An anticancer drug to probe non-specific protein–DNA interactions†

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A visible fluorescence switch of an eminent anti-carcinogen, ellipticine has been used to probe non-specific protein–DNA interaction. The unique pattern of protein–DNA complexation is depicted for the first time through field emission scanning electron microscopy (FE-SEM) images and spectroscopic techniques.

Molecular switches are sharing the spotlight from the recent past due to their potential applications in bio-sensors, memory devices, logic gates and energy storage.1–6 Mainly two mechanisms involved in fluorescence switching, namely, photo-induced electron transfer (PET)7,8 and fluorescence resonance energy transfer (FRET),9,10 require covalent tagging, which appears as a tough challenge for chemists. Moreover, 'PET' or 'FRET' from weakly bound or freely roaming fluorophores are likely to be diffusion controlled, which may not serve the purpose properly.11 If a single fluorophore could execute the fluorescence switching, it could facilitate sample labelling and eliminate the complications of integrating two dyes into desired locations. To date, such a single colour switching probe is scarcely available. Recent reports about the dual emission of ellipticine in few selective solvents trigger the idea of monitoring important biological processes using the fluorescence switching of ellipticine. Ellipticine (Scheme 1) is a naturally occurring plant alkaloid, and has been known as an anti-carcinogen since the 1960s.12,13 Ellipticine and its derivatives effectively intercalate in DNA and inhibit the activity of DNA topoisomerase-II (topo-II), which ultimately restricts the process of DNA replication and hence transcription of RNA.12,14–16

Protein–DNA interaction plays a central role in all aspects of genetic activity, like translation, transcription, repair and packaging. The present work reports a simple but useful visualization of the non-specific interaction between serum proteins17–21 (bovine (BSA) or human serum albumin (HSA)) and calf thymus DNA (CT-DNA) with the help of an ellipticine fluorescence switch. We demonstrate that two different biomacromolecules can shuffle the emission position so effectively that ellipticine can even be promoted as a molecular sensor for these biopolymers. Moreover, we report direct morphological patterns of the protein–DNA self-assembled aggregates through field emission scanning electron microscopy (FE-SEM), which offers valuable physical insight about the probable ternary complexation comprising of protein, ellipticine and DNA.

Emission features of ellipticine in PBS and with increasing concentrations of serum protein are depicted in (Fig. 1). Serum protein (BSA) bound ellipticine mainly emits blue fluorescence at 440 nm, and is attributed to the neutral ellipticine molecules residing at domain IIIA (Fig. 1c) (from molecular docking study, detailed in ESI†). With the gradual addition of DNA, the intensity of blue emission (440 nm) decreases sharply, and a new peak appears in the green region at 512 nm (Fig. 2). A strong intensity decrease at 440 nm along with a freshly appearing green emission at 512 nm infers DNA mediated withdrawal of neutral ellipticine molecules from the ellipticine–BSA complex. The increasing ellipticity of BSA in the circular dichroism (CD) (Note 3, ESI†) spectra with raising DNA concentration suggests that the secondary structure of BSA is not perturbed, rather CT-DNA stabilizes the secondary structure of the protein. This substantiates well with the previous reports about structural stabilization of HSA by CT-DNA.17

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Scheme 1 Different forms of ellipticine in solution.
Inset represents a sample deconvolution. Figure 2 shows the emission intensity of ellipticine with increasing BSA concentrations where $1 \rightarrow 13$ represent $0, 0.25, 0.50, 1, 3, 6, 10, 20, 30, 50, 70, 90$ and $110 \mu M$ BSA. Inset shows the colour switch by BSA addition. Deconvoluted emission intensity of ellipticine with variable BSA concentrations, where $1 \rightarrow 13$ carries the same meaning of (a). Figure 1 shows the magnified view of interactions along with surrounding amino acids.

**Fig. 1** (a) Fluorescence emission intensity of ellipticine with increasing BSA concentrations where $1 \rightarrow 13$ represent $0, 0.25, 0.50, 1, 3, 6, 10, 20, 30, 50, 70, 90$ and $110 \mu M$ BSA. Inset shows the colour switch by BSA addition. (b) Deconvoluted emission intensity of ellipticine with variable BSA concentrations, where $1 \rightarrow 13$ carries the same meaning of (a). (c) Neutral ellipticine bound to domain IIA, where inset shows the magnified view of interactions along with surrounding amino acids.

**Fig. 2** (a) Fluorescence emission spectra of BSA (110 \mu M) bound ellipticine with increasing DNA concentration ($1 \rightarrow 9$ represents [DNA] = $0, 1, 6, 10, 30, 50, 70, 90$ and $110 \mu M$). Inset shows colour transition due to ternary complexation. (b) Deconvoluted emission intensity of ellipticine–BSA with variable DNA concentration, where $1 \rightarrow 9$ carries the same meaning of (a). Inset represents a sample deconvolution.

Henceforth, DNA assisted perturbation of the tertiary structure of BSA may be the possible reason for the displacement of drug molecules from the protein binding cavity. It is also noticeable from Fig. 2 that the intensity at 440 nm does not vanish completely even at the highest concentration of CT-DNA, indicating a small fraction of neutral ellipticine molecules are still left inside the binding pocket of BSA. The DNA driven dissociation constant ($K_D = (2.70 \pm 0.2) \times 10^5 \text{ M}^{-1}$) calculated from the intensity decrement at 440 nm is very close to that of the ellipticine–BSA binding constant (Note 2c, ESI†).

Although in BSA the main emitting species is neutral ellipticine, the deconvoluted emission spectra (Fig. 1b) clearly indicates the presence of protonated ellipticine in BSA. To get a clear insight about the interaction feature of BSA-bound protonated ellipticine with DNA, we have deconvoluted all the spectra (Fig. 2), which shows that BSA bound protonated ellipticine peak appearing at $\sim 475$ nm is gradually red shifted along with progressive decrease of emission intensity. Moreover, a clear iso-emissive point is observed at $507$ nm in the presence of DNA. The red shift along with the iso-emissive point is indicative of the existence of a ternary complex comprising DNA, protein and protonated ellipticine. Using the emission intensities at the respective peak maximum, the binding constant for ternary complexation is calculated to be $(2.20 \pm 0.18) \times 10^5 \text{ M}^{-1}$, which infers the process is highly energy favoured ($\Delta G^c = -5.90 \text{ kcal mol}^{-1}$). It is intriguing to observe that HSA and salmon sperm DNA (Note 5, ESI†) also offer similar trends in emission and changes in binding free energies like the BSA–DNA system, inferring the wide applicability of the present work for wild DNA and globular proteins.

Previously, IR studies revealed that the interaction between CT-DNA and HSA (a homologous protein of BSA) takes place via polypeptide $\text{C} = \text{O}$, $\text{C} – \text{N}$ and $\text{N} – \text{H}$ groups. Moreover, IR studies showed that two major binding sites were located at G–C bases and the backbone phosphate group of DNA. Considering the fact that protonated ellipticine will be attracted by the phosphate backbone of DNA, we believe that an electrostatically formed tertiary complex might be favoured in our case. It is also noticeable that at higher concentration of DNA ($\sim 110 \mu M$), protonated ellipticine emits at $\sim 512$ nm (Fig. 2) which is $\sim 13$ nm blue shifted from DNA-intercalated protonated ellipticine emission at $\sim 525$ nm (Note 4, ESI†). This suggests ellipticine does not intercalate, rather it is involved in ternary complex formation with BSA and DNA. The time-resolved anisotropy results and lifetime measurements are employed to explore the detail of ternary complexation (Note 5, ESI†). Note that we have found $\sim 22.5$ percent increase of hydrodynamic radius (Note 5c, ESI†) from BSA–ellipticine ($r = 35.6 \text{ Å}$) to BSA–ellipticine–DNA system ($r = 43.3 \text{ Å}$). Interestingly, the change in hydrodynamic radius of BSA bound DNA ($\sim 8 \text{ Å}$) is closely matched with the width of DNA base pair ($\sim 9 \text{ Å}$), which infers that BSA predominantly interacts with the major groove region of DNA. This corroborates well with the proposed concept of ternary complexation as phosphates in DNA attract serum proteins to a great extent to bind to the G–C rich major groove of CT-DNA. We can rule out the possibility of ternary complex formation with neutral ellipticine as the 440 nm peak does not exhibit any red shift (Fig. 2). Moreover, the anisotropy...
described by the diffusion limited aggregation model (DLA),22 these radially branched dendritic architectures are best characterized in which DNA nucleation at the tip of the ‘fern leaf’ structure has been retrieved when equimolar DNA and protein is solubilised in PBS. A closer look shows each arm of the flowery structure is extended in a typical fashion on the 2D plane and resembles the ‘fern leaf’ structure. As protein is solubilised in PBS, evaporation of water gives a rapid aggregation, where hydrophobic interaction drives protein molecules together and forms this kind of ‘fern leaf’ structure. A more distinctive self-assembled structure has been retrieved when equimolar DNA has been added to serum protein. An additional nucleation centre at the tip of ‘fern leaf’ is found in the presence of DNA, which extends randomly and results in a dendritic distribution. The radial branched dendritic architecture is an outcome of a diffusion limited process. The results presented in this paper have close resemblance with the recently reported morphology of silk proteins of Bombyx mori and Wako sericin.23,24

In summary, the present work illustrates the interaction scenario of wild DNA and serum protein probed by the fluorescence switch of an anti cancer drug, ellipticine. In protein (serum albumin) it dramatically shifts the equilibrium from protonated (~525 nm in water) to neutral (~440 nm), which reflects through a ~85 nm blue shift in emission. In DNA, only the protonated form of ellipticine intercalates due to the strong electrostatic interaction between the drug and the negatively charged phosphate backbone of DNA, reflected by tremendous hike in intensity at 525 nm (Note 4, ESI†). The very different fluorescence feature of ellipticine with serum albumin and DNA, offers a notion about monitoring the protein–DNA interaction with the help of the fluorescence switch of this drug from green (in water) to blue (in presence of protein) to sea green (in protein–DNA). We found DNA does not disturb but stabilizes the secondary structure of protein through complexation comprising protein and DNA. SEM images depict the formation of radially branched dendritic architecture for protein–DNA interaction, driven by the above mentioned self assembled structure. This kind of fluorescence switch of a biologically important drug molecule provides new strategy to monitor the protein–DNA interaction. Moreover, in the near future this approach can be effectively used to probe different kind of bio-macromolecular interactions in vivo and in vitro.

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