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CHIRAL DISCRIMINATION BETWEEN LEFT-HANDED AND RIGHT-HANDED DNA SUPERCOILS BY ACTINOMYCIN D.

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In the recent few years, the analysis of many different systems has shown that topological equilibria have a main role in the recognition processes, involving DNA. In fact, DNA can variate its twisting and writhing in different ways, interacting with proteins or relatively small molecules in isotopological transformations. The untwisting of DNA is the main consequence of interaction with intercalators such as ethidium bromide, proflavine, actinomycin D etc. (1).

Base sequence specific intercalators, such as Actinomycin D, (ACT), are of particular interest to obtain information on specific interactions involved in recognition processes.

However, despite a large research effort, the molecular structure of the complex between DNA and ACT is still debated.

Some structural aspects are provided by model systems, mainly complexes between ACT and oligonucleotides (2,3), which indicate that the phenoxazone ring intercalates in the double helix at GpC sequences, whereas the two cyclopentapeptide rings are engaged in a couple of hydrogen bonds with guanine.

More recently the structure of ACT-GpC complex, solved by crystallographic analysis, revealed some features of interaction rather different from the classic intercalation mode. On this basis Takusagawa and Berman (4) suggested that the phenoxazone moyety could be intercalated between two guanines belonging to two different sections of DNA double helix so that ACT could mediate a sort of non covalent cross-links between distant regions of DNA.

To obtain experimental evidence in solution, on this structure, we have analyzed the binding of ACT to supercoiled as well as to relaxed pBR322 DNA at very low ACT/DNA molar ratios, freezing the topological state of DNA in the complex by reaction with topoisomerase I. Fig. 1 shows the ΔL_K as a function of ACT/DNA input molar ratios (r) in the case of supercoiled pBR322 DNA.

A peculiar trend can be seen, at r=1/100, ACT stabilizes at least ten DNA supercoils, showing a dramatic difference respect to the value deriving from the generally accepted, DNA untwisting of 28°. Similar results were obtained for complexes between ACT and previously relaxed DNA. In this last case

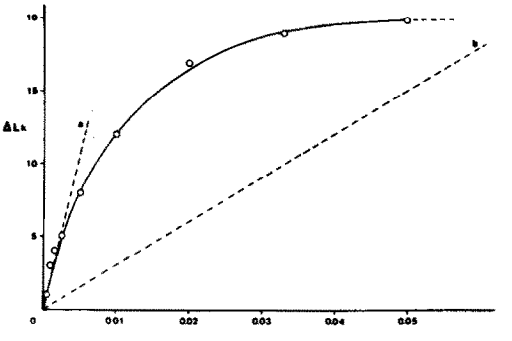
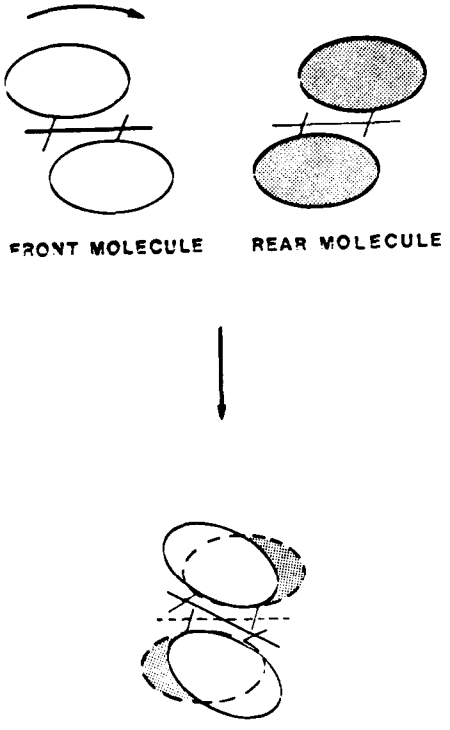


Fig. 1 Variation of the linking number (ΔL_K) of supercoiled pBR322 DNA in complexes with ACT as function of different drug/DNA molar ratios (r). ΔL_K was evaluated from agarose gel electrophoresis with different amounts of ethidium bromide. The dotted line (b) represents the expected trend if ACT intercalates with an angular unwinding of 28°.

measurements in the presence of ethidium bromide demonstrate that the supercoils induced by ACT are left-handed. On the basis of these results, we propose the model illustrated in Fig. 2, which derives the stabilization of the left-handed crossing of negative DNA supercoils from the chirality of ACT (5). The two molecules are intercalated in two different portions of the molecule of DNA. They are faced to each other on the side of cyclopeptide rings. Beginning from a position in which the phenoxazones of the two molecules lie on the same plane, one molecule must rotate in a well defined way to optimize the van der Waals interactions between the cyclopeptide rings, as can be seen in Fig. 2, where the DNA molecule is omitted for clarity.

Electron microscopy visualizations and kinetic analysis are now in progress to obtain further evidences of this model.

Fig. 2 Schematic drawing of the chiral discrimination between left-handed crossing of negative DNA supercoils and right-handed crossing of positive DNA supercoils by two ACT molecules. The terms front and rear are relative to the reader.



References

1) H. M. Sobell, T. D. Sakore, S. C. Jain, A Bauersee, K. K. Baudary, B. S. Reddy and E. D. Lozansky, Cold Spring Harb. Symp. on Quant. Biol., Vol. **XLVII** (1982) 293

2) H. M. Sobell, S. C. Jain, T. D. Sakore and C. E. Nordman, Nat. New. Biol. 231 (1971) 200

3) H. M. Sobell and S. C. Jain, J. Mol. Biol. 68 (1972)21

4) F. Takusawa and H. M. Berman "Some new aspects of actinomycin D - nucleic acid binding". Cold Spring Harb. Symp. on Quant. Biol. Vol. **XLVII** (1982) 315.

5) M. Savino, P. De Santis, L. Leoni, C. Palermo and A. Palleschi "Topology of DNA in its interactions with actinomycin D and with the histones octamer". In "Mechanisms of recognition in Biological Macromolecules. Molecular aspects and applications in Biotecnology." Ed. E. Chiancone and J. M. Chaiken. Humana Press (1987) (in press).