DNA binding and photo-induced DNA cleavage activity of Elsinochrome A in visible light

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Abstract. The interaction of Elsinochrome A (EA) with calf thymus DNA (CT-DNA) has been investigated by UV-vis spectra and fluorescence spectra. The results show that EA can bind with CT-DNA and binding sites are destroyed after irradiation by visible light, which indicates that EA is a promising candidate for photodynamic therapy. In addition, the binding mechanism is studied using fluorescence quenching test and ethidium bromide (EB) replace assay experiments. The results suggest that EA and CT-DNA are binding with a strong force and the major binding mode of EA with DNA could be the electrostatic binding.

Keywords: Elsinochrome A, fluorescence quenching, photodamage, thermodynamics parameter

1. Introduction

Elsinochrome A (Fig. 1), a perylence-quinonoid pigment which isolated from *Elsinoe* spp. [17], has been investigated as a new efficient photosensitizer against cancer and the human immunodeficiency virus (HIV-II) over recent years [4–6,12]. It was reported by C. Li that the triplet state lifetime of EA was much longer than hypocrellins, which made EA possess higher the \( ^1 \text{O}_2 \) quantum yield. It is indicated that EA possesses a higher photosensitization activity than hypocrellins, and it may be a better candidate for photodynamic uses [1,10]. DNA is a very important targeting in photodynamic therapy and the research on the interaction mechanism of EA and DNA such as intercalation, surface binding and so on, which would be helpful in understanding the therapeutic mechanism, and in designing and synthesizing new ramifications which have stronger binding ability to DNA and possess higher phototherapeutic effect on DNA. So far, to our knowledge, the investigations on the interaction mechanism between EA and DNA are lacking.

Herein, we study the binding behavior between EA and CT-DNA by the UV-vis spectra and the results show that EA is intercalated into CT-DNA. The fluorescence spectroscopy results indicate that the fluorescence quenching EA by CT-DNA is a static quenching procedure. Furthermore, intensive CT-DNA photodamage by EA using ethidium bromide (EB) as a fluorescence probe is observed which imply that EA is a promising candidate for photodynamic therapy. In addition, the thermodynamics parameters of EA–CT-DNA suggest that the major binding mode between EA and CT-DNA would be the electrostatic binding.

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2. Materials and methods

2.1. Materials

Elsinochrome A (EA) was extracted from *Elsinoe* spp. and recrystallized twice from chloroform and petroleum ether before use. Ethidium bromide (EB) and calf thymus (CT-DNA) were purchased from Sigma. Dimethyl sulfoxide (DMSO) and other chemical reagents were analytical grade.

2.2. Preparation and characterization

EA was dissolved by DMSO and diluted in deionized water. The CT-DNA solution was prepared by dispersing an appropriate amount of CT-DNA in buffer solution (pH = 6.8) with stirring 12 h below 4°C, and the concentration was calculated according to the absorbance by using $\varepsilon_{260\text{ nm}} = 6600 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ and an average molecular of 338 for a nucleotide.

EB was an efficient probe widely used in biochemical research for visualizing nucleic acids. It was used to assay the interaction of EA with DNA in this work. The solution of EB was prepared by dissolving EB in deionized water, the concentration was determined by UV-vis spectrophotometer based on $\varepsilon = 5450 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$, and the solution was stored in dark.

The UV-vis spectra was conducted by using Varian Cary 5000 spectrophotometer and the fluorescence spectra was measured by PE LS-50B fluorescence spectrophotometer.

3. Results and discussion

3.1. UV-vis spectral measurements

To study the binding property of EA with CT-DNA, we measure the UV-vis and fluorescence absorption of EA with different amount of CT-DNA in PBS solution (pH = 6.8). There are three absorption peaks at 461, 525 and 567 nm of EA, respectively. When increasing the concentration of CT-DNA, the absorption peaks decrease obviously and have a little red-shift (Fig. 2). It is known that the interaction between an intercalator and DNA bases commonly result in hypochromism and a red shift of the transition of the intercalated chromophore [11]. The results indicate that interaction between EA and CT-DNA might be by an intercalation mechanism.
Fig. 2. The changes of the UV-vis absorption spectra of EA with increasing concentrations of CT-DNA (0, 0.04, 0.08, 0.12, 0.16 and 0.20 mM, respectively).

3.2. Fluorescence quenching mechanism

Generally, the fluorescence quenching mechanisms can be described as dynamic quenching, static quenching and the combined static and dynamic quenching. In the case of the combined static and dynamic quenching, the Stern–Volmer plot is characterized by a non-linear behavior with an upward curvature, but dynamic quenching and static quenching were both linear [20]. The polynomial equation is as follows [9]:

$$\frac{F_0}{F} = 1 + (K_D + K_S)[Q] + K_D K_S [Q]^2,$$

where $K_D$ and $K_S$ are the dynamic and static quenching constants, respectively. $F_0$ is the fluorescence intensity in the absence of the quenching reagent, $F$ is the one after adding the quenching reagent, $[Q]$ is the concentration of quenching reagent.

When CT-DNA is added into the EA, the shape and fluorescence peak have no change, however, the intensity have decreased regularly. The results are shown in Fig. 3.

As shown in Fig. 4, $F_0/F = 0.99686 + 0.03095[Q]$ ($R = 0.9996$), $F_0/F$ is linearly proportional to the concentration of CT-DNA, it is suggest that the interaction between EA and CT-DNA are not the combined quenching because the Stern–Volmer plot is a linear. So, followed the classical Stern–Volmer relationship [2,3]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{sv} [Q],$$

where $K_q$ is the quenching rate constant of biomacromolecule, $\tau_0$ is the average lifetime of the fluorescence molecule in the absence of quenching reagent and its value is about $10^{-8}$ s [8], $K_{sv}$ is the Stern–Volmer dynamic quenching constant and $K_{sv} = K_q \tau_0$ [15].

From Stern–Volmer curves, the quenching rate constant $K_q = 3.09 \times 10^{12}$, which is more than the maximum collision quenching rate constants $2.0 \times 10^{10}$ l (mol · s)$^{-1}$, which reveals the process is static quenching rather than the dynamic collision quenching [18].
Fig. 3. The changes of the fluorescence emission spectra of EA by addition of CT-DNA (0, 0.04, 0.08, 0.12, 0.16 and 0.20 mM, respectively, $\lambda_{ex} = 480$ nm).

Fig. 4. The Stern–Volmer curves of fluorescence quenching of EA by addition of CT-DNA.

3.3. *Ethidium bromide* (EB) replacing assay

EB is a well-known duplex DNA intercalator, which can cause fluorescence intensity enhanced upon intercalating in CT-DNA. A simple assay method for DNA cleavage is applied based on ca. 20-fold enhancement of the fluorescence intensity exhibited by EB upon intercalation into DNA [14]. Any process in which the potential EB binding site is destroyed results in a decrease in fluorescence intensity [19].

When EA is added into the solution of CT-DNA-EB, the position and the shape of the fluorescence peak have no change, while the emission intensity increases obviously in Fig. 5, which shows that the interaction occurs between the EA and the CT-DNA-EB system. The static interaction can be attributed to the surface binding association of EA to negatively charged double-strand CT-DNA driven by electrostatic stabilization, considering the positively charged structure of EA. Surface binding of EA to CT-DNA can prevent its fluorescence emission from being quenched by polar solvent molecules and result in the fluorescence increase. Moreover, the surface binding of EA may change the spacer structure of the CT-DNA helix, and consequently expel EB from CT-DNA [21].
3.4. Photo-induced damage DNA

Photodynamic activity mechanisms of EA are measured. EA is used as a phototherapeutic target and EB assay is adopted to follow the photodamage. The fluorescence intensity decreased following the change of time (in Fig. 5). The percentage of binding site remaining at a given time \( t \) is calculated from the following formula [19]:

\[
\text{Binding site remaining (\%)} = 100 \times \left[1 - \frac{(I_0 - I_t)}{(I_0 - I_{buf})}\right],
\]

where \( I_0, I_t, I_{buf} \) denote the integrated fluorescence intensities before irradiation, after \( t \) min of irradiation and of DNA-free buffer, respectively.

It is concluded that 46.55% binding sites are destroyed during a 30-min irradiation with light of the CT-DNA-EB solution, which imply that EA is a promising candidate for photodynamic therapy (Fig. 7).
3.5. Thermodynamics parameter and binding mode

The fluorescence spectra of EA with addition of CT-DNA are also measured at different temperature of 298 and 313 K, respectively (Figs 8 and 9). The binding constants of EA with CT-DNA are determined at 298 and 313 K. There might be several forces between the molecular and biomacromolecular such as hydrogen bond, van der Waals, electrostatic force, hydrophobic force and so on. According to the thermodynamics parameter $\Delta H$ and $\Delta S$, we can judge the main force style between EA and CT-DNA [15].

The dissociation constant at different temperature $K_D$ is obtained from the equation of the static quenching [7]:

$$(F_0 - F)^{-1} = F_0^{-1} + K_DF_0^{-1}[Q]^{-1}.$$
Fig. 9. The emission spectra CT-DNA-EB system (20 mM EB, 10 mM CT-DNA) in the presence of EA at 313 K (0, 0.67, 1.34, 2.68, 5.36, 10.72, 21.44, 42.88 µM, respectively, λ<sub>ex</sub> = 480 nm).

Figure 10 shows the Lineweave–Burk curves at different temperature of 298 and 313 K. The binding constant $K_A$ is obtained by $1/K_D$, and $K_A1 = 1.04 \times 10^5$ (mol/l)<sup>-1</sup>, $R = 0.9974$ (at 298 K); $K_A2 = 0.81 \times 10^5$ (mol/l)<sup>-1</sup>, $R = 0.9994$ (313 K). The relationship of $\Delta H$, $\Delta G$ and $\Delta S$ as follows:

\[
\ln K_2/K_1 = \Delta H(1/T_1 - 1/T_2)/R,
\]

\[
\Delta G = -RT \ln K,
\]

\[
\Delta G = \Delta H - T\Delta S,
\]

when $\Delta H < 0$ or $\Delta H \approx 0$, $\Delta S > 0$, the mainly acting force is electrostatic force; when $\Delta H < 0$, $\Delta S < 0$, the mainly acting force is van der Waals or hydrogen bond and when $\Delta H > 0$, $\Delta S > 0$, the mainly force is hydrophobic [13,16]. The results in Table 1 display that the interaction process is spontaneous. Form $\Delta H < 0$, $\Delta S > 0$, the mainly acting force between EA and CT-DNA is electrostatic force.
Table 1

<table>
<thead>
<tr>
<th>ΔH (kJ/mol)</th>
<th>ΔG (kJ/mol)</th>
<th>ΔS (J/mol·K)</th>
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<tr>
<td>−12.92</td>
<td>−28.62</td>
<td>52.75</td>
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4. Conclusion

In summary, DNA binding and photo-induced DNA cleavage activities of EA are researched by the UV-vis spectra and fluorescence spectra. The results suggest that EA was intercalated into CT-DNA, and CT-DNA fluorescence quenched EA is a static quenching procedure. Using EB as a fluorescence probe assay of CT-DNA, fluorescence intensity increase with increasing of EA. During a 30-min irradiation, about 46.55% of CT-DNA binding sites are destroyed, which suggest that EA is a promising candidate for photodynamic therapy. By calculating the thermodynamics parameter ΔH, ΔG and ΔS we can obtain a conclusion that the major binding mode between EA and DNA would be the electrostatic binding. These results would be helpful in understanding the therapeutic mechanism of EA.

Acknowledgements

This work was supported by the Natural Science Foundation of Jiangsu Higher Education Institutions of China (No. 10KJB150008) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

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