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Coralyne induced self-structure in polyadenylic acid: Thermodynamics of the structural reorganization

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ABSTRACT

Thermodynamics of the induction of self-structure in polyadenylic acid by the cytotoxic isoquinoline alkaloid coralyne was investigated using calorimetry tools. The binding was an exothermic process driven by large negative enthalpy change. The equilibrium constant at $[K^+] = (130 \pm 0.01)$ mM and $T = (298.15 \pm 0.01)$ K was calculated to be $(1.34 \pm 0.07)\cdot 10^6$ M^{-1}. Temperature dependent calorimetric studies suggested weakening of the self-structure at higher temperatures. The self-structure induction process was characterized by complete enthalpy-entropy compensation. The temperature dependence of the enthalpy change yielded negative heat capacity value. The equilibrium constant decreased with increasing $[K^+]$ apparently due to the weakening of the self-structure at higher salt concentrations. Differential scanning calorimetry studies testified for the formation of self-structure in (50 ± 0.01)–(130 ± 0.01) mM $[K^+]$. Parsing of the standard molar Gibbs energy change revealed that the non-polyelectrolytic forces dominated the self-structure induction process.

1. Introduction

Alkaloids possess unmatched chemical diversity and biological relevance suitable for inclusion in drug development programs [1–3]. Protoberberines are one such abundant group of alkaloids having remarkable biological significance including high anticancer potential [1,4–6]. Coralyne (Fig. 1) is a planar crescent-shaped synthetic protoberberine alkaloid analog having a dibenzoquinolizinium skeleton. Coralyne and its derivatives have high antitumor potential with significantly lower toxicity in comparison to other natural members of the isoquinoline family [7,8]. It also acts as a dual poison of topoisomerases I and II [9–12]. Topoisomerase I poisoning and DNA binding ability of coralyne have been interpreted in terms of a model involving intercalation and groove binding depending on the saturation state of the ring system [9,12]. Coralyne is considered as a privileged scaffold that has the ability to interact with more than one biological target [13–22]. The biological properties of coralyne are often linked with its excellent nucleic acid binding ability [13–19]. Coralyne is known to disproportionate duplex poly(dT)·poly(dA) into triplex poly(dT) * poly(dA)·poly(dT), intercalate into T * A·T triplexes and enhance the stability of DNA as well as RNA triple helices [18,19,23,24]. The photo induced DNA damaging property of coralyne is also known [25].

Chaires and colleagues have shown, for the first time, that coralyne can induce self-structure in single stranded polyadenylic acid [poly(A)] [13]. Poly(A) has single stranded helical structure at neutral pH but some small molecules and alkaloids can induce unique self-structure (double stranded) in poly(A) at neutral pH where otherwise only single stranded structure can exist [13,15,26–35]. Such self-structure induction can influence mRNA degradation and stop protein production in the cell. Hence, molecules inducing self-structure can be utilized as RNA based therapeutics. Coralyne promotes homoadenine aggregation [36], which is further testified by the formation of self-structured poly(A) [13,15]. However, details of the thermodynamics of self-structure formation in poly (A) in presence of coralyne have not been investigated. In this study we have elucidated the thermodynamics of coralyne induced self-structure formation in poly(A) under varying salt and temperature conditions using isothermal titration calorimetry and differential scanning calorimetry techniques. Thermodynamic evaluation can yield valuable information on the balance of energetic forces governing the self-structure induction process. This knowledge is essential to understand and optimize the molecular interactions needed for drug development at the early stage of lead identification [37]. The binding reaction between coralyne and poly(A) can be represented as

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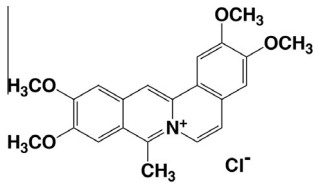

Fig. 1. Molecular structure of coralyne chloride.

$$\text{Coralyne} + \text{poly(A)} \rightleftharpoons \text{Coralyne} - \text{poly(A)complex} \quad (1)$$

The equilibrium constant (K) is given by

$$K = \frac{[\text{Coralyne} - \text{poly(A)complex}]}{[\text{Coralyne}][\text{poly(A)}]} \quad (2)$$

2. Materials and methods

2.1. Materials

Coralyne chloride (coralyne hereafter, ⩾0.95 mass fraction purity, M = 399.87 Da), poly(A) potassium salt (poly(A) hereafter), dipotassium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Poly(A) was dissolved in the autoclaved RNAase free phosphate buffer of pH 7.20. All the experiments were conducted in this buffer containing (130 ± 0.01) mM K^+ at T = (298.15 ± 0.01) K unless mentioned otherwise. This salt concentration was used for the study because coralyne has been shown to induce self-structure in single stranded poly(A) under closely matched conditions [15]. Provenance and mass fraction purities values of the materials used are listed in Table 1. Concentrations of coralyne and poly(A) in terms of nucleotide phosphate were calculated using molar absorption coefficient values of $1.45{\cdot}10^4\ M^{-1}\ cm^{-1}$ at 421 nm and $1.00{\cdot}10^4\ M^{-1}\ cm^{-1}$ at 257 nm, respectively [18,38].

2.2. Isothermal titration calorimetry (ITC)

ITC experiments were performed to thermodynamically characterize the self-structure induction process in a MicroCal VP ITC unit (MicroCal, Inc., Northampton, MA, USA) [38–48]. The ITC unit was calibrated periodically to ensure the accuracy of the calorimetric results. Calibration of the unit for volume of the calorimetric cell, injected volumes and heat exchange was performed and verified with water–water dilution experiments as per criteria of the manufacturer that the mean energy per injection was <5.46 μJ and standard deviation was <0.063 μJ. Chemical calibration of the unit was performed using 2%, 5% and 10% propan-1-ol.

Poly(A) and coralyne solutions were degassed on the MicroCal's Thermovac unit to avoid bubble formation during the course of the experiment. The degassed poly(A) solution was then injected from the rotating syringe into the calorimeter chamber containing the degassed coralyne solution at regular time intervals. Duration of each injection was fixed at 10 s while the delay time between two successive injections was 240 s. After each titration a dilution experiment was performed to obtain the heat of dilution of poly (A). The heat of dilution of poly(A) was subtracted from the corresponding heat of coralyne-poly(A) reaction to obtain the true heat of coralyne-poly(A) complexation. The corrected values were subsequently analyzed using Levenberg-Marquardt non-linear least squares curve fitting algorithm to obtain the equilibrium constant (K), stoichiometry (N) and the standard molar enthalpy change of binding ($\Delta_r H^0$). The standard molar Gibbs energy change ($\Delta_r G^0$) was calculated from the relationship

$$\Delta_r G^0 - RT \ln K \quad (3)$$

where R is the universal gas constant (R = 8.314472 $J{\cdot}K^{-1}{\cdot}mol^{-1}$) and T is the thermodynamic temperature in kelvin. The $\Delta_r G^0$ and $\Delta_r H^0$ values were utilized to obtain $T\ \Delta_r S^0$, the standard molar entropic contribution ($\Delta_r S^0$ is the standard molar entropy change), from the relationship

$$T\Delta_r S^0 = \Delta_r H^0 - \Delta_r G^0 \quad (4)$$

The combined standard uncertainty $u_c(x)$ was determined from the relationship

$$u_c(x) = \left\{(u_1(x))^2 + (u_2(x))^2 + (u_3(x))^2 + \cdots + \text{etc}\right\}^{1/2} \quad (5)$$

where $u_1(x)$, $u_2(x)$, $u_3(x)$, etc. are the individual uncertainties in the measurements.

2.3. Differential scanning calorimetry (DSC)

DSC experiments were performed on a MicroCal VP-DSC unit. At first, both the sample and reference cells were rinsed and filled with phosphate buffer solution and then equilibrated for 15 min. Scanning was performed in the temperature range 306.15 K–373.15 K at a rate of 60 $K{\cdot}h^{-1}$. Typically, 12 buffer scans were needed to obtain a stable reproducible base line (noise <2.1 $\mu J{\cdot}K^{-1}$ and repeatability specification <5.46 $\mu J{\cdot}K^{-1}$). Then on the cooling cycle the sample cell was filled with poly(A) solution followed by coralyne-poly(A) complexes and scanned within the same temperature range. The DSC thermograms were analyzed using Origin 7.0 and the temperature at which maximum heat capacity was observed is known as the transition temperature (T_{fus}). Temperature calibration of the unit was performed with the MicroCal standards whereas calibration of the DSC unit was done using the built-in calibration heaters.

3. Results and discussion

3.1. Thermodynamics of the self-structure formation in poly(A) by coralyne

ITC can be employed to efficiently measure the thermodynamics of ligand-nucleic acid interactions [38–40,47,48]. ITC affords K, N, $\Delta_r H^0$, $\Delta_r G^0$ and $T\Delta_r S^0$ from a single titration. The standard molar heat capacity change ($\Delta_r C_p^0$) is subsequently obtained from the temperature dependence of the $\Delta_r H^0$. The upper panel of Fig. 2 shows the ITC profile for the titration of poly(A) into a solution of coralyne at T = (298.15 ± 0.01) K and [K^+] = (130 ± 0.01) mM. Generally, in ITC the concentration of the solution in the calorimeter cell is lower than that of the solution taken in the syringe. Therefore, poly(A) was titrated from a rotating syringe into the coralyne solution in the calorimeter cell in order to ensure that the concentration of coralyne is kept to a minimum to prevent aggregation of the alkaloid. Each spike in the thermogram corresponds to a single injection. These heats were corrected by

Table 1
Provenance and mass fraction purity of the samples used.

Sample	Provenance	Mass fraction purity[a]
Coralyne chloride	Sigma-Aldrich	0.95
Poly(A)	Sigma-Aldrich	0.98
Dipotassium hydrogen phosphate	Sigma-Aldrich	0.99
Potassium dihydrogen phosphate	Sigma-Aldrich	0.99

[a] The mass fraction purities are based on information provided by the supplier Sigma-Aldrich.