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DETECTION OF THE EFFECTS OF INTERCALATING AND NON-INTERCALATING DRUGS ON DNA STRUCTURE

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INTRODUCTION

We have recently shown that there is no correlation between inhibitory property on the relaxing activity of human DNA topoisomerase type II and the antineoplastic activity of a group of new anthracyclines (1). However, the apparent inhibitory activity of these drugs, as determined by gel electrophoresis analysis, could in part be attributed to their relaxing properties.
To clarify this point, we have developed a novel technique to detect any modification of DNA structure. This presentation describes this technique and its use for studying alterations in DNA structure.

METHODS

DNA substrate preparation. Naturally supercoiled plasmid pAT153 was converted into nicked circular form by treatment with pancreatic DNase I following the protocol of Wang (2).
Ligation assay. The reaction mixture (20 µl containing: 66 mM Tris-HCl, pH 7.6; 6.6 mM $MgCl_2$; 10 mM DTE; 0.66 mM ATP; 100 ng pAT153 DNA; 1 units of bacteriophage T4 DNA ligase from Toyobo) is incubated for 10 min at desired temperature with and without compounds under analysis. Then the reaction is stopped by addition of EDTA (20 mM final concentration). The reaction mixture is boiled for 5 min to denature unligated substrate. Before loading onto the gel added drugs, when used, were extracted twice with 10 volumes of butanol saturated with the running buffer. The samples are run on 1% agarose gel; running buffer: 0.4 M Tris base, 0.2 M CH_3COONa, 20 mM Na_2EDTA, 0.18 M NaCl, final pH 8. Electrophoretic conditions: 1.4 V/cm, 14h, 25°C. Gels are stained for 30 min with 1 µg/ml ethidium bromide, washed twice for 15 min with H_2O. Photographs are taken on P55 films using a 312 nm transilluminator. Negatives are analysed on a Beckman DU-8 gel scanner.

RESULTS AND DISCUSSION

To study DNA-drug interactions, we have modified the assay utilized for studying protein binding to DNA (3). In this assay nicked circular double-stranded DNA is covalently circularized by the action of a DNA ligase, in this case the bacteriophage T4 enzyme. The topological status of the resulting DNA is described by the equation: L= T+W, where L= Linking number, T= Twisting number, W= Writhing number. The ligation of a homogeneus population of circular nicked DNA molecules results in a population of covalently closed circles with an average value of linking number (L_m) which depends on the temperature at the time of closure. Therefore any temperature variation changes the resulting degree of supercoiling of the molecule. When the substrate is ligated at a specific temperature and then run on an agarose gel at a different temperature, it is possible to see a set of topoisomers with a different degree of supercoiling normally distributed around a mean value that is the average linking number. With the experiment of Fig.1 we show how L_m varies proportionally to the temperature of ligation. The relative position of topoisomers distri-

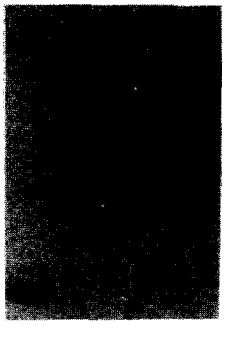


Fig. 1

bution on agarose gel depends also on the T°C between ligation and electrophoresis temperatures. We optimized these conditions in 0°C for ligation and 25°C for electrophoresis. The microdensitometric analysis of bands distribution allows the calculation of the center of distribution and therefore the determination of the T°C needed to modify the L_m of one unit. We find that the relative position of topoisomers is modified also when the substrate is ligated in the presence of an intercalating drug (Fig.2). Therefore the result of the interactions between drugs and DNA can be expressed either as L_m or ΔT°C equivalents. However the correlation between drug concentration and ΔL_m generally deviates from linearity (Fig.3). This deviation is probably the consequence of the loss of the normal distribution which is well visible at high drug concentrations (not shown). For this reason to calculate the specific unwinding (ΔL_m/μM or ΔT°C/μM) we utilized only data obtained at lower drug concentrations.

In addition we find that also non intercalating but DNA binding agents alter the DNA tertiary structure. In fact we obtain a positive result when chemicals with such properties are analysed in our assay. Therefore the assay proved to be usefull for determining any kind of DNA structure alterations resulting in a change in the overall superhelical structure after ligation. An evident advantage of this assay over any other assay utilizing a bidimensional electrophoresis consists in the possibility of analysing many samples on the same gel.

Fig. 2

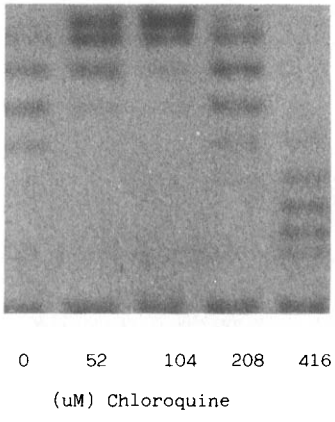
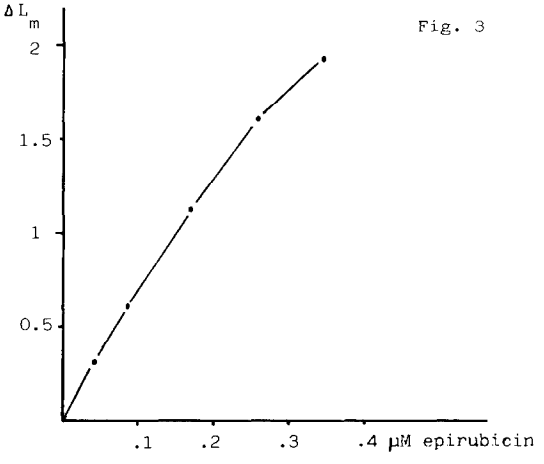


Fig. 3



SUMMARY

We find that the ligation of a circular, nicked homogeneus DNA substrate in the presence of an intercalating agent, followed by the analysis on agarose gel after removal of the drug, allows the quantification of the unwinding properties of the drug. This assay is very informative since it actually detects any alteration of the DNA structure resulting in an overall change of the superhelical structure as in the case of physical and simple chemical agents (such as temperature and salt) and therefore it detects any interaction between drugs and DNA.

After comparative analysis by this assay we found no correlation between the inibition of the relaxing activity of DNA topoisomerase II by anthracycline derivatives and their ability to alter the twist angle of DNA.

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