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Recognition of chromatin by the plant alkaloid, ellipticine as a dual binder

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ABSTRACT

Recognition of core histone components of chromatin along with chromosomal DNA by a class of small molecule modulators is worth examining to evaluate their intracellular mode of action. A plant alkaloid ellipticine (ELP) which is a putative anticancer agent has so far been reported to function via DNA intercalation, association with topoisomerase II and binding to telomere region. However, its effect upon the potential intracellular target, chromatin is hitherto unreported. Here we have characterized the biomolecular recognition between ELP and different hierarchical levels of chromatin. The significant result is that in addition to DNA, it binds to core histone(s) and can be categorized as a 'dual binder'. As a sequel to binding with histone(s) and core octamer, it alters post-translational histone acetylation marks. We have further demonstrated that it has the potential to modulate gene expression thereby regulating several key biological processes such as nuclear organization, transcription, translation and histone modifications.

1. Introduction

Chromatin is the physiological template of eukaryotic genome. DNA-binding anticancer drugs actually target the chromatin template in cells. With the advent of the field, we have made an attempt to re-classify these small molecules as 'single binder' which interacts with DNA only or 'dual binder' which binds to both DNA and core histones [1–5]. Functionally the dual binders could affect the cellular chromatin organization, epigenetic landscape and consequent gene expression programs [1,5].

Ellipticine, ELP (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) (Fig. 1A), a naturally occurring plant alkaloid is a putative anticancer agent. It is isolated from plants belonging to *Apocynaceae and Loganiaceae* families [6,7]. Research in the past few years have shown several targets and mechanisms of its biological action. Of these, interactions with DNA are well established, and include intercalation, topoisomerase II inhibition [8], bio-oxidation and adduct formation with DNA [9]. ELP inhibits some of the key cellular players such as c-Kit and AKT kinase [9] and induces activation of tumor suppressor p53 [9]. Our group has shown that ELP interacts with the human telomeric DNA sequence, $d(TTAGGG)_4$ and inhibits telomerase activity in MDAMB-231 breast cancer cell line extracts [10]. Here, we have studied the interaction of ELP with hierarchical levels of chromatin viz. long chromatin, chromatosome, nucleosome, chromosomal DNA and histone octamer. Our results show that ELP exhibits dual binding mode of interaction at the chromatin level [1–4,11], binding to both histones and DNA. At the structural level, ELP disrupts the integrity of histone octamer and causes DNA release from chromatosome. An enhancement in the hydrodynamic size of long chromatin ensues the association process.

Chromatin structure and function are influenced by post-translational modifications (PTMs) of histone(s) which impact gene expression [12]. These epigenetic modifications are reversible and serve as potential targets for drugs [13]. Interestingly, ELP was found to inhibit some of the important post-translational histone acetylation marks implicated in transcription activation. Histone modifications are key players in regulating chromatin states and

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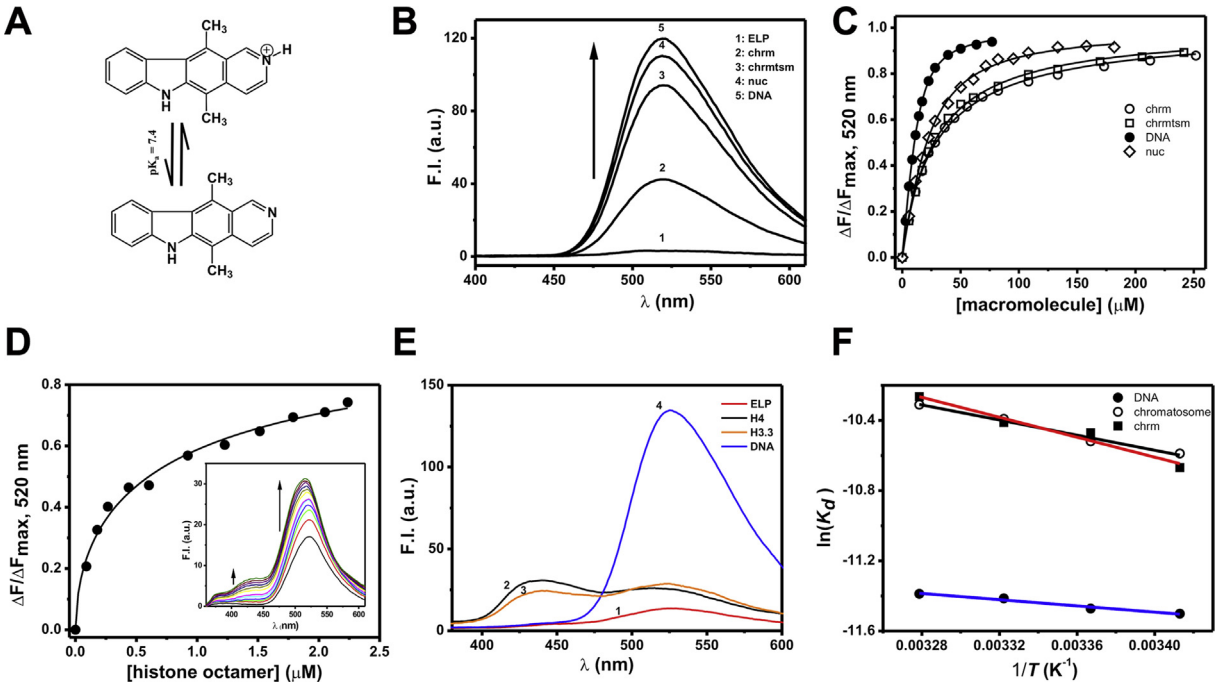

Fig. 1. **(A)** Chemical structure of ELP. **(B)** Emission spectra of 3 μM ELP in absence (curve 1) and in presence of 50 μM chromatosome (curve 2), long chromatin (curve 3), nucleosome (curve 4) and chromosomal DNA (curve 5) in 10 mM potassium phosphate, pH 6.8 containing 15 mM KCl at 25 °C. **(C)** Binding isotherms obtained by non-linear curve fitting analyses (○, long chromatin; □, chromatosome; ●, chromosomal DNA; ◊, nucleosome). **(D)** Binding isotherm for the interaction of ELP with core histone octamer in 10 mM potassium phosphate, pH 6.8 containing 2 M KCl at 25 °C. Inset shows the emission spectra of 2 μM ELP in presence of increasing concentrations of core octamer. **(E)** Emission spectra of 2 μM ELP in absence (curve 1) and in presence of 6 μM histone H4 (curve 2), H3.3 (curve 3) and chromosomal DNA (curve 4) in 10 mM Tris–HCl, pH 8.0 containing 150 mM NaCl at 25 °C. **(F)** van't Hoff plot to determine ΔH and ΔS for association of ELP with long chromatin (■), chromatosome (○) and chromosomal DNA (●). The linear best fits of the experimental data points are represented by the solid lines.

dynamics as well as in gene expression [14]. We have observed that the genes involved in several biological processes are differentially expressed upon ELP treatment. Summing up, histone-binding ability of ELP suggests an additional pathway of action of this biologically important molecule.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma Chemical Company, USA, unless otherwise specified.

2.2. Methods

The technical details and instrument specifications are provided under Supplementary Material.

2.2.1. Preparation of ELP stock

Stock solution of ELP was prepared in freshly distilled DMSO and the concentration was determined spectrophotometrically, using the molar extinction coefficient, $\varepsilon_{295} = 60{,}000\ M^{-1}\ cm^{-1}$ [10].

2.2.2. Preparation of chromatin samples

Chromatin, chromatosome, nucleosome and chromosomal DNA were isolated from rat liver following standard protocol [15] and mononucleotide concentrations of the samples were determined spectrophotometrically using the molar extinction coefficient (ε_{260}) of 6600 $M^{-1}\ cm^{-1}$.

Core octamer was prepared using the method described by Peterson and Hansen [16] and concentration of core octamer was determined using the molar extinction coefficient (ε_{230}) of 507,233 $M^{-1}cm^{-1}$.

2.2.3. Steady state fluorescence measurements

The fluorescence emission spectra of ELP (3 μM) were monitored in presence of chromatin components where macromolecule/ligand ratio was gradually increased. The binding isotherms were obtained from a plot of $\Delta F/\Delta F_{max}$ (at 520 nm, $\lambda_{ex} = 320$ nm) as a function of input macromolecule concentration. The apparent dissociation constant K_d, at a given temperature was determined using non-linear curve fitting analysis [17]. The thermodynamic parameters were determined from van't Hoff analysis [18].

2.2.4. Circular dichroism study

The circular dichroism spectra of chromatin components (50 μM of long chromatin, chromatosome, nucleosome, chromosomal DNA each and 3 μM core histone octamer) were recorded in absence and presence of ELP at 25 °C. The molar ellipticity of macromolecules was plotted against the wavelength. Convex constraint analysis (CCA) was performed to extract the basis spectra and their associated coefficients [19]. As controls, CD spectra of macromolecules were monitored in presence of DMSO (corresponding to the total ELP volume added).

2.2.5. Dynamic light scattering (DLS)

In order to investigate the effect of ELP on the hydrodynamic size, chromatin (120 μM) was treated with ELP in ligand: DNA base ratio of 0, 0.05, 0.10, 0.15 and 0.20 and the hydrodynamic diameters (Z_{av}) were monitored. Similarly, core histone octamer (3 μM) was treated with increasing concentrations of ELP (3, 6 and 9 μM) and the particle size was measured.