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Thermodynamics of the induction of self-structure in polyadenylic acid by proflavine

Anirban Basu *, Gopinatha Suresh Kumar *

Biophysical Chemistry Laboratory, Organic & Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700 032, India

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ABSTRACT

The energetics of self-structure induction in polyadenylic acid in the presence of proflavine has been studied using isothermal titration calorimetry. The self-structure induction process was exothermic and driven by large positive entropy change. The equilibrium constant at $T = (298.15 \pm 0.01)$ K and $[Na^+] = (130 \pm 0.01)$ mM was calculated to be $(1.01 \pm 0.08) \cdot 10^6$ M^{-1}. Salt dependent calorimetric studies revealed that the equilibrium constant increased with increasing $[Na^+]$ in the 50–130 mM range suggesting enhanced binding preference at higher salt concentrations, apparently due to easier self-structure formation at higher $[Na^+]$. Dissection of the standard molar Gibbs energy change clearly established that the self-structure induction was driven by non-polyelectrolytic forces and the polyelectrolytic contribution was relatively small. The equilibrium constant decreased with increasing temperature indicating destabilization of the self-structure at higher temperatures. Negative standard molar heat capacity value obtained from the temperature dependence of the enthalpy change suggested that hydrophobic forces are important for the self-structure induction. Furthermore, a complete enthalpy–entropy compensation phenomenon was also observed.

1. Introduction

An understanding of the interaction of photoactive small molecules with nucleic acids may enable the development of better therapeutics for photodynamic therapy. RNA plays lead roles in many biological processes and in the progression of many diseases. Consequently development of RNA targeted therapeutics has gained remarkable importance in recent times. Polyadenylic acid (poly(A) hereafter) has attracted attention for its key role in maintaining the stability and biological functions of mRNA [1,2]. Eukaryotic mRNAs have a long poly(A) tail at the 3′ end which is added during post transcriptional modification of mRNA. The single stranded (ss) poly(A) tail is known to influence the mRNA stability and maturation, and is essential for the initiation of the translation process. The discovery that neo polyadenylic polymerase (PAP), a human PAP, is significantly over expressed in human cancer cells in comparison to normal cells [3,4] has identified poly(A) as a malignancy specific target [3]. Poly(A) is known to exist as a ss helical structure at neutral pH and as parallel double stranded (ds) helix at pH less than 5.00 [5–7]. Many small molecules have been reported to be able to bind selectively to the ss poly(A) tail in vitro and induce a unique self-structure in poly(A) at pH 7.0 where otherwise only the ss structure can exist [8–19]. Such small molecule recognition and structural reorganization in poly(A) of mRNA in the cells may intervene with the mRNA processing by PAP and may emerge as a novel class of RNA based therapeutics. The mechanism underlying this unique phenomenon of poly(A) self-structural reorganization is still unclear [20,21].

Proflavine (hereafter PF, Fig. 1) is a diamino acridine analog that has been used as a powerful antiseptic earlier [22]. PF is known to have mutagenic effects on DNA and it has been studied for the development of anticancer agents and novel RNA-targeted antiviral drugs [23–26]. PF can form strong intercalative complexes with nucleic acids and therefore studies on the interaction of PF with nucleic acids are pertinent for understanding the mechanism underlying its therapeutic action. PF has previously been shown to bind to poly(A) and induce self-structure at neutral pH and 128 mM $[Na^+]$ [10]. However, detailed thermodynamics of the binding reaction has not been revealed.

Thermodynamic evaluation can provide crucial information on the balance of energetic forces driving binding interactions. This is essential to understand and optimize molecular interactions required for drug development [27]. Therefore, in this study we have elucidated the thermodynamics of self-structure induction

* Address: Biophysical Chemistry Laboratory, CSIR-Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Jadavpur, Kolkata 700 032, India.
E-mail addresses: basu.ani.anirban@gmail.com (A. Basu), gskumar@iicb.res.in (G. Suresh Kumar).

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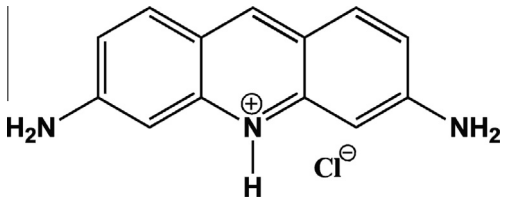

Fig. 1. Molecular structure of PF.

in poly(A) by PF under a variety of conditions of salt and temperature using isothermal titration calorimetry. The binding reaction between PF and poly(A) may be represented as follows

$$\mathrm{PF} + \mathrm{poly(A)} \leftrightarrow \mathrm{PF{-}poly(A)} \cdot \mathrm{complex} \quad (1)$$

The equilibrium constant (K) for the above reaction is

$$K = \frac{[\mathrm{PF{-}poly(A)} \cdot \mathrm{complex}]}{[\mathrm{PF}][\mathrm{poly(A)}]} \quad (2)$$

2. Materials and methods

2.1. Materials

PF ($\geqslant$0.95 mass fraction purity, M = 245.71 Da), poly(A) potassium salt, citric acid and disodium hydrogen phosphate were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Poly (A) was dissolved in the RNAase free and autoclaved citrate–phosphate (CP) buffer, pH 7.00. The sample was dialyzed into the experimental buffer at T = 278.15 ± 0.01 K. All the experiments were performed in CP buffer of pH 7.00 at T = (298.15 ± 0.01) K containing (130 ± 0.01) mM [Na^+] unless mentioned otherwise. This salt concentration was chosen because PF has been shown to induce self-structure in poly(A) under similar salt conditions. The provenance and mass fraction purity values of the samples used are listed in Table 1. Concentration of poly(A) in terms of nucleotide phosphate and PF were determined using molar absorption coefficient values of 1.00×10^4 and 4.20×10^4 $M^{-1}\cdot cm^{-1}$, respectively [18,19,28–30].

2.2. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) experiments were conducted on a MicroCal VP ITC unit. The solutions were extensively degassed on the MicroCal's Thermovac unit prior to titration to prevent bubble formation. The ITC experiments were performed according to the procedure reported earlier [31–36]. Briefly, aliquots of the degassed poly(A) solution were titrated from a preprogrammed rotating syringe into the calorimeter cell containing the degassed PF solution. The duration of each injection was 10 s whereas the delay time between two successive injections was 240 s. Dilution experiments were performed to determine the heat of dilution of poly(A) by injecting identical volumes of the same concentration of poly(A) into the CP buffer alone. The heat of each poly(A)-buffer mixing was deducted from the corresponding heat of PF-poly(A) reaction to obtain the real heat of PF-poly(A) binding reaction. The data, corrected for the heat of dilution, was analyzed using Levenberg–Marquardt non-linear least squares curve fitting algorithm. This analysis afford the equilibrium constant (K), stoichiometry (N) and standard molar enthalpy change accompanying the binding ($\Delta_r H^0$). The standard molar Gibbs energy change ($\Delta_r G^0$) was determined 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 $\Delta_r G^0$ value together with $\Delta_r H^0$ were utilized to obtain $T\Delta_r S^0$, the standard molar entropy change, using the following relationship

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

In order to ensure the accuracy of the calorimetric data, calibration of the ITC 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 ITC unit was done using varying propan-1-ol concentrations of 2%, 5% and 10%. The combined standard uncertainty $u_c(x)$ was calculated using 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. represent the individual uncertainties in the measurements.

3. Results and discussion

3.1. Energetics of the binding reaction using ITC

The binding of PF to poly(A) was studied by isothermal titration calorimetry. The top panel of Fig. 2 depicts the isothermal titration calorimetry profile for the titration of poly(A) into a solution of PF at T = (298.15 ± 0.01) K and [Na^+] = (130 ± 0.01) mM. The heat involved with each injection was corrected by deducting the respective heat of dilution that was determined from a separate control experiment as described in Section 2.2 (curves on the top offset for clarity). The lower panel of Fig. 2 shows the actual heats for PF-poly(A) complexation that were plotted against the molar ratio of poly(A)/PF (χ). In this panel the solid line is the best fit of the experimental data. The thermogram revealed negative peaks in the plot of power *versus* time suggesting exothermic binding with one binding event. The equilibrium constant (K) was calculated to be $(1.01 \pm 0.08) \cdot 10^6$ M^{-1} and the binding stoichiometry (N) was estimated to be 0.362. Therefore, the site size (n), which is the reciprocal of the binding stoichiometry (N), was (2.76 ± 0.06). A similar site size was reported earlier for some other small molecules that induce self-structure in poly(A) [16,17]. The binding was driven by negative standard molar enthalpic contribution and positive standard molar entropic contribution. The $\Delta_r H^0$ value was deduced to be (−9.64 ± 0.03) $kJ\cdot mol^{-1}$ while the $T\Delta_r S^0$ value was calculated to be (24.63 ± 0.05) $kJ\cdot mol^{-1}$. Thus, the process of self-structure induction in poly(A) was predominantly entropy driven at T = (298.15 ± 0.01) K and [Na^+] = (130 ± 0.01) mM. Such entropy dominated binding resulting in self-structure formation in poly(A) has been reported earlier also [16–19]. The strong positive entropic contribution originates from the release of bound cations and alterations in water structure as observed in many earlier small molecule–nucleic acid interactions [37–39]. The negative enthalpy is primarily due to the restricted mobility

Table 1
List of provenance and mass fraction purity of the materials used in this study.

Sample	Provenance	Mass fraction purity[a]
PF	Sigma–Aldrich	0.95
Poly(A)	Sigma–Aldrich	0.98
Disodium hydrogen phosphate	Sigma–Aldrich	0.99
Citric acid	Sigma–Aldrich	0.99

[a] The mass fraction purities are based on the information provided by the supplier Sigma–Aldrich. The purity of PF was verified by TLC and HPLC.