A NEW DNA INTERCALATING DRUG: METHOXY-9-ELLIPTICINE

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Received 16 July 1971

1. Introduction

Methoxy-9-ellipticine (MEL) is an alkaloid extracted from barks of *Ochrosia maculata* (= *O. Borbonica*) [1].

Its formula which is characterized by indol and isoquinoleine moieties is shown in fig. 1. The main feature of this tetracyclic drug is its structural resemblance with the well known DNA intercalating drugs, acridines and phenanthridines; they all share a flat structure and a similar size. Therefore, one could expect that MEL would also be able to form an intercalation complex with double stranded DNA resulting in a local distortion of the DNA molecules and their lengthening [2].

These modifications which involve changes of the hydrodynamic properties of the polymer have been observed by viscometry either on linear [3–5] or on covalently closed circular molecules [6].

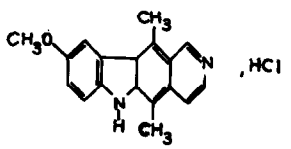

Fig. 1. Methoxy-9-ellipticine (chlorhydrate: PM = 312.5 –log $\Sigma(M)_{309\,nm}$ = 4.58).

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2. Material and methods

Calf thymus DNA [7] was sonicated in a cacodylate buffer $\mu = 0.01$ pH 6.5 (concentration 1 mg/ml) saturated with nitrogen at 4° in a Branson sonifier; repeated ultrasonication cycles were performed 30 sec, each for 10 min.

The final product was made of rods 3.5×10^5 daltons; its T_m and hyperchromicity were identical to those of non sonicated DNA. Its intrinsic viscosity was 1.8 dl/g.

The bacteriophage PM2 and its host bacteria, *Pseudomonas* BAL 31, were kindly given to us by Dr. R.T. Espejo [8]; the PM2 DNA was prepared as closed circles as described by Paoletti and Le Pecq [9]. The final dialysis was performed for both linear and circular DNA against a cacodylate buffer $\mu = 0.05$ pH 6.5.

Control drugs, ethidium bromide (EB) and hydroxystilbamidine (OHSA) were purchased from Boots pure Drugs Co. Nottingham (GB) and May-Baker Dagenham (GB).

MEL was prepared according to a method previously described [1].

Viscometric measurements were carried out in an Oswald capillary viscometer at 20° and 25° (±0.02°) in the cacodylate buffer; shear gradient was 500 sec^{-1}.

Flow times for the equilibrium solutions were about 50–55 sec while the DNA solutions had 5–100 sec longer flow times; the long afflux times made kinetic energy corrections negligible. The reduced viscosity (η_{red}) of sonicated DNA does not depend on its con-

centration [4] in the 150–750 μg/ml concentration range. η_{red} was determined at one DNA concentration for each D/P ([dye]/[nucleotide]) value.

Relative increase in length of rodlike DNA molecules, L/L_0, were obtained through the relationship $L/L_0 = ([\eta]/[\eta_0])^{1/3}$.[4].

For circular DNA, such as PM2 DNA, the magnitude of η_{red} is lowered by the effects of shear gradients [6] which however have no influence on the position of the equivalence point (D/P for which maximum relaxation is observed) or the shape of the titration curve; in this case also, η_{red} is nearly independent of the concentration of DNA.

After addition of a concentrated solution of dye (10^{-3} M), the solutions were mixed with a small flow of air through the dilution bulb of the viscometer. In our concentration conditions, the dye was almost totally bound on DNA [10, 11].

3. Results

A comparative set of data were obtained with MEL, EB and OHSA: EB is well known as a model for intercalating drugs [10, 12] while OHSA is unable to become intercalated although it can modify the polymer structure by an internal binding to it [11].

On sonicated linear DNA, MEL elicited at both $\mu = 0.05$ and $\mu = 1.0$ ionic strength, an important increase of viscosity which was related to the increase of the hydrodynamic volume of DNA (fig. 1b).

This increase was of the same order of magnitude

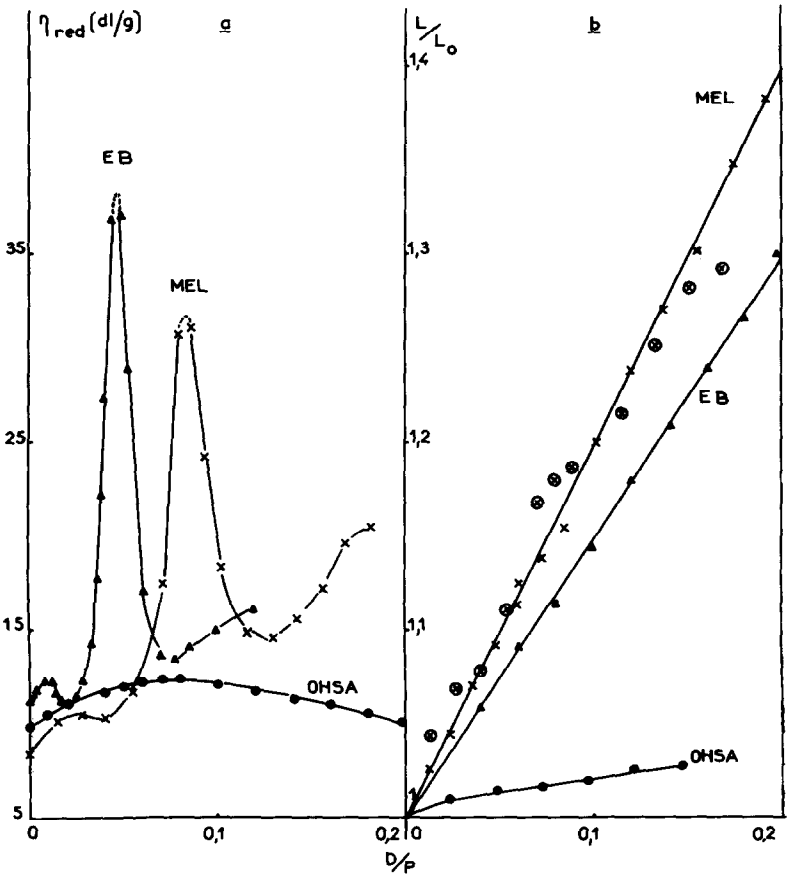

Fig. 2. Viscometry of DNA after addition of methoxy-9-ellipticine (MEL). Cacodylate pH 6.5 $\mu = 0.05$ unless otherwise stated; T: 20° (EB-OHSA) 25° (MEL). (a) Circular closed PM2 DNA: DNA initial concentrations (μg/ml): EB = 850 (▲); OHSA = 560 (●); MEL = 107 (×). (b) Sonicated calf thymus DNA: DNA initial concentration (μg/ml): 495; ionic strength (MEL) $\mu = 0.05$ (×) and $\mu = 1$ (⊕).

as the one due to EB at 20° and it verified one of the predictions of the intercalation model according to which the relationship between L/L_0 and r must be $L/L_0 = 1 + 2r$ [2], r being the number of dye molecules bound per nucleotide.

On the closed circular DNA, the addition of MEL elicited drastic changes of η_{red} which, after a slight modification at the lowest concentrations of the dye, is very much increased for the higher concentrations up to a maximum which is the transition point; finally, η_{red} decreased abruptly (fig. 1a). The interpretation of the phenomenon has already been presented by Revet et al. [6]: the amount of intercalated dye per nucleotide at the transition point is the one just necessary for obtaining a complete relaxation of the DNA circles.

Qualitatively, EB titration yielded the same sequence of events and allowed the determination of the superhelix density σ_0 of PM2 DNA; in our conditions, σ_0 was about 0.032 if one accepts the value of 12° for the unwinding angle of DNA base pairs for each intercalated EB molecule [13]. This value if open to discussion [14].

In contrast, OHSA induced only slight variations of viscosity in both systems as expected from its mechanism of binding on DNA.

4. Discussion

These experiments give an unequivocal answer: MEL does intercalate between the base pairs of DNA at low and high ionic strengths.

The changes of twisting of closed circular DNA is very indicative of an intercalation process but not totally specific for it, as established when the steroidal diamine, irehdiamine [12, 15] was studied; thereforee this test must be ascertained by a direct demonstration of the changes of the morphology of DNA which is provided by the hydrodynamic measurement on rodlike molecules.

Up to date, all intercalating drugs display active pharmacological properties (mostly antitumoral and parasicidal). Once more, MEL obeys this general rule since this intercalating alkaloid has been demonstrated to induce remissions of myeloblastic acute leukemia in man [16]; it displays antitumoral and immunosuppressive activity [17] in mice; morever, it exhibits a very active trypanocidal action in vitro (*trypanosoma cruzi*) [18].

It inhibits DNA, RNA, and protein synthesis [19] in fibroblasts and interferes with the process of maturation of the nucleolar 45 S RNA to ribosomal RNAs in L 1210 mouse lymphoma cells [20].

A complete physico chemical study on the MEL-DNA interaction has been undertaken.

Acknowledgements

This work was aided by CNRS, INSERM and the Ligue National francaise Contre le Cancer. We thank Dr. J.B. Le Pecq and Mr. J.M. Saucier for useful advice and Miss J. Bonnafoux for skilful technical assistance.

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