement with the notion, that in the resistant strain a tight complex of topoisomerase II and DNA-affinity is present and can be detected by this procedure. When normal sensitive HL-60 cells are treated with cytotoxic concentrations of the topoisomerase II targeting drugs amsacrine or etoposide before mechanical disruption and SDS-treatment, the amount of protein-complexed DNA which becomes bound to the filter increases from 10 to 70%. This is indicative of the drug-induced formation of cleavable toposimerase II-DNA complexes. In contrast, in the resistant HL-60/R cells the drug treatment does not add to the pre-existing DNA-protein complex formation. This confirms our notion, that in these cells topoisomerase II, which exists as a tight DNA-complex, is not targeted by the drugs.

Thus, it appears that the conversion of the drug-sensitivity early- to the drug-resistant late-eluting form of topoisomerase II is a pivot point which includes a major alteration of the enzyme function. The physiological role of this alteration is not clear to date and we cannot say whether this process occurs only in HL-60 cells. However, our data indicate that the structural microheterogeneity of topoisomerase II which has been observed in several human cells also by other investigators, has its parallel in a heterogeneity of the enzyme function. This may have consequences for the therapeutical use of topoisomerase II—targeting drugs and our study has some suggestions how these may be experimentally addressed.

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