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Binding of DNA with Rhodamine B: Spectroscopic and molecular modeling studies

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ABSTRACT

Binding of Rhodamine B to Calf thymus DNA (CT DNA) was studied using various biophysical techniques and molecular docking method. Circular dichroic studies revealed that conformation of CT DNA changed moderately and unwind on binding with Rhodamine B. The binding was cooperative in nature. The overall binding constant, evaluated from Benesi Hidebrand plot was seen to be in the range of 10^3 M^{-1}. Salt dependence binding data showed that the binding free energy depends on salt concentration. From the thermodynamics studies, it was concluded that binding process is favored by both negative enthalpy change and positive entropy change. Molecular docking calculations and Quenching experiment confirmed that the dye binds in the minor groove of CT DNA. These results further advance our knowledge on the molecular aspects on the interaction of these types of dyes to nucleic acids.

1. Introduction

The interaction of nucleic acids with small molecules such as drugs, organic dyes and metal complexes has been studied intensively as it provides knowledge on the screening and design of novel and more efficient drugs targeting DNA, and can potentially speed up the drug discovery and development processes [1–4]. The study of anti-carcinogenic medicines and their interactions with DNA are also significantly important to develop new cancer therapy treatments [5,6].

Several types of molecules are known that interact with DNA, *viz.* isoquinoline alkaloids [7,8], indole alkaloids [9,10], pyrrolimidazol polyamides [11,12], synthetic indole derivatives [13], etc. Rhodamine B (RB) (Fig. 1), a xanthene dye is widely used to detect metal ions in luminescent analysis [14–16] and in biotechnology to study fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA [17–19]. Rhodamine B possesses biochemical and pharmacological effects and shows antibacterial activity upon conjugation with peptides [20]. The studies of mutagenicity indicate that the commercial Rhodamine B dye is mutagenic after activation in *in vitro* systems although much of this effect may be due to (unidentified) impurities. Elliot and coworkers investigated the mutagenic potential of urinary metabolites of Rhodamine B and found no mutagenic activity of rabbit urine against TA98 or TA100 with and without activation [21]. Moreover, a urine sample of human subject was also found negative. Two commercial samples of Rhodamine B were both weakly mutagenic in the same system [21]. Brown and group tested two samples of Rhodamine B by Ames test, both with and without activation and both were found positive after activation, but one was significantly weaker than the other and this difference appeared to be associated with an undefined impurity [22]. Nestman and hisngroup also conducted the Ames in which the Rhodamine B was found positive after activation in TA1538 and TA98 [23]. They also found that DNA damages in CHO cells were significantly diminished by purification of the dye [23]. Lewis et al. exposed fibroblast cell samples (from *Muntiacus Muntjac*) to a concentrations of Rhodamine B between 2 and 20 μg/ml for 24 and 48 h respectively and they found that all doses and times indicate the frequency of chromosome aberrations

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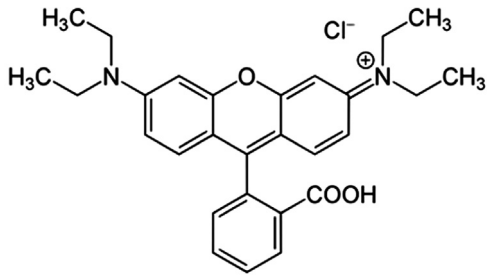

Fig. 1. Structure of Rhodamine B.

was increased in comparison with controls. At the higher doses, there was also an increase of induced aberrations of chromosome [24]. Parodi et al. carried out Ames test to the strains TA98 and TA100 and test for DNA fragmentation in rat liver in vivo with Rhodamine B [25]. Both results were negative. Wuebbles & Felton tested a range of dyes including Rhodamine B by Ames test in strains TA1538, TA98 and TA100 and the results were negative for Rhodamine B both with and without activation [26]. Investigations in Drosophila showed that Rhodamine is genotoxic in both somatic and germ line cells [27]. From the currently available data, it is appropriate to consider that Rhodamine B is potentially genotoxic. From the experiments of Wang et al. and Kui Jiao et al., it was suggested that Rhodamine B is able to bind with DNA sequences [28–30]. The earlier studies of DNA binding ability of Rhodamine B did not provide the details at the molecular level. An understanding at the molecular level is important to characterize the mechanism of ligand–receptor interactions. In this paper, we have investigated the interactions of Rhodamine B with DNA at the molecular level using Spectrophotometric, Fluorimetric, Circular dichroic, Calorimetric and computer docking procedure. These studies provide an understanding of the binding affinity and structural details of Rhodamine B: DNA complex.

2. Experimental

2.1. Materials and methods

CT DNA was purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Its concentration was determined spectrophotometrically using known molar extinction coefficient (ε) (6600 M^{-1} cm^{-1} at 260 nm) expressed in terms of nucleotide phosphates [7]. The ratio of the absorbances at 260–280 nm indicated that the sample was free from protein contamination. Rhodamine B were obtained from Sigma–Aldrich and were used throughout without further purification as no detectable impurities were found by thin layer chromatography and ^{1}H NMR spectroscopy. Rhodamine B solutions were freshly prepared each day and were always kept protected in dark to prevent any light induced photochemical changes. Molar extinction coefficients (ε) of Rhodamine B were determined in our laboratory and the value is 91,675 M^{-1} cm^{-1} (at 554 nm). This value was used to determine the concentration of Rhodamine B by absorbance measurements. No deviation from Beer's law was observed in the concentration range at 0–100 μM for Rhodamine B used in our study.

All the experiments were conducted in Citrate-Phosphate (CP) buffer medium (10 mM [Na^+]) at pH 7.0 containing 5.0 mM Na_2HPO_4. pH of the medium was adjusted using citric acid [31]. Glass-distilled deionized water and analytical grade reagents were used throughout. The pH values of the solutions were measured with a calibrated Orion-Ross combined electrode system (Model 81-02). All buffer solutions were filtered through Millipore filters (Millipore, India Pvt. Ltd, Bangalore, India) of 0.45 μm before use.

2.2. Absorbance spectral studies

Absorbance spectra were measured on a Shimadzu Pharmaspec UV-1700 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) equipped with a thermoelectrically controlled cell holder (model TCC 240A) under stirring at 25 ± 0.1 °C in quartz cells of 1.00 cm path length.

2.3. Fluorescence spectral studies

All Fluorescence spectral studies were measured on a Hitachi spectrofluorimeter (Hitachi Corporation, Tokyo, Japan) equipped with a thermoelectrically controlled cell holder in matched quartz cells of 1.00 cm path length under stirring at 25 ± 0.5 °C.

2.4. Determination of affinity constants

All calculations regarding the binding of RB with CT DNA were evaluated by Benesi Hildebrand plot [32,33], Scatchard analysis and Hill plot using the following equations (equations (1)–(3))

2.4.1. Benesi Hildebrand plot

$$1/\Delta A = 1/(K_a \cdot \Delta A_0)[\mathrm{DNA}] + 1/(\Delta A_0) \quad (1)$$

where ΔA is absorbance/Florescence intensity change, K_a is the binding constant, and ΔA_0 is maximum absorbance/Florescence intensity change. The binding constant can be obtained by plotting $1/\Delta A$ vs 1/[DNA] and then dividing intercept by slope.

2.4.2. Scatchard analysis

The amount of free and bound dye was determined as follows. During the UV/Vis experiment, known amount of the dye was added to DNA solution (70 μM). This quantity was used to calculate the expected absorbance at wavelength maximum, $A_{exp} = lC_t\varepsilon_{max}$. From the difference between A_{exp} and the observed absorbance (A_{obsd}), the amount of bound drug can be calculated as $C_b = \Delta A/\Delta\varepsilon = (A_{exp} - A_{obsd})/l(\varepsilon_f - \varepsilon_b)$. The amount of free drug was determined by the difference, $C_f = C_t - C_b$. The extinction coefficient of the completely bound drug was determined from the slope of Benesi–Hildebrand plot. In the fluorescence study, C_b was calculated from the relation $C_b = C_t(I - I_o)/(V_o - 1)I_o$, where C_t is the known total drug concentration, I is the observed fluorescence, I_o is the fluorescence intensity of identical concentration of drug in absence of DNA and V_o is the experimentally determined ratio of the fluorescence intensity of totally bound drug to that of free alkaloid. Free drug concentrations (C_f) were obtained from the relationship $C_t = C_b + C_f$. The binding ratio r is defined as $r = C_b/[\mathrm{DNA}]_{total}$.

Binding data obtained from spectrophotometric and spectrofluorimetric titrations were converted into Scatchard plot of r/C_f versus r. All the Scatchard plots revealed positive slopes at low r values as observed in cooperative binding isotherms and hence were analyzed using the following McGhee–von Hippel equation [34,35]:

$$\frac{r}{C_f} = K_i(1 - nr) \times \left(\frac{(2\omega + 1)(1 - nr) + (r - R)}{2(\omega - 1)(1 - nr)}\right)^{(n-1)} \left(\frac{1 - (n + 1)r + R}{2(1 - nr)}\right)^2 \quad (2)$$

where, $R = \{[1 - (n + 1)r]^2 + 4\omega r(1 - nr)\}^{1/2}$, K_i is the intrinsic binding constant to an isolated binding site, n is the number of base pairs excluded by the binding of a single ligand molecule and ω is