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An insight into the interaction of phenanthridine dyes with polyriboadenylic acid: Spectroscopic and thermodynamic approach

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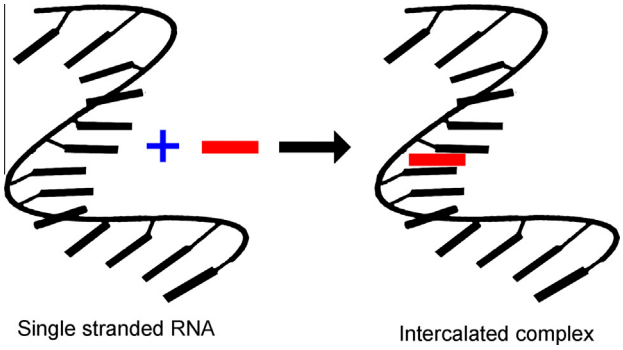
HIGHLIGHTS

- Two dyes ethidium bromide and propidium iodide bind to single stranded polyriboadenylic acid.
- Binding affinity is greater for propidium iodide than ethidium bromide.
- Binding are characterized by both negative enthalpy and entropy changes.
- Ionic strength dependence of binding revealed lesser electrolytic contribution to the binding process.

GRAPHICAL ABSTRACT

We have investigated the interaction of two phenanthridine ligands namely ethidium bromide and propidium iodide with single and double stranded polyriboadenylic acid using various spectroscopic techniques. Both of them were found to bind with the single stranded polymer, while no interaction was observed with the double stranded one. Thermodynamic studies revealed that in both the cases the binding were characterized by negative enthalpy and negative entropy changes. Ionic strength dependence studies revealed a lesser electrolytic contribution to the total Gibbs free energy change in both the cases compared to the non-electrolytic contribution. Our results may be of potential use in the design of specific phenanthridine derivatives and understanding the structure activity relationship for improved therapeutic applications.



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ABSTRACT

Interaction of two phenanthridine dyes, namely ethidium bromide (EB) and propidium iodide (PI) with polyriboadenylic acid was investigated using various spectroscopic techniques. They were found to bind only with the single stranded form of the polymer, while no affinity was observed for the double stranded form. Enhanced binding observed for PI compared to EB may be attributed to the presence of external alkyl chain in PI. Thermodynamic studies showed negative enthalpy and negative entropy changes for the binding of both the dyes. Salt dependent studies revealed a lesser electrolytic contribution compared to the nonelectrolytic contribution to the total Gibbs free energy change in each case. This indicated importance of hydrophobic and van der Waal's interaction for the binding process. Overall, the binding data and detail energetics of interaction presented here would be helpful in the design of phenanthridine based molecules that interact with specific RNA structure.

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Introduction

Nucleic acid structures have been the focus of study since long time because of their central importance within the machinery of life. The polymorphism of nucleic acids in *vivo* presents numerous opportunities for new therapeutic agents. Accordingly, the design of small molecules that interact with noncanonical nucleic acid structures represents an active area of rational drug design [1–3]. With the discovery of RNA viruses that are responsible for a number of fatal human diseases like hepatitis b, AIDS, cancer, etc., the characterization of retroviral genome, there is considerable interest in developing compounds that interact with RNA of these viruses and exert their antiviral activity. In the last few years there has been a significant shift to study and understand the fundamentals of small molecule interactions with various RNA structures in order to develop RNA targeted therapeutic agents [4].

Among the single stranded nucleic acids, polyriboadenylic acid [hereafter poly(A)] is of particular biological relevance due to its role in mRNA functioning and gene expression [5,6]. Since the discovery that Neo-PAP (a human poly(A) polymerase) is over-expressed in some human cancer cells [7], it has been suggested that the poly(A) tails of mRNA may represent a malignancyspecific target [8,9]. Poly(A) has been established to exist as a single stranded helix stabilized by pair-wise stacking interaction between adjacent bases at physiological pH and temperature [10,11]. Helical conformation of poly(A) gets gradually converted to random coil with increasing temperature. This polymer adopts a parallel stranded double helical conformation at pH $\leqslant$ 4.5, with a single groove in which the adenine–adenine base pairs are significantly tilted from the plane perpendicular to the helix axis [12]. The molecular structure of the double stranded poly(A) [hereafter ds poly(A)] has been established from X-ray crystallographic analysis and has been proved to be quite different from those of double stranded RNA double helices [11]. Polyadenylation process also plays a significant regulatory role in the production of alternative forms of proteins [13,14]. A possible biological role for ds poly(A) structure has been proposed by Zarudnaya et al. [15] and other group [16]. They have suggested the involvement of such structure in intracellular process as termination of mRNA–poly(A) synthesis and auto regulation of poly(A) binding protein synthesis.

Ethidium bromide (hereafter EB; Fig. 1A) is an aromatic compound. Its core heterocyclic moiety is generically known as a phenanthridine, an isomer of which is the fluorescent dye acridine. It is a flat molecule that resembles a DNA base pair. Because of its chemical structure, it can intercalate (or insert) into double stranded DNA and also to triple stranded DNA [17]. Because of the binding to DNA, EB is a powerful inhibitor of DNA polymerase [18]. EB has been commonly used since 1950s in veterinary medicine to treat trypanosomosis in cattle, a disease caused by trypanosomes [19]. Studies have been conducted in animals to evaluate EB as a potential antitumorigenic chemotherapeutic agent [20]. It has been shown that EB acts as a topoisomerase I poison, just like several anticancer drugs used in human [21].

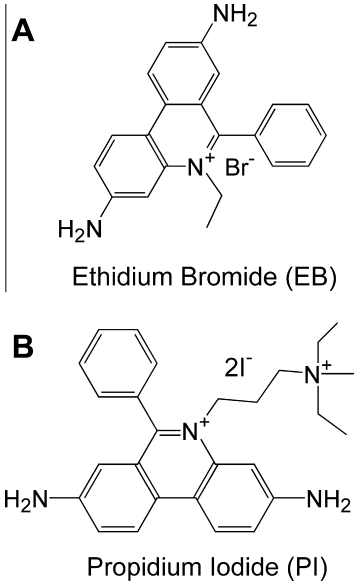

Fig. 1. Chemical structures of EB (A) and PI (B).

Propidium iodide (3,8-diamino-5-(3-diethylmethylamino)propyl)-6-phenyl phenanthridinium diiodide; hereafter PI; Fig. 1B) is another DNA intercalating agent and a fluorescent molecule. It is structurally similar to EB except having a long carbon chain. PI binds to DNA by intercalating between the bases with little or no sequence preference [22]. PI also binds to RNA and a typical use of PI in plant biology is to stain the cell wall [23].

DNA binding aspect of EB and PI is well established [24,25]. Interaction of these dyes with synthetic deoxy homopolymers like *poly dA*.poly dT, *poly dA*.poly dU etc have also been reported [26]. But binding studies of these compounds with single stranded RNA are scarce. In the last few years there has been a significant shift to study and understand the fundamentals of small molecule interactions with various RNA structures in order to develop RNA targeted therapeutic agents. Keeping in mind the importance of the above facts, the focus of our present study was to understand the complete molecular details of the interaction of EB and PI with ss poly(A) from spectroscopic and thermodynamic studies. Further the interaction of these compounds with ds poly(A) structure was undertaken for comparative study. This model study will help us for the design of phenanthridine based antiviral molecules that are pharmacologically important.

Experimental methods

Materials

Poly(A), EB and PI were purchased from Sigma Chemical Co., St. Louis, MO, USA. They were used without any further purification. Concentration of nucleic acid was determined by UV measurements using molar extinction coefficient of 10,000 M^{-1} cm^{-1} at 257 nm [27]. Each day Solutions of EB and PI were prepared freshly. Concentrations of the EB and PI were calculated using molar extinction coefficients of 5680 M^{-1} cm^{-1} at 480 nm [28] and 5900 M^{-1} cm^{-1} at 493 nm respectively [29]. The compounds obeyed Beer's law in the concentration range employed in the study. All the stock solutions were prepared in 10 mM citrate–phosphate (CP) buffer of pH 7.1 containing 5 mM Na_2HPO_4. The double stranded form of poly(A) was prepared by slowly adding single stranded poly(A) [hereafter ss poly(A)] solution into CP buffer of pH 4.5 under stirring and followed by 2 h incubation at 20 °C for the transition to be completed [30]. The formation of ds poly(A) was verified by circular dichroism (CD) and UV spectral studies [31]. Salt dependent studies were performed in the same buffer containing different concentrations of Na^+ ions. Deionized and triple distilled water was used throughout for the experiments.

Instrumentation and methods

Absorption spectrophotometric measurements

All the UV–VIS absorbance studies were made on a Shimadzu model UV-1800 spectrophotometer (Shimadzu Corporation, Japan) in matched quartz cell of 1 cm path length. A thermo programmer was attached to it to maintain the temperature of the spectrometer