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Thermodynamical study of interaction of histone H1 chromosomal protein and mitoxantrone anticancer drug

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ABSTRACT

Using ultraviolet spectroscopy technique, we have investigated the interaction of anticancer drug, mitoxantrone with calf thymus histone H1 chromosomal protein in 100 mM phosphate buffer, pH 7.0, at temperatures 300 and 310 K. UV spectroscopy results show interactions between mitoxantrone and histone H1 with a positive cooperative binding process which was confirmed by Scatchard plot. According to the obtained results, it is concluded that histone H1 can be considered as a target for mitoxantrone binding at the chromatin level.

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1. Introduction

The fundamental repeat unit of eukaryotic chromatin is the nucleosome [1–3]. The nucleosome is composed of a core particle consisting of DNA and a histone octamer, and linker DNA that connects the core particles and is associated with one H1 linker histone [4]. The linker histones are involved in both the nucleosome structure and the formation of a higher order chromatin structure. Two major types of organization have been described for the nucleosomes chain. On one hand, the solenoid as presented by Thoma et al. [5] can be defined as a one-start helix in which consecutive nucleosomes follow each other, and the linker DNA between the nucleosomes is oriented toward the center of the solenoid fiber [6]. The main characteristic of the second model is the Zig–Zag arrangement of nucleosomes, i.e. the linker DNA connects back and forth two rows of nucleosomes. This results overall in a two-start, double-helical structure [7,8].

So far there is no experimental data which would allow defining the exact position of linker histones either in the solenoid or in the Zig–Zag model. However, experimental evidences suggest that the linker histone has a major impact on the compactness and DNA topology upon formation of higher order chromatin structures [9,10].

Recently, considerable attention has been focused on the interaction of anticancer anthracycline antibiotics with histone H1, suggesting that H1 can be considered as a macromolecular receptor for those drugs [11–14].

Mitoxantrone (Novatrone™) is a synthetic anticancer agent that was resulted from a concerted effort in the late 1970s to develop a less cardiotoxic form of the anthracycline antibiotics (daunomycin and doxorubicin). It is used primarily for the treatment of advanced breast cancer, non-small cell lung cancer, melanoma, lymphoma, and leukemia and more recently for prostate cancer [15–17]. The structures of the four anthracyclines (doxorubicin, daunorubicin, epirubicin and idarubicin) approved for clinical use, chemical structure of mitoxantrone and model of mitoxantrone dimer have been shown in Fig. 1. Both absorption and emission spectra of mitoxantrone in aqueous solution depend on the drug concentration. Mitoxantrone in concentrations below 1.0 μM exists almost only in monomeric form while in higher concentrations, dimerization and trimerization occurs [18,19]. This classic intercalating agent accumulates in nucleus where it functions as a topoisomerase II inhibitor [20]. However, in addition to the mentioned mechanism, its oxidation products have been shown to form a covalent complex with DNA in vitro [21,22]. Detection of drug–DNA adducts in mitoxantrone-treated promyelocytic HL-60 (which contain myeloperoxidase) [23] and other tumor cells [24] has subsequently provided direct support for the notion that mitoxantrone–DNA adducts may contribute to the mechanism of action of mitoxantrone. In vitro transcription analysis revealed that adduct levels at 5-methyl-CG sites were enhanced 2–3-fold compared with non-methylated CG sequences. Methylation of cytosine at single CpG sites on oligonucleotides also showed that adduct levels were enhanced 3-fold by the presence of the methylated cytosine [25].

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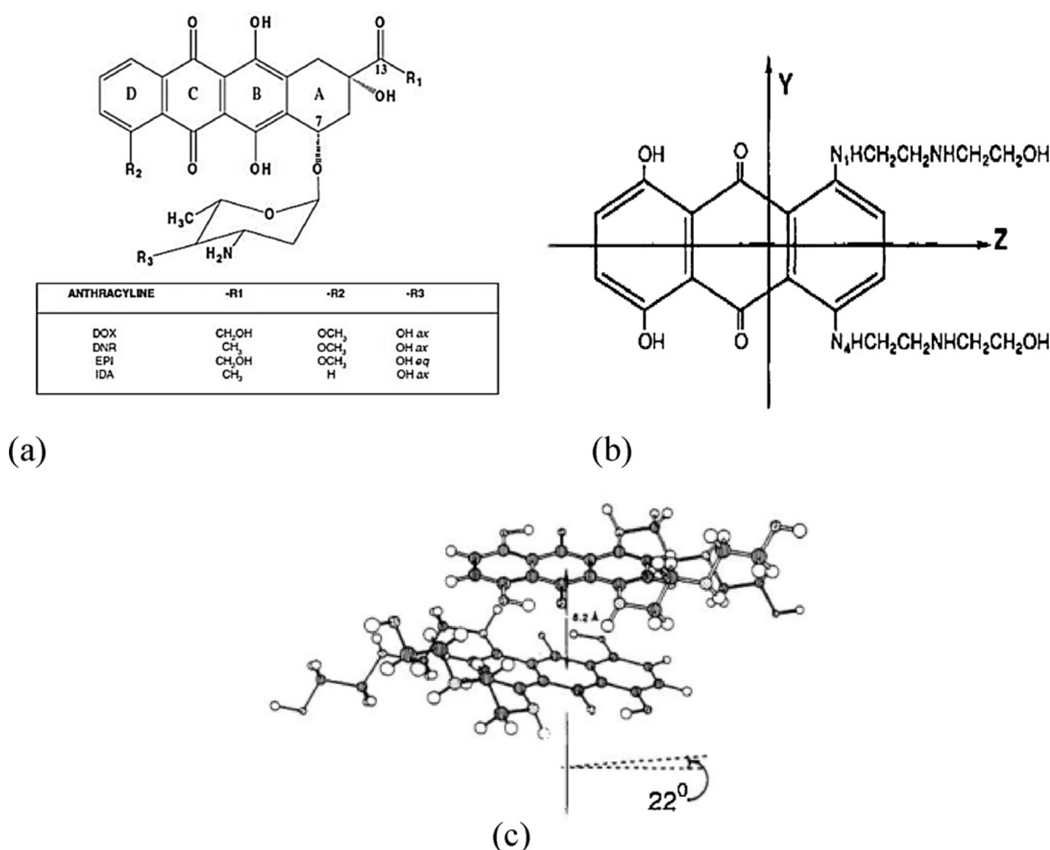

ANTHRACYLINE	-R1	-R2	-R3
DOX	CH_2OH	OCH_3	OH *ax*
DNR	CH_3	OCH_3	OH *ax*
EPI	CH_2OH	OCH_3	OH *eq*
IDA	CH_3	H	OH *ax*

Fig. 1. Structures of the four anthracyclines approved for clinical use. DOX, doxorubicin; DNR, daunorubicin; EPI, epirubicin; IDA, idarubicin. *ax*, axial; *eq*, equatorial (a), chemical structure of mitoxantrone (b) and model of mitoxantrone dimer (c) [18,19].

An interesting result from the H1 knockout studies is the link between DNA methylation and histone H1 [26]. Removal of H1 in plants or mammals can cause both inhibition and enhancement of DNA methylation at specific genomic locations [26,27]. While effects of DNA methylation on H1 binding to chromatin have been extensively studied in vitro, the available data on this issue are controversial [28] which is possibly due to carrying out the experiments using total H1 from different sources with variation in the relative amount of H1 variants. Affinity of an individual variant for methylated DNA might be masked by the presence of other subtypes. In vitro transcription from DNA methylated templates has been shown to be indeed more efficient in the presence of isolated H1c in comparison with other mouse H1 variants [29]. Additionally, poly-ADP-ribosylated H1e exhibits an inhibitory effect on DNA methylation [30].

In the present study considering the link between DNA methylation and histone H1 and 2–3-fold enhancement of mitoxantrone–DNA adduct formation at 5-methyl-CG sites in comparison with non-methylated CG sequences, we have examined mitoxantrone binding to the purified calf thymus histone H1 in solution using ultraviolet spectroscopy technique.

2. Experimental

2.1. Materials

Mitoxantrone was purchased as a solution with concentration of 2.0 mg ml^{-1} from Iranian Red Crescent and used without further purification. Stock solution of mitoxantrone was prepared in sterile double distilled water, stored at 4 °C in the dark and was diluted to desired concentrations with 100 mM phosphate buffer, pH 7.0. Histone H1 was purchased from Sigma Chemical Company and further purified by membrane ultrafiltration. The purity of the protein was assayed by polyacrylamide gel electrophoresis. The purified histone H1 was divided into several portions, stored at −20 °C and used within six months. All measurements were carried out in 100 mM phosphate buffer, pH 7.0.

2.2. Interaction of mitoxantrone with histone H1

Appropriate concentrations of mitoxantrone (0.0–1.0 μM) were incubated with two different concentrations of histone H1 (0.02 and 0.03 μM) for 90 min at 300 and 310 K in the dark.

2.3. Absorption spectroscopy

All measurements were carried out in 100 mM phosphate buffer, pH 7.0. Histone H1 (0.02 and 0.03 μM) and various concentrations of mitoxantrone were mixed at 300 and 310 K and incubated in the dark for 90 min. Temperature 300 K was chosen because of experimental temperature in the laboratory, and 310 K was chosen because of healthy human body's temperature. Also, in order to use van't Hoff equation, the differences between T_1 and T_2 should be relatively small [31]. Absorbance changes were followed using Rayleigh UV-2100 Spectrophotometer at wavelength of 276 nm.

3. Results and discussion

Binding isotherms for mitoxantrone on interaction with histone H1 at 300 and 310 K (Fig. 2a), along with molar entropies and molar Gibbs free energies of interaction between histone H1 and mitoxantrone as a function of number of ligands bound per protein

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