A study of the interaction of calf thymus DNA with Co(III)-salen complex

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Keywords: Cobalt-salen, DNA-binding, Fluorescence quenching, Binding constant.

1. Introduction

A large percentage of chemotherapeutic anticancer drugs are compounds that interact with DNA directly or prevent the proper relaxation of DNA (through the inhibition of topoisomerases). To understand the molecular basis of drug–DNA interactions in details, various physicochemical and biochemical techniques are employed to determine the mechanism of interaction between DNA and drug agents such as spectrophotometric, circular dichroism, spectrofluorometric, melting temperature, isothermal titration calorimetry (ITC) and viscosimetric techniques. Together, these assays are powerful tools to determine the mechanism of previously discovered molecules, and will be crucial to the discovery of the next generation of DNA-binding anticancer drugs [1].

Various types of metallo-salens have been developed over the period of last two decades and their DNA binding and damaging properties have been studied in details for example [SalenAl^{III}] complex, Fe (Salen)Cl, Ni(II) or Mn(III), Cr(III), Cu(II) Salen complexes [2-5].

In this study, The interaction of schiff-base complex of novel Co^{III}complex, [Co(Hsalen)(CH_3OH)(CH_3O)]$^+CH_3COO^-$, where H_2salen denotes $C_{16}H_{16}N_4O_4$ with calf thymus DNA (CT-DNA) has been investigated in vitro by physicochemical methods such as fluorescence spectrometry and ultra-violet (UV) spectrometry techniques to obtain the intrinsic binding constant (k_b) value and to found that this complex can bind to DNA or not?

2. Methods

The cobalt complex was presented by department of chemistry of Ferdowsi University. Experiments were carried out in buffer Tris-HCl, pH 7. Solutions were prepared with distilled deionized water.

Calf thymus DNA was extracted from calf thymus. The stock solution of DNA obtained, was prepared by dissolving DNA in 10 mM Tris-Hcl buffer at pH 7.5. The solution gave a ratio of ≥1.8 at A_{260}/A_{280}, indicating that the DNA was sufficiently free from protein. The concentration of DNA was determined by monitoring the UV absorbance at 260 nm using ε_{260} = 6667 cm^{-1}. The stock solution was stored at -20°C.

Absorbance spectra were recorded using a Shimadzu UV-2550 double beam spectrophotometer. The absorbance measurements were performed by keeping the concentration of the complex constant (20 μM) while varying the DNA concentrations from 0.5 μM to 75 μM. The absorbance at 223, 241 and 259 nm was recorded after each addition of DNA.

Fluorescence measurements were made using Shimadzu-RF-1501 spectrofluorophotometer. The concentration of the cobalt complex was fixed (5μM) while varying concentration of the CT-DNA (4μM to 123μM). The cobalt (III) complex in the presence of DNA was excited at 315 nm and the fluorescence spectra were recorded between 350 and 550 nm. The Stern-Volmer quenching constant (Kc_{DNA}) was calculated with the help of the Stern-Volmer equation.

3. Results and discussion

3.1. Electronic spectra

The UV absorption spectra of Co-salen with and without the addition of calf thymus DNA are shown in Fig.1. An increase in the intensity (hyperchromism) with a red shift were observed with the addition of (0.5 μM to 75 μM) DNA. If the salen-co interact with DNA, leading to spectral changes. Absorption titrations were carried out by keeping the concentration of the salen-co constant while adding a solution of the CT-DNA in increasing amounts in both cuvettes until the saturations in hyperchromism were observed. The saturation in hyperchromism is quantitatively shown by plotting the A/A_0 vs [DNA], where A_0 and A are the absorption intensities of the Co-complex in the absence and presence of increased concentrations of DNA, respectively (Fig. 2).

The intrinsic binding constant K_b was determined for cobalt complex according to Eq. 1 [6].

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_b-\varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, $\varepsilon_a=A_{obsd}/[compound]$, ε_f=the extinction coefficient for the free compound and ε_b=the extinction coefficient for the compound in the fully bound form, respectively.

A plot of [DNA]/ $(\varepsilon_a-\varepsilon_f)$ vs. [DNA] gives K_b as the ratio of the slope to intercept.

The binding constant, K_b, for the cobalt-complex has been estimated to be $0.5\pm0.25 \times 10^6\ M^{-1}$ by the half-reciprocal plot method (Fig. 3).

3.2. Emission spectra

The addition of CT-DNA to Salen-Co solution resulted in a fluorescence quenching. $K_{C_{DNA}}$ quenching of CT-DNA molecule was calculated as being $0.9\times10^3 M^{-1}$ according to Eq. 2.

$$F_0/F = 1 + K_{C_{DNA}}[DNA] \qquad (2)$$

Where F_0 and F refer to the fluorescence intensities in both the absence and presence of CT-DNA, $K_{C_{DNA}}$ is the Stern-Volmer quenching constant and [DNA] is the concentration of CT-DNA in mol L^{-1}.

A plot of (F_0/F)-1 vs. [DNA] indicated the $K_{C_{DNA}}$ values that it was estimated from this linear plot (Fig. 4).

4. Conclusions

In this study, we unravel the DNA interaction of Co-salen complex. The binding behavior of complex with DNA was characterized by absorption titration and fluorescence. The DNA-binding constant in connection with other experimental observations show that Co-salen can bind to CT-DNA avidly and this complex may be effect the biological activity of CT-DNA.

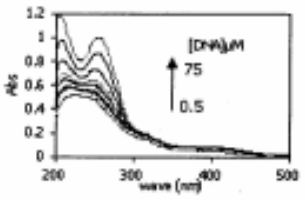

Fig 1: The UV absorption spectra of Co-salen 20 μM with and without the addition of calf thymus DNA from 0.5-75 μM

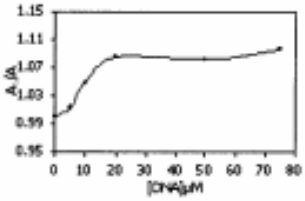

Fig 2: Abs titration of the Co-complex 20 μM in the absence and presence of increased concentrations of calf thymus DNA from 0.5-75 μM

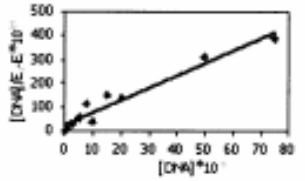

Fig 3: Half-reciprocal plot for binding of Co-complex 20 μM with CT-DNA from 0.5-75 μM

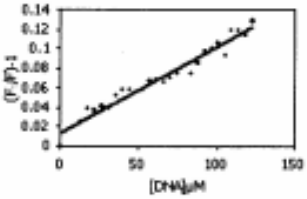

Fig 4: Stern-volmer plot for binding of Co-complex 5 μM with calf thymus DNA from 4-123 μM

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