Concentration-dependent mode of binding of drug oxatomide with DNA: multi-spectroscopic, voltammetric and metadynamics simulation analysis

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ABSTRACT

The interaction between antihistaminic drug oxatomide (OXT) and calf thymus DNA (CT-DNA) has been investigated in a physiological buffer (pH 7.4) using UV-Vis, fluorescence, 1H NMR and circular dichroism spectral techniques coupled with viscosity measurements, KI quenching, voltammetry and in silico molecular modeling studies. OXT binds with CT-DNA in a concentration-dependent manner. At a lower [Drug]/[CT-DNA] molar ratio (0.6-0.1), OXT intercalates into the base pairs of CT-DNA, while at a higher [Drug]/[CT-DNA] molar ratio (13-6), the drug binds in the minor grooves of CT-DNA. The binding constants for the interaction are found to be in the order of 10^3 M^-1, and the groove binding mode of interaction exhibits a slightly higher binding constant than that of intercalative mode. Thermodynamic analysis of binding constants at three different temperatures suggests that both these modes of binding are mainly driven by hydrophobic interactions (ΔHθ > 0 and ΔSθ > 0). Voltammetric investigations indicate that the electro-reduction of OXT is an adsorption controlled process and shifts in reduction peak potentials reiterate the concentration-dependent mode of binding of the drug with CT-DNA. The free energy landscape obtained at the all-atom level, using metadynamics simulation studies, revealed two major binding forces: partial intercalation and minor groove binding, which corroborate well with the experimental results.

1. Introduction

Oxatomide belongs to the diphenylmethylpiperazine family, is a first-generation, well-tolerated antihistaminic drug and is claimed to prevent histamine release from mast cells and basophils. The mechanism of action of OXT is reported to be probably similar to that of some other lipophilic H1-histamine receptor antagonists. OXT, a primary lipophilic compound, shares the ability to inhibit mast cell histamine release with an acidic polar sodium cromoglycate (Marone et al., 1999). Truneh et al. have reported the concentration-dependent effect of OXT on histamine secretion from mast cells and found that at high concentrations, OXT induces histamine release. In contrast, at low concentrations, it prevents induced exocytosis A (Truneh et al., 1982). Richards et al. have shown that over 90% of OXT was bound to plasma protein in human blood when studied in the concentration range of 0.1 to 1 μg/mL. Further, the effect of OXT on histamine release from human lung cells is also concentration-dependent (Richards et al., 1984). Domae et al. have demonstrated that OXT promotes human eosinophil apoptosis, DNA fragmentation and suppresses IL-5-induced eosinophil survival in a concentration-dependent manner and may contribute to the resolution of tissue eosinophilia in allergic inflammation (Domae et al., 2003). Hence, it is presumed that investigation of the mode of binding of OXT with DNA would shed some light on the mechanism of its overall activity. Furthermore, unraveling such mechanistic information would help us in rational drug design and discovery. Therefore, the main objective of the present endeavor is to investigate the mechanism of interaction of OXT with CT-DNA using various spectral (UV-Vis, fluorescence, 1H NMR and circular dichroism), electrochemical and molecular modeling techniques. Interestingly OXT was found to bind with CT-DNA in a concentration-dependent mode.

2. Experimental section

2.1. Materials

The chemicals used in the present study are commercially available analytical grade. Ethidium bromide (EB) was purchased from Sigma Aldrich, India, and was used as received. The calf thymus DNA (CT-DNA) was obtained from Genie, whose purity was checked by monitoring the absorbance ratio at 260/280 nm. Oxatomide drug was obtained, as a gift sample, from a locally available pharmaceutical company, and its purity was checked using 1H NMR (Supporting Information Figure S1) and m.p. (Theoretical: 153–155 °C; Found: 153 °C). The structure of oxatomide is shown below.
The stock solution of the drug was prepared using DMSO. The stock solutions of CT-DNA and EB were prepared using Millipore water. Freshly prepared solutions were used for all the spectral measurements. All the solutions used in the experiments were adjusted with HEBES buffer solution (pH 7.4).

2.2. Methods

2.2.1. UV-Vis spectroscopic method
UV–Vis spectroscopy is the commonly used method to investigate the interaction of small molecules with DNA. Generally, when small molecules bind with DNA, changes in absorbance and/or wavelength of absorption maximum are observed. The magnitude of such changes is correlated with the strength of the interaction between them. The UV–Vis spectra were recorded using a JASCO (V630) double beam spectrophotometer equipped with 1 cm quartz cuvettes at room temperature. The experiment was carried out by the fixed concentration of OXT (100 µM) with varying CT-DNA concentration (0–120 µM). Likewise, another experiment was done using a fixed amount of CT-DNA (22 µM) titrated with varying concentrations of OXT (0–110 µM) in both cases, the total volume of the solutions was made to 2 mL using HEBES buffer (pH 7.4).

2.2.2. Viscosity measurement
To clarify the binding mode of OXT, based on the lengthening of CT-DNA helix, kept fixed concentration (different for both ratio) of CT-DNA (33 µM) with an increasing amount of OXT (0–22 µM) using Ostwald viscometer at 25 °C. The flow time was measured by using a digital stopwatch, and the time was taken from the average of the individual three measurements. Total volume 20 mL was made by using HEBES buffer (pH 7.4). The resulted data were plotted as ($\eta / \eta_0$)$^{1/3}$ versus [OXT]/[CT-DNA] ratio, $\eta$ and $\eta_0$ were the relative viscosity in the absence and presence of OXT.

2.2.3. Fluorescence studies
A variety of molecular interactions such as ground state complex formation, excited state reactions and energy transfer can decrease small molecules’ fluorescence intensity when they interact with DNA. To establish the mechanism of interaction of OXT with CT-DNA, the fluorescence intensity of the drug in the absence and presence of increasing amounts of CT-DNA was measured in HEPES buffer (pH 7.4). Fluorescence emission spectra were recorded using JASCO FP 8500 in a range of (300–500 nm) upon excitation of 233 nm with an increasing amount of (0–102 µM) at different temperatures (298 K, 308 K, 318 K). Initially, the fluorescence spectra of OXT (25 µM) were recorded by adding an increasing concentration of CT-DNA (0–102 µM).

The mode of drug–DNA interaction can be decoded through competitive displacement assay with well-known DNA binding dyes whose binding modes have already been established. In the case of EB displacement assay, an experiment mixture containing EB (5 µM) and CT-DNA (30 µM) was titrated with increasing amounts of OXT (0–110 µM) in the range of 250–800 nm with an excitation wavelength of 250 nm. A similar set of Hoechst (5 µM)-CT-DNA (30 µM) system was also titrated with increasing amounts of OXT (0–110 µM) upon excitation of 350 nm. Potassium iodide quenching experiments were carried out by adding KI (3.2 mM) to the OXT alone, and OXT-CT-DNA and excitation wavelength was 233 nm.

2.2.4. Circular dichroism study
Exogenous substances’ influence on the conformation of DNA molecules can well be observed using circular dichroism (CD) spectral studies. Therefore, CD spectra of CT-DNA were recorded in the absence and presence of different concentrations of OXT in the HEPES buffer (pH 7.4). CD spectra of CT-DNA (5 µM) and OXT-CT-DNA systems with increasing concentration of OXT (0–250 µM) were recorded using a JASCO (J810) spectrometer in the range of 220–310 nm at room temperature. Before initiating each experiment, the compartment of the spectrometer was deoxygenated with nitrogen gas and kept in the nitrogen atmosphere during the experiments. Background spectrum of buffer solution (HEBES buffer, pH 7.4) was recorded and subtracted from CT-DNA spectra. The CD spectrum of free OXT showed no signals in the wavelength region. We cannot observe any apparent change in the CD spectra of CT-DNA at very low [Drug]/[CT-DNA] ratios.

2.2.5. Voltammetric studies
In recent years, much attention has been paid to the voltammetric studies on interaction of small molecules with DNA. It would enable us to evaluate and predict the mechanism of such bindings. Voltammetric experiments were carried out, using a CHI-643B, Austin, TX electrochemical workstation, using a three-electrode system viz. glassy carbon electrode (working electrode), Pt wire (counter electrode) and Ag/AgCl (reference electrode). Cyclic voltammetry was performed in 0.05 M Tris-HCl buffer solution (pH 7.3) containing 50 µM OXT at different scan rates (50–250 mV). Voltammetric experiments were performed in the 0.05 M Tris-HCl buffer solution (pH 7.3) containing 50 µM OXT by adding varying CT-DNA concentrations (0–430 µM).
2.2.6. ¹H NMR spectral studies
¹H NMR spectra were attained on Bruker NMR spectrometer (400 MHz) in DMSO-d₆ as a solvent, and the chemical shifts are expressed in ppm. ¹H NMR spectra of free OXT (46 μM) and OXT with CT-DNA (1 mM) were recorded and compared.

2.2.7. Molecular modeling
The mode of binding of OXT with DNA was imitated using the AutoDock 4.2 software. For this purpose, flexible OXT and rigid DNA (PDB id: 1BNA) were used, and DNA was enclosed in a grid having 0.642 Å spacing, and all other parameters were assigned the default values given by the software. The energetically more favourable docked pose was taken to the meta dynamic simulations (MD) using the Desmond Maestro platform. The complex was first soaked in an orthorhombic water box containing 11,659 water molecules, and 22 sodium ions were used to neutralize the system, followed by energy minimization and simulations. The molecular dynamic simulation was carried out up to 100 ns at 300 K and 1 bar pressure. The two collective variables used for metadynamic simulation are: (i) distance between the center of mass (COM) of four bases of DNA and the COM of ligand, and (ii) the angle φ between the fixed vector AB and vector AC, the distance between the COM of four bases of DNA and that of the ligand as shown in Figure 1 (Mukherjee et al., 2008). Before MD simulations, the system was minimized and pre-equilibrated using the default relaxation routine implemented in Desmond. Subsequently, the system was simulated with the default parameters, and 1000 frames of trajectories were recorded at 200 ps intervals. The free energy landscape was viewed with a metadynamics simulation analysis module. The root mean squared deviation (RMSD) of the DNA backbone was calculated against the initial structure for the OXT–DNA complex.

3. Results and discussion

3.1. UV–Vis spectral studies
UV–Vis spectra of OXT (100 μM) in the absence and presence of increasing concentrations of CT-DNA (0–120 μM) were recorded in HEPES buffer (pH 7.4) and are shown in Figure 2. Free OXT showed absorption bands at 233 and 279 nm due to π-π* transitions. On adding increasing amounts of CT-DNA to the drug solution, initially up to 37 μM concentration of CT-DNA ([Drug]/[CT-DNA] = 2.7), the absorbance of the band at 279 nm decreased very slightly (hypochromic effect), suggesting groove binding mode of interaction (Blue curves). Whereas, beyond this concentration of CT-DNA (at [Drug]/[CT-DNA] < 2.7), the absorbance of the band increased (hyperchromic effect) with a blue shift (~4 nm) in its position of absorption (Green curves), suggesting intercalative binding mode of interaction between the drug and CT-DNA (Sirajuddin et al., 2013; Tan et al., 2007). These observations indicated a concentration-dependent mode of binding between them.

3.2. Viscosity measurements
This concentration-dependent mode of binding of OXT with CT-DNA has been confirmed using viscosity measurements. It is considered a robust test to identify the method of binding of small molecules with DNA in solution. The relative viscosities of CT-DNA in the presence of different concentrations of OXT have been measured in HEPES buffer (pH 7.4). The concentrations of the drug have been selected in such a way to have [Drug]/[CT-DNA] ratios between 0.1–0.6 (Figure 3) and...
Effect of increasing amounts of CT-DNA (2–20 μM) on relative viscosity of CT-DNA (33 μM) at [Drug]/[CT-DNA] = 0.6–0.1.

Figure 3. Effect of increasing amounts of OXT (2–20 μM) on relative viscosity of CT-DNA (2 μM) at [Drug]/[CT-DNA] = 6–10.

Figure 4. Effect of increasing amounts of OXT (10–20 μM) on relative viscosity of CT-DNA (2 μM) at [Drug]/[CT-DNA] = 6–10.

As seen from Figure 3, when [Drug]/[CT-DNA] ratio is very low (0.1 – 0.6), the viscosity increased significantly, suggesting intercalative mode of binding between them. When small molecules intercalate between the base pairs of DNA, they cause increase in separation of base pairs and overall lengthening of DNA and, consequently, lead to an appreciable increase in viscosity. Conversely, when the [Drug]/[CT-DNA] ratio is relatively high (6 – 10), the change in viscosity of CT-DNA is very minimal (Figure 4), which indicated that at this concentration range OXT binds to CT-DNA through groove binding mode of interaction (Ma et al., 2013; Ponkarpagam et al., 2020). These observations confirmed the concentration-dependent mode of binding of OXT with CT-DNA.

3.3. Potassium iodide quenching studies

To further confirm the concentration-dependent mode of binding of OXT with CT-DNA, its fluorescence quenching in the absence and presence of CT-DNA has also been investigated using KI as a quencher. It is well established that I⁻ ions are well-known quenchers of fluorescence of small molecules in an aqueous solution. However, the approach of I⁻ ions towards DNA molecule is restricted due to repulsion between I⁻ ions and negatively charged phosphate groups of the backbone of DNA. Therefore, small molecules that are intercalated into the base pairs of DNA are well protected from I⁻ ions, while that are present in grooves of DNA are less protected so that I⁻ ions can effectively quench their fluorescence even in the presence of DNA (Zhang et al., 2011). The Stern–Volmer plots of quenching of fluorescence of OXT by I⁻ ions in the absence and presence of CT-DNA are depicted in Supporting Information Figures S2–S4. The Stern–Volmer quenching constants, Ksv, were calculated using the following equation (Qais et al., 2017).

\[
\frac{F_0}{F} = 1 + Ksv[Q]
\]

where \(F_0\) and \(F\) are the fluorescence intensities of the drug in the absence and presence of the quencher, respectively. When the [Drug]/[CT-DNA] ratio is low (0.1 – 0.6), the \(Ksv\) values for Drug/KI and Drug/KI/CT-DNA were found to be 110 and 158 M⁻¹, respectively. In this [Drug]/[CT-DNA] ratio, UV–Vis spectral and viscosity measurement studies suggested intercalational mode of binding of OXT with CT-DNA. So, we would expect an appreciable decrease in \(Ksv\) value, but an unexpected increase was observed. Such an unexpected rise in \(Ksv\) value can be explained by taking into account of the role of ionic strength. There might be an increase in ionic strength in the medium upon the addition of KI, which results in the release of OXT from Drug/CT-DNA adduct. And I⁻ ions quenched the fluorescence of the released OXT molecule (Rehman et al., 2014). Additionally, even in groove binding mode, DNA can protect small molecules to some extent but certainly to a lesser extent compared to intercalation (Sarwar et al., 2015). However, when the [Drug]/[CT-DNA] ratio is relatively high (5 – 8), the \(Ksv\) values for Drug/KI and Drug/KI/CT-DNA were found to be 110 and 100 M⁻¹, respectively. Such a meager relative reduction in \(Ksv\) value (9%) indicated that OXT might interact with CT-DNA through groove binding mode. This conclusion corroborates well with the results of electronic spectral studies and viscosity measurements, as described above.

3.4. Competitive binding assay with EB

As UV–Vis spectral studies indicated a concentration-dependent mode of binding of OXT with CT-DNA, intending to substantiate the indication, the competitive binding assay has been carried out using EB, a well-known intercalator. EB is one of the most sensitive fluorescence probes, which intercalates within DNA base pairs, and the resulting complex fluoresces strongly. Any small molecule that binds to DNA through intercalative mode will displace EB from the base pairs of DNA, and thus, quenches the fluorescence of EB/ DNA complex to a greater extent (Sarwar et al., 2015) As seen from Figure 5, EB/CT-DNA complex emits strongly at 602 nm in HEPES buffer solution (pH 7.4). Upon addition of increasing amounts of OXT into the complex, there is a steady and continuous reduction in fluorescence intensity,
and beyond a given concentration of \textit{OXT}, the fluorescence intensity falls sharply to completion (Supporting Information Figure S5). Initially, up to 8.5 \textmu{M} of the drug ([Drug]/[CT-DNA]= 2.9), the fluorescence of EB/CT-DNA complex gets quenched with a concomitant increase in emission at 311 nm, which nearly corresponds to the emission of free \textit{OXT} (Blue lines). This may be because the added \textit{OXT} might bind to CT-DNA loosely so that it showed an increase in emission at 311 nm and a relatively weaker quenching in fluorescence of EB/CT-DNA complex. This could be explained based on the observations made by Wilhelm et al. during their study on the interaction of drug daunomycin with DNA through molecular dynamics and free energy studies. They have proposed a multistep drug intercalation mechanism for the interaction, wherein the initial step is the minor groove binding step (Wilhelm et al., 2012). Therefore, under such circumstances, the loosely bound \textit{OXT} molecule can exhibit its fluorescence at 311 nm and the intensity of which increased with an increase in \textit{OXT} concentration up to 8.5 \textmu{M}, beyond which there is no such fluorescence at 311 nm when the molecule intercalated completely into the base pairs of DNA (Green lines).

### 3.5. Fluorescence spectral studies

As seen from Supporting Information Figure S6, added CT-DNA quenched the drug’s fluorescence in a concentration-dependent manner. This observation is in line with those made in UV–Vis spectral, viscosity measurement and iodide ion quenching studies. As the mode of binding of \textit{OXT} with CT-DNA was found to be a concentration-dependent process, fluorescence quenching studies have also been carried out at two different [Drug]/[CT-DNA] ratios, i.e., at 0.6–0.1 (Supporting Information Figures S7–S9) and 13–6 (Supporting Information Figures S10–S12) ratios in HEPES buffer (pH 7.4) at three different temperatures. In both cases, the addition of increasing amounts of CT-DNA to the drug solution quenched the fluorescence of the drug linearly. From the fluorescence data, \(K_{SV}\) values were calculated using Equation (1) (Supporting Information Figures S13 and S14), and the data obtained are collected in Table 1. As seen from the table, at a low [Drug]/[DNA] ratio, the \(K_{SV}\) values were found to increase with an increase in temperature, indicating that the drug quenched the fluorescence of \textit{OXT} via a static quenching mechanism (Zhang et al., 2012). The \(K_{SV}\) values, at a high [Drug]/[DNA] ratio, showed a non-obvious variation with an increase in temperature. Wang et al. have reported that such a variation in \(K_{SV}\) values might be due to the static quenching process (Wang et al., 2014). Thus, at both low and high [Drug]/[CT-DNA] ratios, CT-DNA quenched the fluorescence of the drug via a static quenching mechanism.

### 3.6. Thermodynamic parameters and nature of binding forces

Using the fluorescence titration data, the binding constants for the interaction of \textit{OXT} with CT-DNA have been calculated, at low and high [Drug]/[CT-DNA] ratios, by the following double logarithmic equation (Ma et al., 2013; Ponkarpagam et al., 2020).

\[
\log (F_0 - F/F) = \log K_0 + n \log [Q] \quad (2)
\]

where \(F_0\) and \(F\) are the fluorescence intensities of \textit{OXT} in the absence and presence of CT-DNA, respectively. \([Q]\) is the concentration of CT-DNA and \(K_0\) is the binding constant. The binding constant values were calculated from the linear plots of \(\log (F_0/F/F)\) versus \(\log [Q]\) (Supporting Information Figures S15 and S16). The \(K_0\) values, thus, obtained at different temperatures are also collected in Table 1. The magnitude of \(K_0\) values indicated that the drug binds strongly with CT-DNA.

To delineate the nature of the binding force which holds the drug with CT-DNA, the thermodynamic parameters were calculated from the temperature-dependent \(K_0\) values using van’t Hoff (Equation (3)) and Gibbs–Helmholtz (Equation (4)) equations (Zhang et al., 2013)

\[
\log K_0 = -\frac{1}{T} \Delta H^0 + \frac{\Delta S^0}{2.303R} \quad (3)
\]

\[
\Delta G^0 = \Delta H^0 - T \Delta S^0 \quad (4)
\]

The enthalpy change (\(\Delta H^0\)) and entropy change (\(\Delta S^0\)) were computed from the slope and intercept, respectively, of the linear plot of \(\log K_0\) versus 1/\(T\) (Supporting Information Figures S17 and S18). The thermodynamic parameters thus obtained are also given in Table 1. It is evident from the data that at both low and high [Drug]/[CT-DNA] ratios, the values of free energy change (\(\Delta G^0\)) are negative, indicating spontaneous interaction between the drug and CT-DNA. Further, positive values of enthalpy and entropy changes (\(\Delta H^0 > 0\) and \(\Delta S^0 > 0\)) suggested that hydrophobic
interactions dominate the binding of OXT with CT-DNA (Gharagozlou & Boghaei, 2008). In general, drug molecules bind with DNA through four nonbonding interactions, viz. H-bonds, van der Walls forces, electrostatic interactions and hydrophobic interactions. In both intercalative and groove modes, drug molecules bind with DNA mainly via hydrophobic interactions (Ma et al., 2012). Thus, in the present study, although the mode of binding of OXT with CT-DNA varies in a concentration-dependent manner, the primary binding forces acting between them are hydrophobic interactions.

3.7. Circular dichroism spectral studies

As seen from Figure 6, free CT-DNA exhibited a negative peak at 246 nm due to right-handed helicity and a positive peak at 272 nm corresponding to base stacking (Shahabadi et al., 2010; Yang et al., 2017). Upon addition of 5 μM ([Drug]/[CT-DNA] = 0.08) and 10 μM ([Drug]/[CT-DNA] = 0.16) OXT to CT-DNA, the basic shape of the CD spectrum remained unaltered. Interaction of the drug with CT-DNA slightly increased the intensity of 246 nm peak and decreased the intensity of 272 nm peak with an isoelliptic point at 258 nm. However, addition of 200 μM ([Drug]/[CT-DNA] = 3.3) and 250 μM ([Drug]/[CT-DNA] = 4.2) drug to CT-DNA decreased the intensity of positive peak alone to a larger extent. The observed decrease in intensity of positive peak with an increase in [Drug]/[CT-DNA] ratio may be due to a transition from extended DNA double helix to the more compact \( \psi \) structure. Further, drug molecules’ hydrophobic interaction with DNA would weaken base stacking and consequently decrease the intensity of positive CD peak (Shahabadi et al., 2010).

3.8. \(^1\text{H} \) NMR spectral studies

The mode of binding of small molecules to the DNA can easily be ascertained using \(^1\text{H} \) NMR technique. The partial \(^1\text{H} \) NMR spectra of OXT in the absence and presence of CT-DNA are depicted in Figure 7. In general, in groove binding, there are slight or no palpable shifts in the proton magnetic resonances upon binding with DNA. As seen from the figure, in free OXT, the signals due to aromatic protons appeared in the region of 6.85 to 7.48 ppm. Upon addition of CT-DNA to OXT, in the ratio [OXT]/[CT-DNA] = 0.046, the signal at \( \delta_H \) 7.417 ppm experienced an upfield shift and appeared at \( \delta_H \) 7.376 ppm (with a \( \Delta \delta_H \) of 0.041 ppm). In addition to that, the signals at \( \delta_H \) 7.103 and 6.963 ppm merged to produce a new signal at \( \delta_H \) 7.014 ppm. The observed up-field chemical shift and line-broadened signal of the aromatic protons of OXT upon interaction with CT-DNA suggested partial intercalation of the aromatic ring of the molecule into the base pairs of the CT-DNA at this low [OXT]/[CT-DNA] ratio. Such changes in the \(^1\text{H} \) NMR signals have also suggested a slow rate of exchange between various DNA binding sites and the unbound state (Zhong et al., 2004). However, we cannot record \(^1\text{H} \) NMR spectra at higher [OXT]/[CT-DNA] ratios due to the solubility issue.

3.9. Voltammetric studies

As seen from Figure 8, at a scan rate of 50 mV, during the reverse scan, a single cathodic peak at \( -0.59 \) V was observed for the electro-reduction of amide moiety of OXT (Ponkarpagam et al., 2020). No oxidation peak was observed.
during scanning in the positive direction, which suggested that the electro-reduction is irreversible. This is further confirmed by the negative shift of the peak potential with an increase in scan rate. The reduction peak current \( i_{pc} \) of OXT varies linearly with scan rate from 50 to 250 mV (Supporting Information Figure S19; \( r = 0.998 \)), indicating that the electro-reduction is controlled by the adsorption step (Laviron et al., 1980).

Differential Pulse Voltammograms of 50 \( \mu \)M of OXT in Tris-HCl buffer (pH 7.3) in the absence and presence of different CT-DNA concentrations were recorded and depicted in Figure 9. The surface of the electrode was re-polished before every recording. As seen from the voltammograms, the oxidation peak current of OXT decreased with an increase in CT-DNA concentration with a shift in peak potential, indicating that binding of OXT with CT-DNA resulted in the formation of an electrochemically nonactive complex. Further, with the addition of CT-DNA, the peak potential was found to shift to more negative potential up to 180 \( \mu \)M concentration of CT-DNA, i.e., up to [Drug]/[CT-DNA] ratio of 0.27 (Curves a – e). Such a peak potential shift indicated that the OXT drug interacted with CT-DNA by groove binding mode (Shahabadi et al., 2010). Upon addition of further amounts of CT-DNA to OXT solution ([Drug]/[CT-DNA] = 0.21 – 0.11), the peak potential has started to shift to more positive potential (Curves f – j), indicating that the drug interacts with CT-DNA through intercalative mode of binding (Carter et al., 1989; Kalanur et al., 2009). This observation is in line with the concentration-dependent mode of binding of OXT with CT-DNA as established by UV–Vis, fluorescence and viscosity measurement studies.

### 3.10. Molecular modeling

Figure 10 showed the most probable binding mode of OXT molecule with DNA, which indicated that the drug enters into the minor groove of DNA. Further, the drug/DNA complex was stabilized by H-bonding and hydrophobic interactions, consistent with the experimental results. Since OXT possesses a flexible structure that allows torsional rotation of the molecule to fit into the helical curvature of the groove of DNA. The relative minimum binding energy of the docked OXT/DNA complex was found to be \(-1.93\) kcal/mol. Negative binding energy indicated a higher binding potential of OXT with DNA, resulting in a more stable complex formation between the drug and DNA. In the present study, the preceding discussions indicated that the drug binds to DNA in a concentration-dependent mode. At higher [Drug]/[DNA] ratios, the suggested mode of binding of the drug with DNA is groove binding. The experimental free energy change for this binding was found to be \(-5.56\) kcal/mol at 298 K. The observed difference in free energies between experimental and docking simulation methods may be due to the solvent-free environment in the in silico studies. A competitive displacement docking also was carried out using EB, and the docked structure is shown in Supporting Information Figure S20. The results showed that the drug binds to minor grooves, and EB intercalates into the DNA base pairs. The docking simulation results showed a mutual complement
between spectroscopic analysis and molecular docking studies (Husain et al., 2015; Ponkarpagam et al., 2020; Ur-Rehman et al., 2015).

The metadynamics simulation of the system revealed the formation of a stable complex with thermodynamic equilibrium throughout the simulation period. The RMSD variance of all the atoms with simulation time with respect to the initial frame, as given in Figure 11, represented the stability of the complex formation. The number of hydrogen bonds was found to have fluctuated between 1 and 7 for the OXT–DNA complex (Figure 12). The free energy contour obtained from the metadynamics simulations of OXT–DNA system revealed the existence of three different conformational states of the complex with respect to the chosen simulation variables. As shown in free energy contour in Figure 13, an exchange between unbound state and minor groove/intercalation bound states seems to be possible from their least free energy values ranging from $-4.26$ to $-8.66 \text{kcal/mol}$. It is also interesting to note that at one form of the complex, a phenyl ring of OXT was intercalated between the residues. At the same time, the remaining part of the drug molecule was positioned at the minor groove region of DNA (Figure 14(a)). This inference of partial intercalation was also very well supported by $^1$H NMR results where there are significant changes only in chemical shifts of the aromatic protons. Figure 14(b,c) indicated the minor groove binding of OXT by interacting with G16 and A17 residues possessing pi–pi stacking interactions and hydrogen bonds mediated by water molecules, solvent and ions. By observing the variations in the angle $\phi$, ranging from 38 to 102 degrees and also from reported observations (Sasikala and Mukherjee, 2013), the ligand must be at the minor groove region at two of three of the local minima and with a partially intercalated state at another local minimum of its energy profile.

### 3.11. Comparison with similar reports

A review of the literature revealed that only very few molecules bind to DNA in a concentration-dependent manner. (Wang et al., 2016) have investigated the nanomechanics of fluorescent dyes on DNA by magnetic tweezers and found...
that YOYO-1, DAPI and DRAQ5 dyes bind to DNA in a concentration-dependent manner. The same research group, during their study on the characterization of the binding mechanism of fluorescent dyes to DNA by magnetic tweezers, has reiterated that the dye DRAQ5 binds to DNA through groove binding mode below a threshold concentration of 0.5 μM beyond which it intercalates to DNA (Wang et al., 2017). Krautbauer et al. (2002) have successfully employed single-molecule force spectroscopy to discriminate the mode of binding of small molecules with DNA. They found that berenil binds to DNA in the minor grooves at low concentrations, while at higher concentrations, it intercalates into the base pairs of DNA. Kreft et al. (2018) have observed a concentration-dependent bimodal binding behaviour of drug mitoxantrone with DNA based on their studies using magnetic tweezers. The drug binds to DNA as an intercalator and groove binder simultaneously at low concentrations (up to 3 μM) and a mere intercalator at high concentrations. In the present study we guessed that OXT intercalates to the base pairs of CT-DNA at lower [Drug]/[CT-DNA] ratios, while binds to the minor grooves at higher ratios, ruling out any bimodal mode of binding.

Figure 14. (a) Partial intercalation by phenyl ring of OXT along with hydrogen bonds, pi–pi stacking interaction. (b) Minor groove binding of OXT with water-mediated hydrogen bonds and (c) The complex structure showing minor groove binding of OXT and the interactions between DNA and OXT.
4. Conclusion

The binding of OXT with CT-DNA has been investigated by various spectral techniques coupled with viscosity measurements, iodide ion quenching, voltammetry and molecular docking studies. Results of UV–Vis spectral studies suggested a concentration-dependent mode of binding of OXT with CT-DNA, which is well supported by viscosity measurements and voltammetric studies. OXT molecule was able to intercalate into the base pairs of CT-DNA at relatively lower [Drug]/(CT-DNA) molar ratios while binds in the minor groove at higher [Drug]/(CT-DNA) molar ratios. The dynamic and energetic properties of these two modes of bindings are confirmed by using metadynamics simulation studies. In both these circumstances, the drug binds strongly with CT-DNA, as evidenced by the magnitude of binding constants (10³–10⁵ M⁻¹). The calculated thermodynamic parameters (positive entropy and enthalpy changes) revealed that hydrophobic interactions played a predominant role in binding the drug with CT-DNA. A review of OXT’s pharmacodynamic properties and therapeutic efficacy, published by Richard et al., revealed that in vitro models of histamine release from human mast cells showed that at lower doses OXT inhibits histamine release and at higher doses stimulates histamine release (Richards et al., 1984). The results obtained in the present study, i.e., the concentration-dependent mode of binding of OXT with CT-DNA may shed some light on further studies on the drug’s concentration-dependent histamine release from human lung cells and may facilitate the design of similar antihistimic drugs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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