RNA targeting by small molecules: Binding of protoberberine, benzophenanthridine and aristolochia alkaloids to various RNA structures

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Studies on RNA targeting by small molecules to specifically control certain cellular functions is an area of remarkable current interest. For this purpose, a basic understanding of the molecular aspects of the interaction of small molecules with various RNA structures is essential. Alkaloids are a group of natural products with potential therapeutic utility, and very recently, their interaction with many RNA structures have been reported. Especially noteworthy are the protoberberines and aristolochia alkaloids distributed widely in many botanical families. Many of the alkaloids of these group exhibit excellent binding affinity to many RNA structures that may be exploited to develop RNA targeted therapeutics. This review attempts to present the current status on the understanding of the interaction of these alkaloids with various RNA structures, mainly highlighting the biophysical aspects.

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1. Introduction

Elucidation of the interaction of small molecules with nucleic acid has been an active area of interest in many laboratories around the world for a long time. In such studies DNA has gained prominence over RNA due to the very fact that the former is considered the genetic material of the cell. A large number of studies and reviews on the DNA binding of a variety of molecules have been published in the last several decades (Waring 1981; Denny 1989; Graves and Velea 2000; Hurley 2002; Martinez and Chacon-Garcia 2005; Maiti and Suresh Kumar 2007; Maiti and Suresh Kumar 2009; Bhadra and Suresh Kumar 2011). At present the interaction of small molecules with the DNA is a well-characterized area with direct correlations of the binding to many biological activities. Many readers might be aware of the original seminal studies of Lerman and Waring (Lerman 1961; Waring 1981) in this respect, describing the elongation of the DNA helix on intercalation of planar conjugated aromatic molecules like acridine and ethidium, and the work of Zimmer, Dervan, Lown, Neidle, and many others (Kopka *et al*. 1985; Zimmer and Wahnet 1986; Wemmer and Dervan 1997; Reddy *et al*. 1999; Neidle 2001) describing the groove binding of isohelical molecules like netropsin, distamycin, hoechst and related synthetic molecules in the minor groove of the DNA. Thus, intercalation and groove binding modes have been the two major kinds of non-covalent DNA interactions characterized for small molecules. In the course of these active DNA binding investigations spanning half a century, RNA was almost a neglected molecule in terms of such binding studies, as its role in gene expression and other cellular activities were thought to be minimum and was considered as an intermediate molecule having a role only in the transcription process. This has also been partially due to the fact that RNA has diverse and complex structures that were difficult to characterize compared to the uniform double helical structure of DNA. But recently RNA molecules have gained remarkable prominence due to the emerging knowledge of their potential and critical roles in many cellular activities and functions (Nelson *et al*. 2003; Esau and

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Monia 2007). The role of RNA in the progression of many diseases particularly in viral infections like HIV, AIDS and hepatitis C has led to growing interest in RNA as a potential target for therapeutic intervention (Gallego and Varani 2001; Foloppe *et al*. 2006; Liu *et al*. 2008; Fulle and Gohlke 2010). Furthermore, the recent discovery of a number of micro-RNA molecules (Nelson *et al*. 2003; Esau and Monia 2007) and unravelling of their various biological functions including their roles in the progression of cancer has led to a paradigm shift for investigating RNA as a target for therapeutic intervention. Consequently, there has been renewed interest recently in deciphering RNA structures and also in understanding the binding aspects of small molecule therapeutics to various RNA structures (Harford 1995; Tor 1999; Wilson and Li 2000; Hermann 2002; Vicens and Westhof 2003). RNAs have diverse structures that could fold to a multitude of conformations, and each of these may be potential targets of small molecules. Hence, interaction studies with various RNA structures are necessary to understand the basic fundamentals of RNA–ligand interaction. Aminoglycosides are a group of small molecules studied with many RNA structures (Walter *et al*. 1999; Kaul and Pilch 2002; Vicens and Westhof 2003; Chao and Chow 2007). Nevertheless, the use of aminoglycosides as drugs is limited due to high toxicity and moreover it is not clear whether the results of aminoglycoside–RNA interaction could be used as fundamentals of small molecule–RNA interaction. Therefore, it was felt that discovery of new molecules are essential for deciphering at first the molecular aspects of binding to various RNA structures for subsequent development as RNA targeted drugs. In this review, we discuss recent progress on the understanding of natural product alkaloids binding to various RNA structures. The focus will be on the biophysical aspects of berberine, palmatine and coralyne of the isoquinoline, sanguinarine of the benzophenanthridine and aristololactam-β-D-glucose of the aristolochia group binding to various RNA structures. The reader is also referred to some of the related reviews that have appeared recently on the various aspects of the RNA binding of these alkaloids (Maiti and Suresh Kumar 2007; Giri and Suresh Kumar 2008a, 2009, 2010a, b; Bhadra and Suresh Kumar 2011).

2. Structures of RNAs

Most cellular RNA molecules are single stranded. They may form secondary structures such as stem-loop, hairpin, etc. Unlike the relatively simple double helical structure of DNA, RNA molecules have diverse and complex secondary and tertiary structures. In the biological system, transfer RNAs (tRNA) are the key molecules that carry amino acids for protein synthesis. Typically, tRNAs have three-dimensional, L-shaped and secondary cloverleaf structures with 76 nucleotides (Kim *et al.* 1974; Robertus *et al.* 1974). One arm of the L-shaped structure ends in the 3′-ends with the single-stranded sequence, ACCA, where the terminal OH of the adenosine residue is the site of aminoacylation by tRNA synthetases. The other arm, at its end, has the trinucleotide anticodon that interacts with the codon in the messenger RNA (mRNA) on the ribosome. All eukaryotic mRNAs have a long poly(A) tail at the 3′-end that is added during a post-transcriptional modification. The poly(A) tail serves to stabilize the mRNA, prevent its degradation and influences the translation process. Functionally, polyadenylation of the mRNAs is linked to the termination of the transcription process.

Major types of small RNA molecules include small nuclear RNA (snRNA), involved in mRNA splicing, small nucleolar RNA (snoRNA) that directs the modification of ribosomal RNAs (rRNA) and micro-RNA (miRNA) and short interfering RNA (siRNA) that regulate gene expression. Ribosomal RNA plays a pivotal role in the selection of transfer RNAs (tRNA) and in catalysis of peptide bond formation by ribosomes (Noller 1991). miRNA and siRNA molecules have about 20–25 nucleotides; they have similar functions but differ in biogenesis. miRNAs are produced from transcripts that form stem-loop structures, while siRNAs are produced from long double-stranded RNA precursors.

3. Importance of natural alkaloids

Alkaloids are a group of nitrogen-containing compounds produced by many plants during metabolism. Many alkaloids have lead roles in medicinal chemistry and biomedical applications. They have been used extensively in folk medicine in China, Korea and Iran and in Ayurvedic medicines in India since several years due to their low toxic nature even at high doses. More recent studies have revealed a variety of medicinal properties including potential anticancer properties for many of them that are exploitable for further research and drug development (Grycova *et al*. 2007; Wink 2007; Maiti and Suresh Kumar 2010). Protoberberines represent one of the largest groups that include the famous berberine (figure 1a), palmatine (figure 1b) and the synthetic analogue coralyne (figure 1c). They have similar structure with small differences in the substituents on the isoquinoline moiety. These substitutions result in significant change in their spectroscopic properties and biological activities. Berberine and palmatine are non-planar, but coralyne due to partial saturation has a planar structure. The biological activities of the protoberberines have been reviewed recently (Grycova *et al*. 2007; Wink 2007). The most important benzophenanthridine alkaloids are sanguinarine, chelithrine, fagaronine and nitidine. Sanguinarine exhibits an interesting structural conversion from the charged iminium ion to neutral alkanolamine form (figure 1d and f). Sanguinarine also has a long history of use in folk medicine worldwide. The more recently discovered

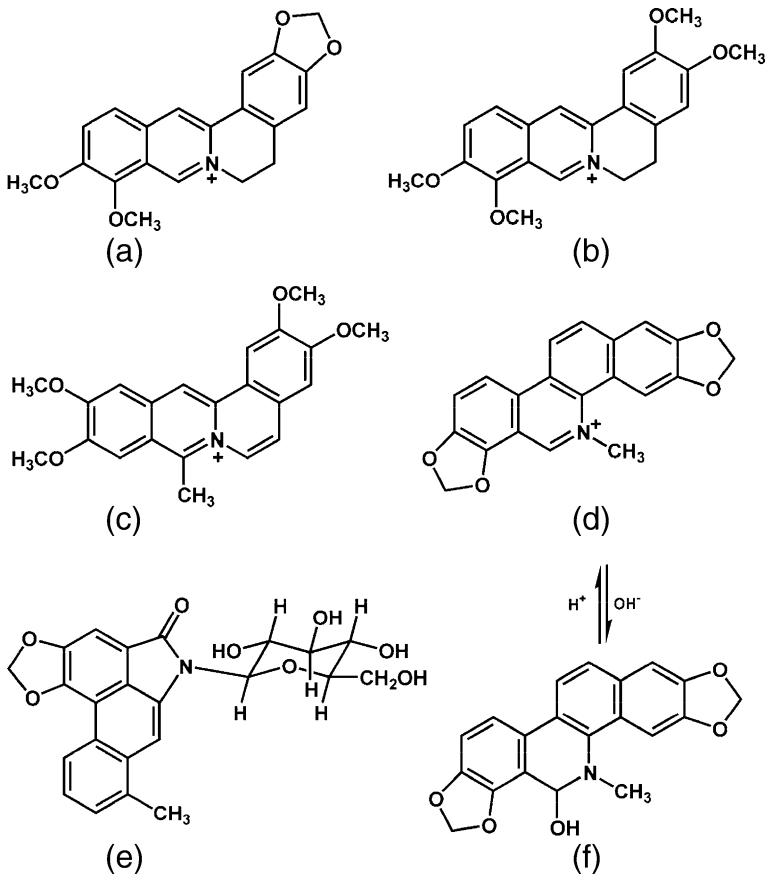

Figure 1. Chemical structure of alkaloids: (**a**) berberine, (**b**) palmataine, (**c**) coralyne, (**d**) sanguinarine (iminium), (**e**) aristololactam-β-D-glucoside and (**f**) sanguinarine (alkanolamine).

aristolochia group of alkaloids have an important member with an attached sugar moiety, aristololactam-β-D-glucoside (ADG) (figure 1e), which has close resemblance to the clinically used anticancer agent daunomycin (Das *et al.* 2011a, b). The aristolochia group of alkaloids possess anticancer activities that have not yet been explored (Cassady *et al*. 1990)

4. Binding of alkaloids to tRNA^{phe}

The cloverleaf structure of tRNAs is shown in figure 2. The crystal structure of tRNA^{phe} is known since 1974 (Robertus *et al*. 1974; Byrne *et al*. 2010). The binding of berberine, palmatine and coralyne to phenylanaline-specific transfer RNA (tRNA^{phe}) structures have been recently studied by various biophysical techniques (Islam *et al*. 2007, 2008, 2009a). A typical absorption spectral titration data of berberine with increasing concentration of tRNA^{phe} is presented in figure 3, which clearly revealed hypochromic (decrease in absorption intensity) and bathochromic (shift of the wavlength maximum to longer side) effects (Islam *et al*. 2007). Similar features were observed for palmatine and coralyne also. Job plot analysis revealed the stoichiometry to be around 10 nucleotides for berberine and palmatine, and 5 nucleotides for coralyne.

The fluorescence spectra of the alkaloids have been reported to enhance in the case of berberine and palmatine and quench in the case of coralyne on interaction with tRNA^{phe} (Islam *et al*. 2007). The binding data evaluated from such studies using Scatchard plots (Scatchard 1949) fitted to the McGhee-von Hippel equation (McGhee and von Hippel 1974) revealed strong binding of the alkaloids to tRNA. All the three alkaloids bound tRNA cooperatively. The affinity values were 1.15×10^5 M^{-1} for berberine, 1.05×10^5 M^{-1} for palmatine and 6.70×10^5 M^{-1} for coralyne. A typical cooperative Scatchard plot with positive slope at low binding ratios for the binding of berberine to tRNA is presented in the inset of figure 3. The binding of these alkaloids also produced changes in the secondary structure as revealed from changes in the circular dichroism (CD) spectrum of tRNA with the development of induced circular dichroism for the bound alkaloids that were otherwise optically inactive.

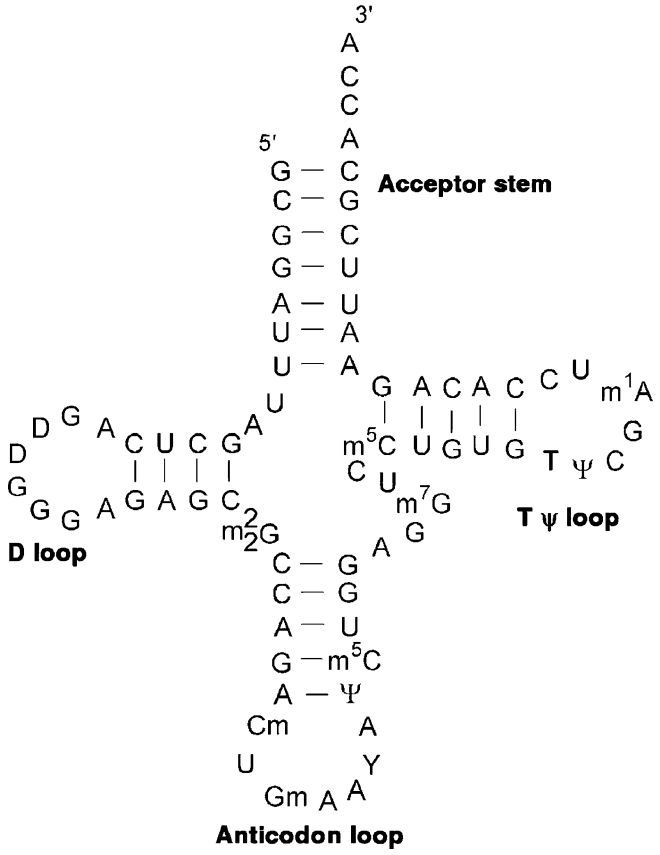

Figure 2. Cloverleaf structure of $tRNA^{phe}$. Reprinted with permission from Islam *et al*. (2007) with permission from Elsevier.

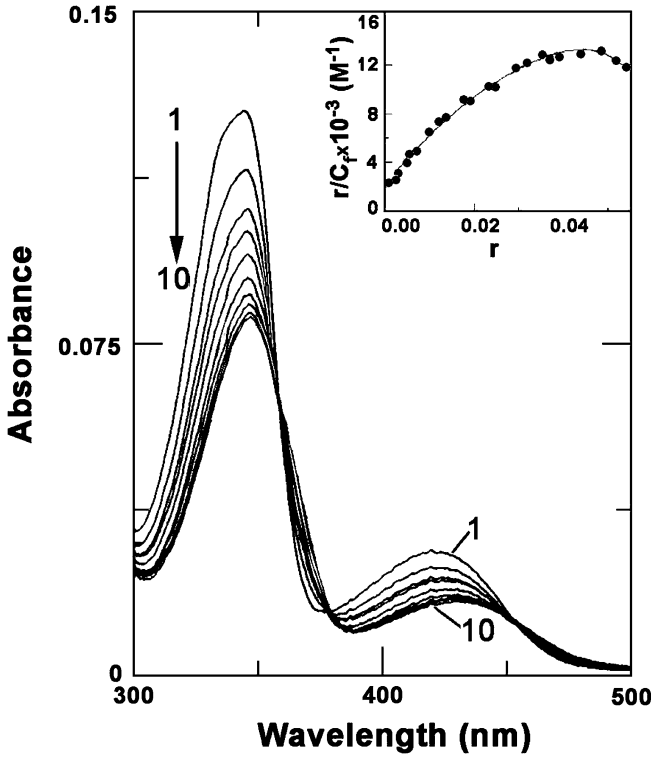

Figure 3. Representative absorption spectral titration of berberine with increasing concentration of $tRNA^{phe}$. Inset represents cooperative Scatchard plot. Reprinted with modification with permission from from Islam *et al*. (2007) with permission from Elsevier.

The binding mode of the alkaloids to tRNA was probed by ferrocyanide quenching experiments revealing that there is significant intercalative component in the complexation. The quenching experiment is a good probe for intercalative binding as the quencher anion cannot penetrate the negative charges of the helix and the fluorescence of the strongly bound molecules will not be affected like that of the free molecules. While structural studies have provided good evidence for strong binding of all the three alkaloids, the binding of the planar coralyne was always stronger than that of buckled berberine and palmatine. This was supported by results from calorimetric experiments (Islam *et al*. 2007, 2008, 2009a, b). Isothermal titration calorimetric experiments revealed that the binding affinity of coralyne to tRNA was remarkably higher than berberine and palmatine. The free energy of binding was almost similar in each case around 7.0 kcal/mol. In all the cases the binding was favoured by negative enthalpy and positive entropy changes. Another important aspect of the interaction revealed from calorimetric studies was the role of electrostatic interactions. All these alkaloids are positively charged, but the analysis of the partition of the Gibb's energy ($\Delta G°$) in terms of electrostatic (ΔG^{pe}) and non-electrostatic components (ΔG^{t}) revealed that the electrostatic contribution to the binding has been much smaller. In case of berberine and palmatine the contribution from the electrostatic interaction was elucidated to be about 15%, while for coralyne it was about 3.2% only. The overall results suggested that the alkaloids berberine and palmatine bound tRNA by mostly partial intercalation, while coralyne bound more strongly and by a true intercalation process. The binding was also substantiated by the molecular modelling studies (Islam *et al*. 2009a, b). A docked posture of coralyne bound to $tRNA^{phe}$ is depicted in figure 4.

The binding of ADG to $tRNA^{phe}$ was studied in comparison with daunomycin (Das *et al*. 2011b). ADG binds to tRNA non-cooperatively with a binding affinity of the order of 10^4 M^{-1}. This affinity was one order weaker compared to the binding of the isoquinolines alkaloids. The binding was shown to result in only very weak conformational changes in tRNA structure as evidenced from CD studies with no induced CD developed for the bound alkaloid molecules. For ADG a 1:2 binding was reported from Job plot analysis. Here also the electrostatic contribution to the binding Gibb's energy was evaluated to be only about 15%. Thermodynamics of the

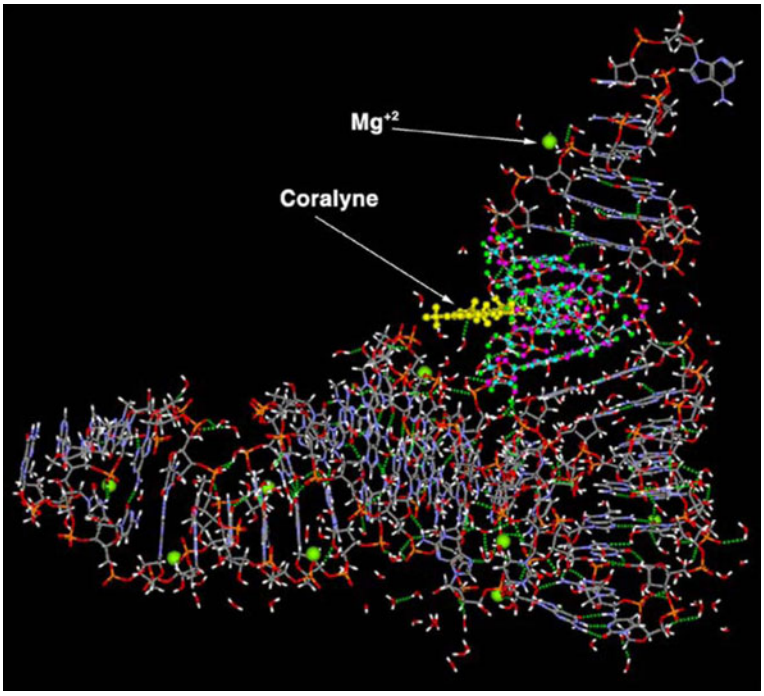

Figure 4. A docked posture of coralyne bound to $tRNA^{phe}$ (Islam *et al*. 2009a). Reproduced by permission of the Royal Society of Chemistry (*www.rsc.org/molecularbiosystems*).

binding of ADG to tRNA has been predominantly entropy driven with a small favourable enthalpy contribution. The heat capacity ($\Delta C_p°$) of binding of ADG–tRNA has been small and negative (−47 cal/ mol K) and was suggested to be indicative of structure-specific binding.

Studies on the structural aspects and thermodynamics of the interaction of the iminium and alkanolamine forms of sanguinarine have been performed with tRNA very recently (Hossain *et al*. 2012a). The charged iminium form was shown to bind to tRNA cooperatively, while the neutral alkanolamine form did not exhibit any binding affinity. Nevertheless, it was shown that in presence of substantial tRNA concentration, a conversion from the alkanolamine to iminium form occurred resulting in concomitant binding of the latter form. This was further confirmed from CD experiments also. The binding of iminium also resulted in the generation of induced CD for the bound alkaloids. The binding affinity of the iminium form to tRNA evaluated from spectroscopy and isothermal titration calorimetric studies was of the order of 10^5 M^{-1}. It was revealed from calorimetric studies that the binding was driven largely by negative enthalpy ($-\Delta H°$) with a smaller but favourable positive entropy contribution ($+T\Delta S°$). It was further shown that although the binding of the iminium was dependent on the [Na^+] concentration, a dominant non-electrostatic contribution was apparent in the free energy of the binding. A small heat capacity value (−184 cal/mol K) and an enthalpy–entropy compensation phenomenon in the energetics of the interaction were shown to characterize the binding of the iminium form to $tRNA^{phe}$.

5. Binding to model single-stranded RNAs

The binding aspects of the alkaloids berberine, palmatine and coralyne to single-stranded structures of polyguanylic acid [poly(G)], polycytidylic acid [poly(C)], polyurylidic acid [poly(U)] and polyinosinic acid [poly(I)] have been reported from multiple biophysical experiments (Islam and Suresh Kumar 2008, 2009). Berberine and palmatine have been shown to bind non-cooperatively exhibiting high affinity of the order of 10^5 M^{-1} to poly(G) and poly(I) from spectroscopy. Both the alkaloids bind non-cooperatively but very weakly to poly(C) and poly(U) as evaluated from absorption and fluorescence studies. The high binding affinity to poly(G) and poly(I) was also demonstrated unequivocally from competitive dialysis experiment. Calorimetric studies revealed the binding to poly(G) and poly(C) to be exothermic and favoured by negative enthalpy and positive entropy changes. Studies also reported significant conformational changes in the A-form CD spectrum of poly(I) on binding by palmatine with the bound alkaloid molecules acquiring induced CD spectra. The change with berberine

was smaller, proposing that small differences in the alkaloid structure to be responsible for these significant differences in the conformational aspects of the binding.

Coralyne binding to these single-stranded polynucleotides have revealed higher binding affinity to poly(G) and poly(I) and very weak binding to poly(U) and poly(C) as elucidated from spectroscopic experiments and competitive dialysis assay (Islam and Suresh Kumar 2009). A typical competitive dialysis result of coralyne with these four polynucleotides reported is presented in figure 5. The binding of coralyne to poly(G) and poly(I), like that of berberine and palmatine, has been reported to be non-cooperative with affinity values of the order of 10^5 M^{-1}. The binding to poly(U) and poly(C) was weak and of the order of 10^3 M^{-1} as revealed from Scatchard analysis of the absorbance data and also from an analysis of the competition dialysis data. Coralyne perturbed the structure of poly(I) and poly(U) to a higher extent, and poly(G) marginally, as evidenced from CD results. Induced CD was also reported to develop for poly(G), poly(I) and poly(U), which was suggested to be due to the effective coupling of the transition moments of the bound coralyne molecules with that of the chirally arranged RNA bases. Thermodynamic characterization of the binding of coralyne has been studied from ITC experiments. The affinity values from ITC results have confirmed the results from spectroscopy. Furthermore, the interactions were characterized to be exothermic and accompanied by negative enthalpy and positive entropy changes. The studies also suggested that although positively charged the binding has almost 85% contribution from non-electrostatic forces. This is similar to what has been reported for the binding to tRNA. The heat capacity change of binding was also estimated from temperature-dependent calorimetric results that suggested the involvement of multiple weak non-covalent interactions in the complex formation. Furthermore, it was also suggested that a partial enthalpy–entropy compensation phenomenon occurs in the interaction of coralyne with the single-stranded RNA conformations of poly(G) and poly(I).

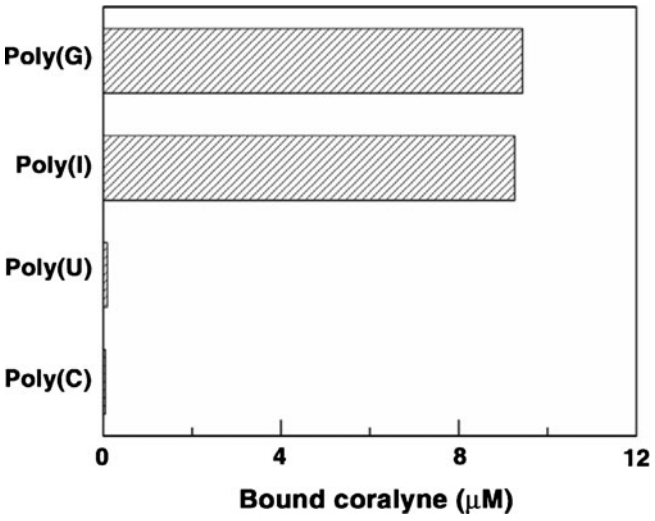

Figure 5. Competitive dialysis result of coralyne with these four single-stranded polynucleotides. The concentration of coralyne bound to each RNA polymer is shown as a bar graph. Reprinted with permission from Islam and Suresh Kumar (2009) with permission from Elsevier.

6. Binding to double-stranded RNAs

Double-stranded (ds) RNA is now been considered as an important regulator of gene expression in many eukaryotes. It triggers different types of gene silencing that are collectively known as RNA silencing or RNA interference (Meister and Tusch, 2004; Fire 2007). A key step in this pathway is the processing of ds RNAs into short RNA duplexes that further guide RNA silencing by specific and distinct mechanisms. Due to the prominence of ds RNAs, agents that can specifically bind to ds RNA structures can be potentially useful to interfere with its function. The binding of berberine, palmatine and coralyne with three model ds RNA, viz. poly (A).poly(U), poly(I).poly(C) and poly(C).poly(G), has been recently characterized (Islam *et al.* 2009b). These RNAs have different base pairing schemes (figure 6). The studies revealed that all the three alkaloids bound these RNAs cooperatively. The binding affinity of berberine and palmatine was higher for poly(A).poly(U) followed by poly(I).poly(C) and lowest for poly(C).poly(G), as evaluated from absorbance and fluorescence studies. Again, for coralyne also, the affinity varied as poly(A).poly(U)>poly(I).poly (C)>poly(C).poly(G). Results from thermal melting studies of the complexes were used to characterize the binding. It has been observed that the melting temperature of poly(A).poly(U) and poly(I).poly(C) enhanced with the three alkaloids, but the highest stabilization was observed with coralyne. Optical melting and differential scanning calorimetry data were also used to evaluate the binding affinities, which showed overall binding affinities to be of the order of 10^6 M^{-1} and highest for coralyne binding. The mode of binding of these alkaloids to ds RNAs was also probed by ferrocyanide ion quenching experiments and viscosity studies. It was observed that the anionic quencher was not able to penetrate the helix to displace the bound alkaloid molecules and the quenching constant from the Stern-Volmer plots revealed lower values for coralyne complexes with AU and IC polynucleotides followed for the complexes of the CG polynucleotide, suggesting that the bound coralyne is sequestered from the solvent indicating intercalation. Viscosity results revealed that a length enhancement of 1.88, 1.27 and 1.07 nm occurred for coralyne complexes compared to 0.19, 0.17 and 0.04 nm for berberine and 0.41, 0.22 and 0.18 nm for palmatine complexes with poly(A).poly(U), poly(I).poly(C)

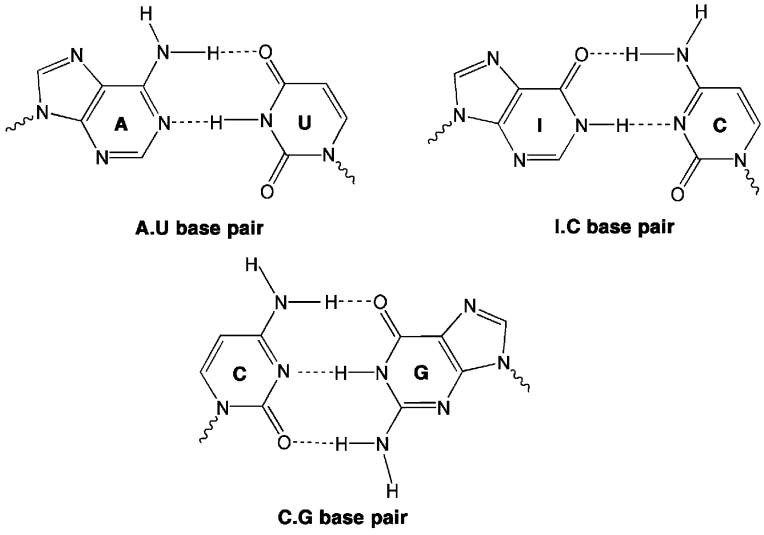

Figure 6. Hydrogen bonding schemes of A:U, I:C and C:G base pairs. Adapted with permission from Islam *et al.* (2009b), © 2009, American Chemical Society.

and poly(C).poly(G). From these results it was concluded that coralyne truly intercalated while berberine and palmatine partially intercalated to these ds RNA structures. The study also reported the circular dichroism features of the binding from extensive CD experiments where it was observed that the conformation of poly(A).poly(U) was perturbed by all the three alkaloids, while that of poly(I).poly(C) was perturbed only by coralyne. The CD spectrum of poly(C).poly(G) was revealed to be unaffected in presence of these alkaloids.

Thermodynamics of the binding of berberine, palmatine and coralyne to these ds RNA structures have also been investigated in extensive details along with the dependence of salt and temperature (Islam *et al*. 2009b). All the systems reported exothermic reaction except poly(C).poly (G) where the heat of binding was very weak. The binding was characterized by negative enthalpy and positive entropy changes in each case. The affinity values from ITC experiments suggested that the binding of coralyne to poly(A).poly (U) was the strongest with a binding affinity of 3.02×10^6 M^{-1} and a binding site size of 2.03 base pairs. The enthalpy change for this binding reaction was −5.86 kcal/mol and the entropy contribution was 2.88 kcal/mol. This was closely followed by the binding of palmatine to poly(A).poly(U) ($K=1.23\times10^6$ M^{-1}) and coralyne to poly(I).poly(C) ($K=1.17\times10^6$ M^{-1}). It is noteworthy that in all the systems the free energy change was reported to be in the range 7–8 kcal/mol. From salt-dependent calorimetric studies it was reported that the number of ions released on the binding of a single alkaloid molecule was 0.91, 0.98 and 0.78 for berberine, palmatine and coralyne binding to poly(A).poly(U), 0.98, 0.97 and 0.83 for berberine, palmatine and coralyne binding to poly(I).poly(C), and 0.99 and 0.87 for palmatine and coralyne binding to poly(C).poly(G). Partitioning of the free energy change between the non-polyelectrolytic and polyelectrolytic contributions suggested that there is a remarkably large non-polyelectrolytic contribution to the binding forces in each case undermining the role of electrostatic forces in the complexation of the positively charged alkaloids.

From the temperature-dependent calorimetric studies and from the plots of variation of $\Delta H°$ with temperature, the heat capacity values for the complexation of these alkaloids with ds RNA polynucleotides have been evaluated and reported (Islam *et al*. 2009b). The $\Delta C_p°$ values for berberine, palmatine and coralyne were −102, −194 and −335 cal/mol K for binding to poly(A).poly(U), −55, −108 and −138 cal/mol K for binding to poly(I).poly(C), and −85 and −93 cal/mol K for binding of palmatine and coralyne to poly(C).poly(G). The finite nature of the $\Delta C_p°$ values and the magnitude in range 100–500 cal/mol K were suggested to be generally observed for ligand–nucleic acid interactions. Furthermore, the significant differences in the $\Delta C_p°$ values have been explained to be due to the differences in the release of structured water from the RNA on binding of the alkaloids. Another important aspect observed in the thermodynamic data of the binding in these systems was the enthalpy–entropy compensation. It was suggested that the change of enthalpy with entropy showed linear variation depicting complete or at least partial compensation behaviour in these interactions. Overall the studies suggested a higher binding affinity for these alkaloids to the ds RNA structures compared to tRNA and ss RNAs.

Interaction of sanguinarine with ds RNA structures of poly(I).poly(C) and poly(A).poly(U) was first reported from spectroscopic studies by Sen and Maiti (2002). Based on Scatchard analysis limited to the narrow region of bound

alkaloid, a non-cooperative binding was proposed. From the magnitude of the binding parameters, a higher binding affinity of sanguinarine to poly(I).poly(C) structure than to poly (A).poly(U) structure was also suggested. The main limitation of this study was that it was conducted at pH 7.4 where both iminium and alkanolamine forms of the alkaloid prevailed in more or less equal proportions. A detailed study employing various spectroscopic, viscometric and calorimetric experiments was performed by Roy Chowdhuri *et al*. (2010) on three model ds RNAs. This study was performed at a pH of 6.5 where only the binding moiety, viz. the charged iminium form of the alkaloid existed. Similar to the binding of the isoquinolines alkaloids, sanguinarine also has been shown to exhibit cooperative binding with all the three ds RNAs. The binding affinity was higher for poly(A).poly(U), but poly(I).poly(C) had closer values. The slightly higher preference for the AU sequences over the IC sequences was also clearly evident from competition dialysis assay. Fluorescence quenching and hydrodynamic studies revealed that the alkaloid binds ds RNAs by intercalation. The length enhancement values for rod-like RNAs on intercalation were 0.91, 0.88 and 0.69 nm, respectively, for poly(A).poly(U), poly(I).poly(C) and poly (C).poly(G) polynucleotides. The binding of sanguinarine stabilized the helical structure of AU and IC duplexes by 19°C and 18°C, respectively. The binding constants calculated from the melting data revealed affinity values of 7.10×10^5 M^{-1} and 5.40×10^5 M^{-1} for poly(A).poly(U) and poly (I).poly(C) at 20°C. These values were of the same order and close to the values deduced from spectroscopic studies.

Circular dichroic perturbation of the RNA structures on sanguinarine complexation was found to be higher for poly (A).poly(U) compared to the other two RNAs. Also, the alkaloid acquired induced CD with a single positive band with maximum around 320 nm. The CD data also suggested that sanguinarine intercalated more strongly to AU and IC polynucleotides than that with the CG polynucleotide.

Calorimetric studies elucidated the energetics of the binding of sanguinarine to these RNAs. The binding in each case was characterized by exothermic heats. The heat data showed only one binding event. The binding to poly (A).poly(U) was shown to be driven largely by negative enthalpy and a small positive entropy change. With the IC and CG sequences, the binding was favoured by both enthalpy and entropy contributions. The large negative enthalpy of the binding observed particularly in the AU system was suggested to be generally typical for intercalative interaction of small molecules with nucleic acids. The strong positive entropy term was suggested to be due to the disruption and release of water molecules on intercalation of sanguinarine into the IC and CG RNA helices. The ΔC_p° values for sanguinarine binding to poly(A).poly(U), poly (I).poly(C) and poly(C).poly(G) were found to be 135, 134 and 28 cal/ mol K, respectively.

Another important phenomenon that was shown to be associated with the sanguinarine–ds RNA binding was the enthalpy–entropy compensation in AU and IC sequences. In CG sequences no such enthalpy–entropy compensation phenomenon was observed. The values of the slope that is $\delta\Delta H^\circ/\delta(T\Delta S^\circ)$ of the plot of ΔH° as a function of $T\Delta S^\circ$ were 1.2, 0.93 and 0.25 with poly(A).poly(U), poly(I).poly(C) and poly(C).poly(G), respectively. It was thus suggested that there is almost complete compensation in cases of poly(A).poly(U) and poly(I).poly(C) (figure 7) leading to finite values of ΔC_p° and was pointed out to be a common phenomena in many drug–nucleic acid and ligand–protein interactions. This was interpreted in terms of the release of structural water molecules from the interacting surfaces and formation of weaker H-bonds in the bulk water. Such kind of phenomena was shown to be absent in the interaction of sanguinarine to poly(C).poly(G) revealing the involvement of different energetics in the binding process.

7. Binding to triple helical RNA, poly(U).poly(A)*poly(U)

Triple helical nucleic acid structures have aroused a resurgence of interest in nucleic acid chemistry and biology as a tool for controlling a particular gene expression via triplex formation, known generally as the antigene strategy. A triplex structure formation was first reported in the year 1957 by Alexander Rich and colleagues (Felsenfeld *et al*. 1957) in an RNA triplex poly(U).poly(A)*poly(U) (the dot and star represents Watson–Crick and Hoogsteen base pairing). It may be likely that RNA triplex formation may also be an important structural motif of small RNAs that the therapeutic agents may target for gene regulation. Consequently, small molecule binding and stabilizing triplexes have attracted great attention and significance. Although a number of studies of stabilizing triplexes by various alkaloids have been reported in the literature (Lee *et al*. 1993; Latimer *et al*. 1995; Moraru-Allen *et al*. 1997), these studies were limited to mostly DNA triplexes. Studies on natural alkaloids stabilizing RNA triplexes have been reported only recently from our laboratory (Ray *et al*. 1999; Das *et al*. 2003; Sinha and Suresh Kumar 2009). Ray *et al*. have studied the binding of ADG to poly(U).poly(A)*poly(U) and found that the alkaloid destabilised the third strand of the triplex. Das *et al*. reported a higher affinity and stabilization for sanguinarine and berberine to poly(U).poly(A)*poly(U) over the duplex poly(A).poly(U) (Das *et al*. 2003). The interaction of berberine, palmatine and coralyne with the RNA triplex poly (U).poly(A)*poly(U) was studied extensively using various biophysical and calorimetric techniques recently (Sinha and Suresh Kumar 2009). Strong binding of all three alkaloids to the triplex was revealed from hypochromic and bathochromic effects in their absorption and considerable changes in the fluorescence spectra. All the three alkaloids bind non-

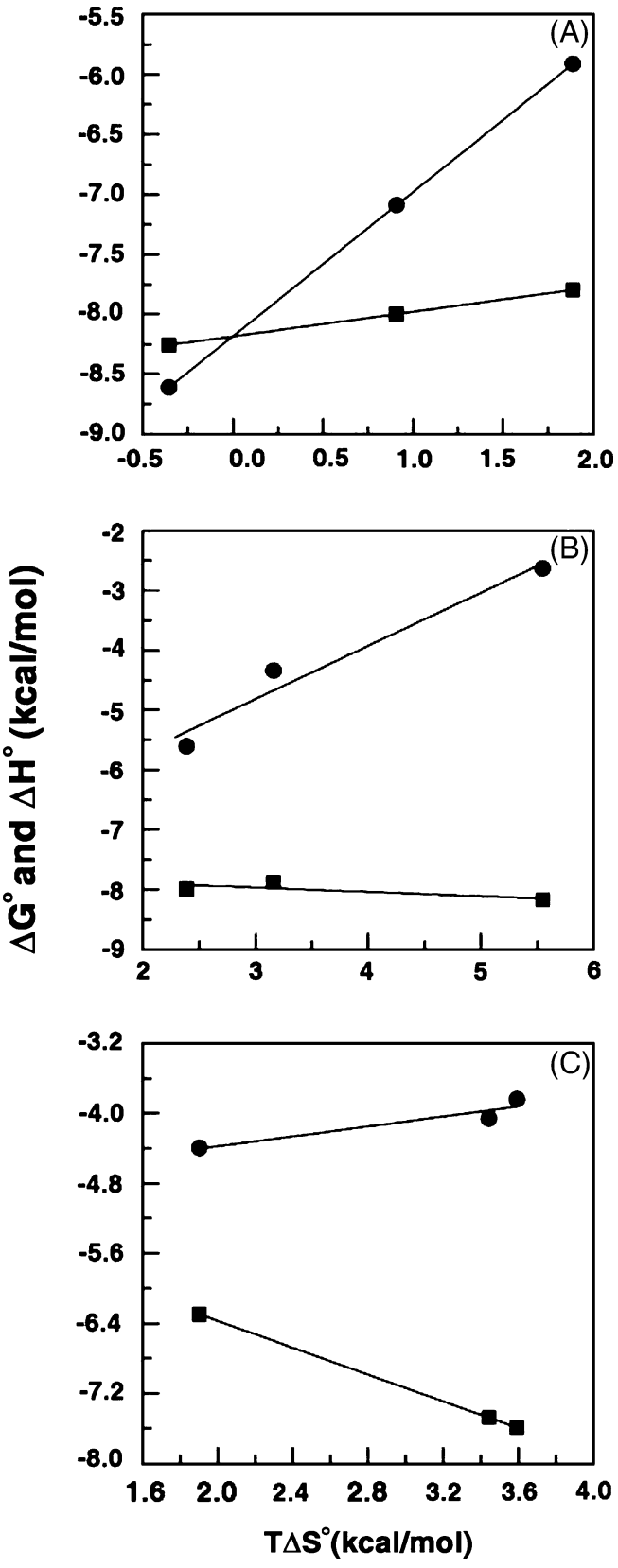

Figure 7. Plot of $\Delta G°$ (closed square) and $\Delta H°$ (closed circle) versus $T\Delta S°$ for the binding of sanguinarine with (**A**) poly(A).poly (U), (**B**) poly(I).poly(C), and (**C**) poly(C).poly(G). Almost complete compensation is observed in poly(A).poly(U) and poly(I).poly (C). Roy Chowdhuri *et al.* (2010). Reproduced by permission of the Royal Society of Chemistry (*www.rsc.org/molecularbiosystems*).

cooperatively to the triplex as evidenced from the Scatchard plots derived from absorbance and fluorescence data. The affinity of berberine and palmatine was of the order of 10^5 M^{-1}, while that of coralyne was one order higher as inferred from spectroscopic studies. The binding affinity values reported were ($1.6\pm0.4\times10^5$ M^{-1}), ($8.0\pm0.3\times10^5$ M^{-1}) and ($4.0\pm0.1\times10^6$ M^{-1}), respectively, for berberine, palmatine and coralyne. Thus, the binding affinity was remarkably higher for coralyne compared to those of palmatine and berberine revealing a higher affinity of coralyne with the triplex. The alkaloids were also shown to stabilize the Hoogsteen base-paired third strand of the triplex without affecting the stability of the duplex. The enhancement of the triplex dissociation temperature (ΔTm_1) was 12.4°C for coralyne compared to 10.2°C for berberine and~11.0°C for palmatine. The melting profiles of the triplex in presence of the alkaloids are presented in figure 8. Fluorescence quenching and viscosity studies were utilized to advance convincing evidence for the partial intercalation of berberine and palmatine and a true intercalative binding of coralyne to the triplex. This was further supported from the significant polarization of the emission spectra of the complex and the energy transfer from the base triplets to the alkaloids. Circular dichroism studies suggested that the conformation of the triplex was perturbed significantly by the binding of the alkaloids, being more in presence of coralyne compared to berberine and palmatine. Further evidence was also provided from the generation of strong induced optical activity in the triplex bound coralyne molecules. Isothermal titration calorimetric studies revealed that the binding to the triplex was favoured by a predominantly large negative enthalpy change ($\Delta H°=-5.42$ kcal/mol) with small favourable entropy contribution ($T\Delta S°=2.02$ kcal/mol) for berberine, favoured by almost equal negative enthalpy ($\Delta H°=-3.93$ kcal/mol) and entropy changes ($T\Delta S°$) 3.89 kcal/mol) for palmatine and driven by predominant entropy contributions ($\Delta H°=-1.84$ and $T\Delta S°=7.44$ kcal/mol) for coralyne. Typical ITC thermograms of berberine, palmatine and coralyne binding to the triplex RNA are presented in figure 9. The results of this study revealed that the alkaloids berberine and palmatine bound to the triplex by partial intercalation, while coralyne exhibited a true intercalative interaction. High binding affinity and intercalation of coralyne to the RNA triplex was also confirmed by Garcia, Secco and colleagues (Biver *et al.* 2009). The work also showed that coralyne induced triple helical formation in duplex poly (A).poly(U). These results advance our knowledge on the

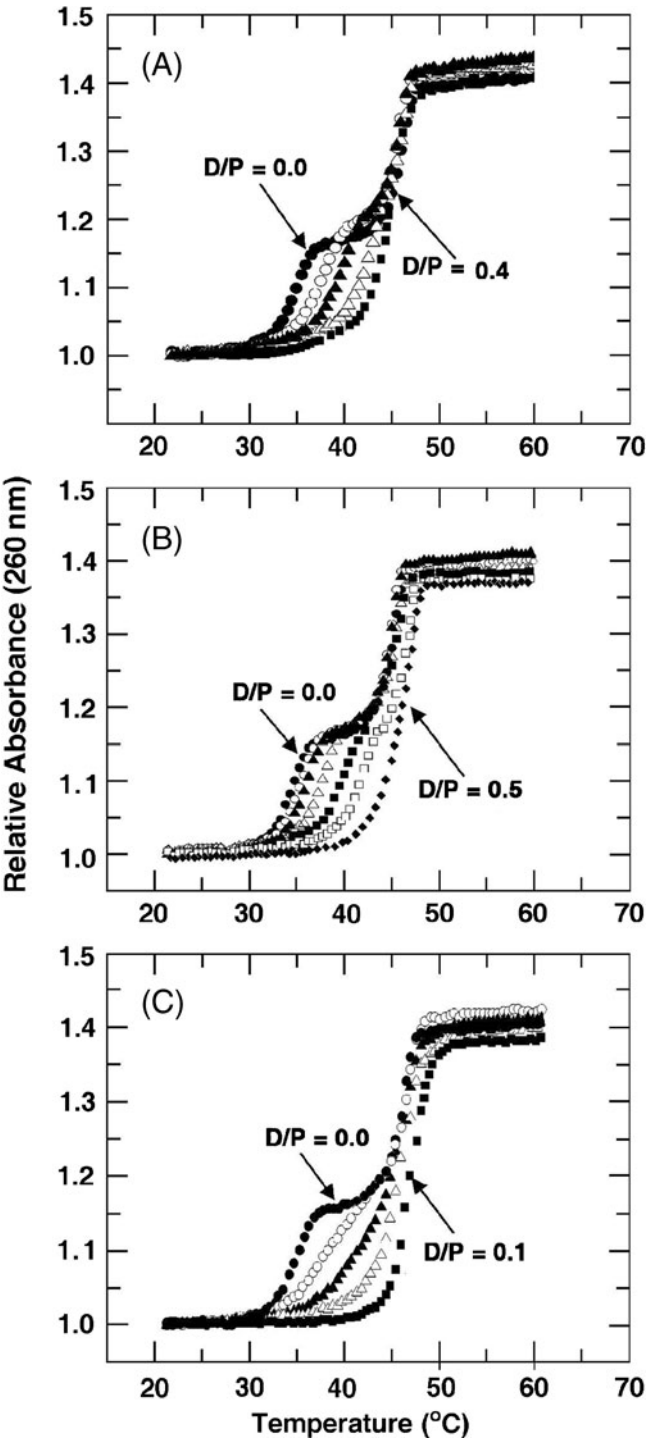

Figure 8. The melting profiles of the triplex in presence of the alkaloids. (**A**) berberine (**B**) palmatine (**C**) coralyne. Reprinted with permission from Sinha and Suresh Kumar (2009) © 2009, American Chemical Society.

binding of small molecule protoberberine alkaloids that are specific binders of RNA structures, particularly triplexes.

8. Binding to poly(A)

In eukaryotes all mRNAs have a single-stranded (ss) polyadenylic acid tail that consists of about 200–250 adenosine residues at the 3′-end. The poly(A) tail is an important determinant in the maturation and stability of mRNA and promote the mRNA's translational efficiency. A schematic representation of the transcription and translation process in eukaryotic genome is presented in figure 10. Polyadenylation of the mRNAs is catalysed by the enzyme poly(A) polymerase (PAP), and it has been observed that in human cancer cells there is an unusual level of overexpression of neoPAP, the poly(A) polymerase enzyme that catalyses poladenylation (Topalian *et al*. 2001, 2002). Thus, if small molecules could be developed that can bind specifically to poly(A) tail, then they may turn out to be suitable agents that could control mRNA function and in turn gene regulation, leading to a new therapeutic route for cancer prevention. Alternatively, the termination of the polyadenylation process may be effected by the formation of double-stranded (ds) poly(A) structures. It is known that poly (A) has the unique capability of transforming from single- to double-stranded conformation in a narrow pH range (Rich *et al*. 1961; Petrovic and Polavarapu 2005). Recently many small molecules have been reported to induce such single- to double-strand conformational change at physiological pH (Xing *et al*. 2005; Giri and Suresh Kumar 2007; Centikol and Hud 2009; Islam *et al*. 2011; Hossain *et al*. 2012b). The first report on the high binding of berberine to ss poly(A) was that of Maiti and colleagues, where a higher binding compared to double-stranded DNA was reported (Nandi *et al*. 1990; Yadav *et al.* 2005). Although these investigators detected high affinity, no self-structure formation was observed. Similarly, more recently, a strong binding of palmatine to ss poly(A) was also reported (Giri *et al*. 2006a, b). The first small molecule reported to induce a self-structure was coralyne (Xing *et al*. 2005). Sanguinarine, the natural alkaloid, binding to poly(A) was suggested to induce self-structure as evidenced from absorbance and CD melting, and DSC experiments (Giri and Suresh Kumar 2007). A typical absorption pattern and CD melting profile of sanguinarine–poly(A) complexation is presented in figure 11. The energetics of the interaction was also studied in details from calorimetric experiments. Subsequently, a number of studies on various intercalators and groove binders have been undertaken in order to understand the molecular basis of self-structure formation (Giri and Suresh Kumar 2008a, b, c, 2009, 2010a, b; Hossain *et al*. 2012b). It was argued that cooperative binding, intercalation and planarity may be very important factors in self-structure induction by small molecules. It was believed that berberine and palmatine due to their buckled structure are not able to intercalate

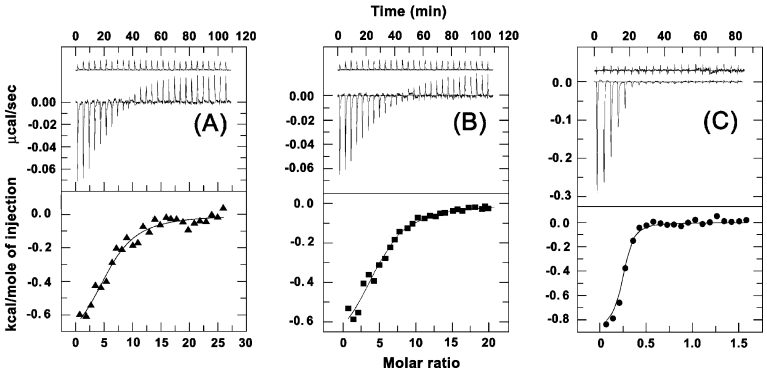

Figure 9. ITC thermograms of (**A**) berberine, (**B**) palmatine and (**C**) coralyne binding to poly(U).poly(A)*poly(U) triplex. Reprinted with permission from Sinha and Suresh Kumar (2009) © 2009, American Chemical Society.

and induce such structural organization in poly(A). But, by a novel dilution method, Hud's laboratory reported that berberine also induced such duplex structure although spectroscopic evidences such as CD and UV melting was not presented (Centikol and Hud 2009). More recently from careful experimental methods involving CD melting, absorbance melting and DSC studies, it was proved that berberine and many 9-substituted analogues induced self-structure formation in poly(A) (Islam *et al*. 2011). Surprisingly, aristololactam-β-D-glucoside was shown not to induce self-structure in poly(A) (Das *et al*. 2011a). Its binding to ss poly (A) was non-cooperative and weak (affinity of the order of 10^4 M^{-1}). It was also reported that all the protoberberine alkaloids that bound with high affinity to ss poly(A) had much lower binding affinity (10^4 M^{-1}) to the low-pH-induced ds poly(A) structure (Giri and Suresh Kumar 2008a, c). Although at present many alkaloids of the protoberberines group and other molecules are suggested to induce self-structure in ss poly(A), the exact reason for such structural organization and the factors that control such structural rearrangements are unclear. Nevertheless, poly (A) binding and self-structure induction by small molecules opens up new avenues for modulating gene expression and the development of newer RNA targeted anticancer agents.

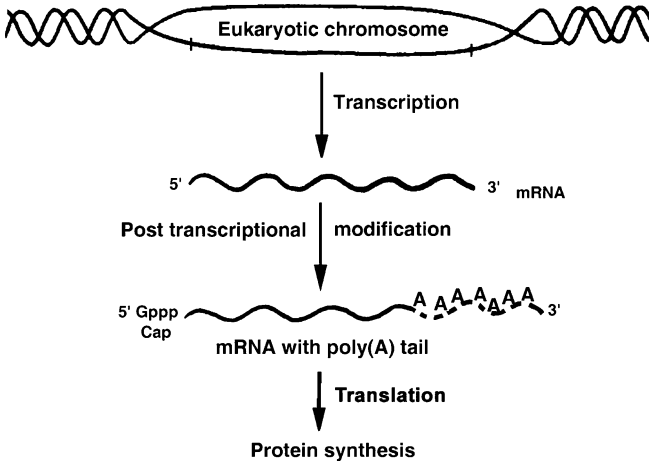

Figure 10. A schematic representation of the transcription and translation process in eukaryotic genome. Reprinted with permission from Bhadra and Suresh Kumar (2011) © 2010 Wiley Periodicals, Inc.

9. Conclusion and perspectives

In the last decade herbal medicines have gained considerable attention over the others. Consequently medicinal plants continue to play a significant role in the life and health of humans. Isoquinoline and aristolochia alkaloids represent a remarkably interesting group of natural products extensively distributed in many botanical families with wide-ranging biological applications that are yet to be understood and exploited for human utility. In order to fully explore their medicinal potential and develop them as futuristic drugs of effective utility, their interaction with biological macromolecules must be understood. A number of studies on the DNA binding aspects are now known, but binding to RNAs and proteins are still not clearly understood. RNA being the current focus for therapeutic drug targeting, this review presented a consolidated account of the rather small literature of these alkaloids interaction to various RNA structures. It has been found from the studies that these alkaloids bind strongly to various RNA structures, stronger than its binding to ds DNAs. In particular, the results in terms of the importance of planarity for stronger interaction and the remarkable role of non-polyelectrolytic forces in the interaction of these

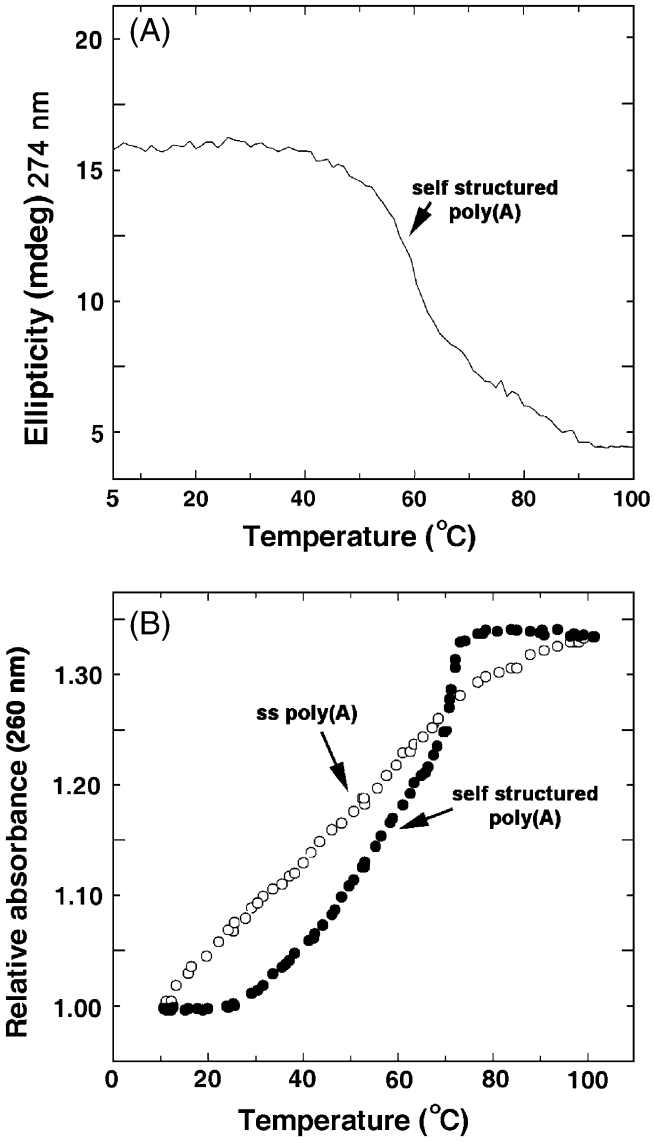

Figure 11. Absorption (**A**) and CD melting (**B**) profiles of self-structured poly(A) induced by sanguinarine. Reprinted with modification with permission from Giri and Suresh Kumar (2007), with permission from Elsevier.

charged molecules are interesting and intriguing. The stronger preference for the ds RNAs against the ss RNAs and the self-structure formation in poly(A) described for many isoquinolines alkaloids may turn out to be a potential route for specific binding to RNA *in vivo* and thereby control of the gene expression. In conclusion, the differential binding affinities, cooperativity, importance of planarity, etc., revealed in the RNA binding data may be useful for the design of newer and more effective anticancer and antiviral agents.

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