BBA - General Subjects 1862 (2018) 1107–1114

Contents lists available at ScienceDirect

BBA - General Subjects

journal homepage: www.elsevier.com/locate/bbagen

# A cooperative transition from the semi-flexible to the flexible regime of polymer elasticity: Mitoxantrone-induced DNA condensation

C.H.M. Lima, G.O. Almeida, M.S. Rocha*

*Laboratório de Física Biológica, Departamento de Física, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil*

ARTICLE INFO

*Keywords:*
DNA condensation
Mitoxantrone
Polymer elasticity
Force spectroscopy

ABSTRACT

We report a high cooperative transition from the semi-flexible to the flexible regime of polymer elasticity during the interaction of the DNA molecule with the chemotherapeutic drug Mitoxantrone (MTX). By using single molecule force spectroscopy, we show that the force-extension curves of the DNA-MTX complexes deviate from the typical worm-like chain behavior as the MTX concentration in the sample increases, becoming straight lines for sufficiently high drug concentrations. The behavior of the radius of gyration of the complexes as a function of the bound MTX concentration was used to quantitatively investigate the cooperativity of the condensation process. The present methodology can be promptly applied to other ligands that condense the DNA molecule upon binding, opening new possibilities in the investigation of this type of process and, more generally, in the investigation of phase transitions in polymer physics.

## 1. Introduction

The interactions of the DNA molecule with ligands such as proteins and drugs is a field important to many areas of knowledge, from the comprehension of basic intracellular processes to the application in medical sciences, especially in cancer chemotherapies. Along the past years, single molecule techniques such as optical and magnetic tweezers, as well as atomic force microscopy, have promoted a major step in the understanding of such interactions [1–6]. In fact, these techniques have opened the possibility of manipulating single DNA-ligand complexes, allowing the determination of force-extension curves (FECs) from which the mechanical properties of the complexes (and the physical chemistry of the interaction [1]) can be extracted. This type of approach is usually known as single molecule force spectroscopy (SMFS).

When using SMFS to study the interactions of the DNA molecule with drugs or proteins, the straightforward approach employed to determine the mechanical parameters of the complexes formed is to fit the experimental FECs to the worm-like chain (WLC) model [7–9]. Nevertheless, such model has its intrinsic limitations, for example, it is valid only for semi-flexible polymers. The bare DNA molecule has a peculiar chemical structure, with two strands forming a double-helix that sets important properties to the biopolymer such as a well-defined negative charge density and a bending stiffness which places DNA in the class of semi-flexible polymers [7,9-11]. When a ligand binds to the double-helix, however, it can change the local bending stiffness as well as the local charge density. Thus, if these changes make the DNA-ligand complex much stiff or soft, the WLC model may fail and the mechanical parameters obtained from the fitting of the FEC are maybe not much realist. Fortunately, a large number of DNA ligands change only slightly the DNA persistence length upon binding. Thus, the complex formed is still a semi-flexible polymer, which justifies the use of the WLC model to analyze the data. Nevertheless, this is not a general property, and there are some ligands that can change drastically the mechanical properties of the DNA molecule upon binding. Maybe the most known examples are ligands that condense the DNA molecule, such as the polyamines spermine and spermidine, and the histone proteins. These cationic ligands considerably reduce the effective persistence length of the complexes formed at low ionic strengths [12,13], facilitating the DNA condensation process. Small drug molecules that induce substantial changes on the DNA persistence length upon binding, however, are much less common.

While SMFS was largely employed in the past to study DNA condensation induced by various compounds such as polyamines and proteins [12,14-17], the DNA complexes formed with Mitoxantrone (MTX) were never characterized at single molecule level. Here we report a SMFS characterization of this interaction using optical tweezers to stretch the DNA-MTX complexes in the entropic regime (forces < 10 pN). While higher forces (tens of pN) may bring information on the structure and structural transitions of these complexes, inside cells the DNA molecule is submitted to very small net forces, on the order of a few picoNewtons [18]. Therefore, to characterize the DNA-drug

* Corresponding author.
*E-mail address:* marcios.rocha@ufv.br (M.S. Rocha).

https://doi.org/10.1016/j.bbagen.2018.01.019
Received 27 November 2017; Received in revised form 17 January 2018; Accepted 30 January 2018
Available online 02 February 2018

interaction under conditions closer to that found inside living cells, which is important from the pharmacological point of view, here we employ forces as small as possible to perform the SMFS experiments.

MTX is currently used to treat various cancers such as acute myelogenous leukemia, breast cancer, non-Hodgkin's lymphoma, and others. Its cytotoxic effects consist of disrupting the DNA synthesis and repair, being also a topoisomerase II inhibitor. Concentrations below 1 $\mu$M are sufficient to inhibit cell growth by about 50% in vitro [19], which shows the efficiency of the drug in inhibiting cell proliferation. Here we show that, for these very low concentrations the drug behaves as a classical intercalator upon binding to the double-helix. For higher concentrations, however, it strongly condenses the DNA molecule, inducing a cooperative transition from the semi-flexible to the flexible regime of polymer elasticity. To the best of our knowledge, this is the first work that fully identifies and characterizes such kind of transition on the DNA elasticity caused by a small drug molecule, although MTX-induced DNA condensation was previously reported in bulk experiments [20]. In addition, the methodology developed here opens new possibilities in the investigation of the DNA condensation process and, more generally, in the investigation of phase transitions in polymer physics.

## 2. Materials and methods

The optical tweezers used here to perform the SMFS consist of a 1064 nm ytterbium-doped fiber laser operating in the $TEM_{00}$ mode (IPG Photonics) mounted on a Nikon Ti-S inverted microscope with a 100× NA 1.4 objective.

Our samples consist of λ-DNA molecules (New England Biolabs) end-labeled with biotin in a Phosphate Buffered Saline (PBS) solution. One end of the DNA molecules is attached to a microscope coverslip surface, which is coated with streptavidin, while the other end is attached to a streptavidin-coated polystyrene bead with a diameter of 3 $\mu$m (Bangs Labs). In order to investigate the effects of the ionic strength on the DNA-MTX interaction, the measurements were carried under two different PBS buffers, whose compositions are detailed in Table 1.

MTX was purchased from Sigma-Aldrich (Cat. M6545) and used without further purification. The results reported below for the mechanical properties are averages over six FECs repeated for each drug concentration. All the error bars were calculated as the standard error of the mean from the six FECs. The experiments were carried out at room temperature (23°C). All additional details about the experimental methods and procedures can be found in ref. [21]. Fig. 1 shows the chemical structure of the MTX molecule.

## 3. Results and discussion

For simplicity, here we discuss separately the results obtained under the different ionic strengths. A comparison between the results obtained under these two conditions is then performed.

### 3.1. High ionic strength (I = 154 mM, [Na] = 150 mM)

Fig. 2 shows some typical FECs measured for the DNA-MTX complexes, obtained for MTX concentrations in the range $C_T \leqslant 7.5$ $\mu$M. For very small drug concentrations (*e.g.* 0.6 $\mu$M shown in Fig. 2), we measured a slight increase on the contour length. Nevertheless, as the drug concentration increases in the sample, the DNA molecule starts to condense. Such effect can be noted in the data of Fig. 2 from the strong decrease measured on the contour length of the DNA-MTX complexes as the drug concentration increases. This result corroborates with previous bulk studies which reported that MTX condenses the DNA molecule [20,22,23].

**Table 1**
Composition of the different PBS buffers used.

| [Na] / Ionic strength | NaCl | $Na_2HPO_4$ | $NaH_2PO_4$ |
|---|---|---|---|
| 150 mM/154 mM | 140 mM | 4.375 mM | 1.25 mM |
| 10 mM/14 mM | 0 | 4.375 mM | 1.25 mM |

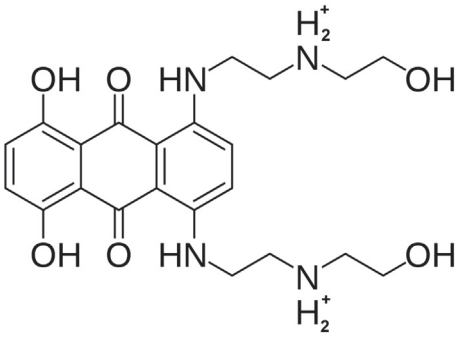


**Fig. 1.** Chemical structure of the MTX molecule.

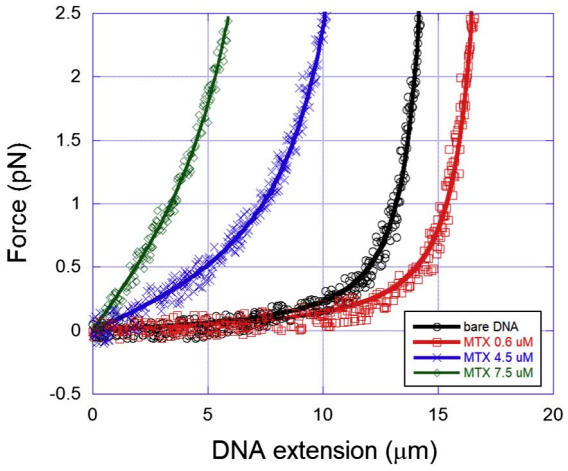


**Fig. 2.** *Points*: typical FECs measured for the DNA-MTX complexes at various drug concentrations, for the regime $C_T \leqslant 7.5$ $\mu$M. *Solid lines*: fittings to the Marko-Siggia WLC expression [7].

The WLC fittings were performed here using the Marko-Siggia expression for the entropic force [7] and are also shown in the figure as solid lines. These fittings could be performed only until a MTX concentration $C_T = 7.5$ $\mu$M, because for higher concentrations the FECs become straight lines, completely differing from the typical WLC behavior. Thus, for [Na] = 150 mM, the DNA-MTX complexes behave as semi-flexible polymers only at the concentration range $C_T \leqslant 7.5$ $\mu$M.

Fig. 3 shows more FECs, now obtained for higher MTX concentrations ($C_T > 7.5$ $\mu$M). As anticipated, the FECs are now straight lines and cannot be fitted to the WLC model. This result strongly indicates that the MTX-promoted DNA condensation process is correlated to a transition on the polymer elasticity regime. In other words, the DNA-MTX complexes become flexible polymers for $C_T > 7.5$ $\mu$M, allowing the condensation process.

In the theoretical framework, Halperin et al. [24] and Polotsky et al. [25] have predicted that the FEC of a completely condensed (totally collapsed) polymer should present three different regimes: (a) the force initially increases linearly with the extension, corresponding to the globule deformation; (b) the force then presents a plateau corresponding to the initiation of the unfolding process, *i.e.*, the change of the globular polymer into a linear one; and finally (c) the force increases again linearly with the extension when the polymer chain becomes linear. As can be seen in Fig. 3, we have not detected any force plateau in our experimental FECs: only the linear regime occurs here. This result is probably due to the fact that our DNA-MTX complexes are