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DNA UNWINDING INDUCED BY NALIDIXIC ACID BINDING TO DNA

S. Tornaletti and A.M. Pedrini

Istituto di Genetica Biochimica ed Evoluzionistica del C.N.R. - Pavia, Italy.

INTRODUCTION

Quinolones are a family of antibacterial drugs whose bactericidal activity is due to inhibition of DNA synthesis. Nalidixic acid, the first member of this class of drugs synthesized (1), has been shown to inhibit the supercoiling and relaxation reaction of DNA gyrase (2). It is thought that DNA gyrase inhibition by nalidixic acid is due to interference with the breakage and reunion reaction catalyzed by this enzyme although the mechanism of action is not well understood. Shen and Pernet (3) have recently shown, by membrane filtration and equilibrium dialysis, that at the inhibitory concentrations, 4-quinolones bind preferentially to DNA than to the enzyme. Thus, these authors have suggested that the inhibitor of the DNA rejoining step of the DNA gyrase mediated strand breakage and rejoining is the DNA-drug complex. The experiment reported below confirms the binding of nalidixic acid to DNA and shows the effect of such binding on the helical structure of the molecule.

MATERIALS AND METHODS

Negatively supercoiled plasmid pAT153 DNA was mixed with increasing concentrations of nalidixic acid in a solution containing 50 mM Tris-HCl pH 7.5, 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT. After incubation for 5 minutes at room temperature, samples were relaxed to equilibrium with human DNA topoisomerase I. Nalidixic acid (NAL) and the enzyme were removed by extraction with neutralized phenol. After ethanol precipitation, pAT153 DNA was electrophoresed at room temperature on 1% agarose gel. Electrophoresis, gel staining and photograph were as described (4).

RESULTS AND DISCUSSION

Based on the assumption that interactions of DNA with either low-M_r or macromolecular ligands involve at least some degree of helical perturbation, we have studied whether NAL binds to DNA by analyzing the conformational changes it ought to produce to the helical structure of DNA. We have explored such possibility by applying the gaussian center method (5).

If a covalently closed circular DNA is fully relaxed by topoisomerase I, a family of topoisomers differing only in their linking number is generated. They can be resolved by agarose gel electrophoresis in a set of bands each differing from the adjacent one by one unit in the linking number. The relative masses of the different topoisomers conform to a gaussian

curve which median can be determined from the intensity of each band. When the relaxation of the covalently closed DNA is carried out in the same conditions, but in the presence of a ligand which alters the DNA structure, the relative amount of each topoisomer is modified. After removal of the ligand, the distribution of the species will still conform to a gaussian curve but different from the preceding one by its median. The change in the position of the median between the two experiments characterizes the change in the linking number at the time of ring closure and therefore the topological winding of the DNA induced by the ligand.

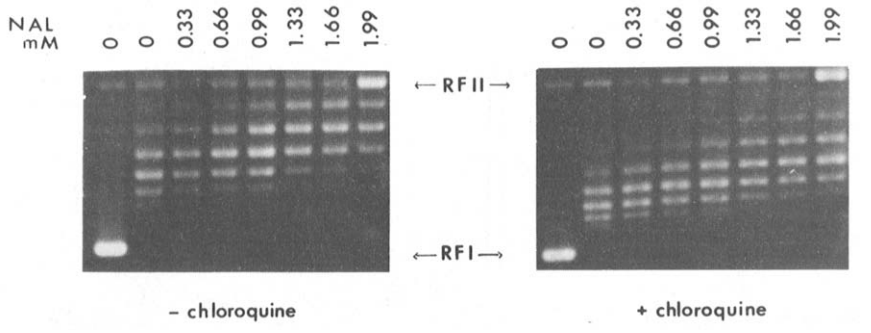

Figure 1. Agarose gel electrophoretic analysis of nalidixic acid induced unwinding of plasmid pAT153 DNA: electrophoresis buffer was: (left) 0.08 M Tris-phosphate pH 8.0, 0.008 M EDTA; (right) the same buffer supplemented with 0.95 ug/ml chloroquine. Lanes A-M contained supercoiled pAT153 DNA processed as all the other samples; lanes B-N contained no drug; the other lanes contained increasing concentrations of NAL, from 0.33 mM up to 1.99 mM.

The effect of NAL-DNA interaction studied with this method is shown in Fig. 1. As it can be seen, increasing concentrations of nalidixic acid produced a change in the electrophoretic pattern of the topoisomer bands consisting in a upward shift of the treated samples. Because of the reaction and electrophoresis conditions, the covalently closed DNA was positively supercoiled during electrophoresis, as indicated by the downward shift of DNA samples run in the presence of chloroquine compared with those without the unwinding drug. Therefore DNA samples closed in the presence of NAL were less positively supercoiled than the control sample, indicating that binding of the drug to DNA caused DNA unwinding. By utilizing a different method we have confirmed the observation reported by Shen and Pernet (3) that 4-quinolones bind to DNA and shown that drug-binding involves some degree of DNA conformational changes.

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