Isoquinoline Alkaloids and their Binding with Polyadenylic Acid: Potential Basis of Therapeutic Action

Prabal Giri*,# and Gopinatha Suresh Kumar*

Biophysical Chemistry Laboratory, Indian Institute of Chemical Biology, CSIR, Kolkata 700032, West Bengal, India

Abstract: After fifty years of DNA targeting through intercalators and groove binders and related studies now the current focus is in RNA targeting. Polyadenylic acid [poly(A)] tail of mRNA has been recently established as a potential drug target due to its significant role in the initiation of translation, maturation and stability of mRNA as well as in the production of alternate proteins in eukaryotic cells. Isoquinoline group of alkaloids have their importance in contemporary biomedical research and drug discovery programme due to extensive pharmacological and biological activity. Very recently some small molecule alkaloids of the isoquinoline group have been found to bind poly(A) with remarkably high affinity leading to self structure formation. These alkaloids have a high binding affinity towards single stranded poly(A) whereas their binding with double stranded poly(A) is weak. Among the alkaloids discussed here, berberine and coralyne are found to be capable inducing self-structure in poly(A). All the binding phenomena are characterized by electrostatic interaction between RNA and the alkaloids and the mode of binding was revealed as either as full or partial intercalation. This review focuses on the structural and biological significance of poly(A) and the recent developments in the use of plant alkaloids and their synthetic analogs to control the structure and function of this RNA for the development of new alkaloid based molecules specifically targeted to poly(A) structures.

Keywords: Polyadenylic acid, binding, alkaloids, spectroscopy, calorimetry.

INTRODUCTION

Alkaloids are nitrogen-containing bases produced mostly by higher plants during metabolism. They occupy a lead position in the area of applied chemistry and also play an indispensable role in medicinal chemistry. Without having knowledge of the chemical role of alkaloids and their biological mode of action, people were using them as drugs. With the development of science, chemists, physicists, and biologists are not only isolating more and more naturally occurring alkaloids but are also trying to prepare synthetic alkaloids with more potential biological activity. The mode of action of many alkaloids in current clinical use for the treatment of cancer, genetic disorders, and microbial and viral diseases is believed to be based on their highly specific but non-covalent and reversible intercalative binding to nucleic acid structures and subsequent modification of the genetic material [1-3]. Needless to say, isoquinoline alkaloids that are most widely distributed in several botanical families have a long history of use world-wide in folk medicine. They exhibit myriad therapeutic applications [4]. The naturally occurring berberine, palmatine and the synthetic coralyne are the representatives of the isoquinoline group so far known to be medically important. These alkaloids are among the most widely distributed natural alkaloids of the isoquinoline series and they are also the main active components of some Chinese herbal medicines such as *Rhizoma coptidis* and *Cortex phellodendri* [5]. Berberine and palmatine (Fig. **1**, top panel) are distributed in many plants like Chinese herb huanglian *(Coptis chinesis*), goldensal (*Hydrastis canadensis), Turkish berberis* and the roots of species belonging to the Malagasy genus *Burasaia*, *Menispermaceae* [6,7]. Berberine and palmatine bear the same tetracyclic structure but differ in the nature of the substitutents on the benzo ring (ring A, Fig. **1,** top panel), being methylene dioxy for berberine and dimethoxy for palmatine. Unlike berberine and palmatine with buckled structure (due to partial saturation in the ring B, Fig. **1,** top panel), coralyne is a planar molecule. The biological activities of these isoquinoline alkaloids have been recently reviewed [8-13]. The physicochemical characteristics of these alkaloids presented in Table **1** provide an understanding of the similarity and differences in their chemical structures and properties.

The biological importance of the nucleic acids raises the necessity of investigations on their structural and conformational aspects through interaction with ligands. Recent progress in molecular biology and biotechnology has created many opportunities for the development of nucleic acids-based therapeutics for the treatment of genetic and acquired diseases [14-18]. DNAs and more recently RNAs have been the focus of such drug targeting. More recent discovery of the many micro-RNAs and knowledge of their critical roles in essential cellular activities have led to a paradigm shift from DNA to RNA as the focus of drug targeting to control genetic activity. The recent anti-viral drug design is based on targeting RNA molecules, especially to unique structural

*Address correspondence to these authors at the Biophysical Chemistry Laboratory, Indian Institute of Chemical Biology (IICB), Kolkata 700032, West Bengal, India; Tel: +91 33 2472 4049; Fax: +91 33 2472 3967;
E-mail : gskumar@iicb.res.in
Tel: +91 33 2499 5798/ 5723; Fax: +91 33 2472 3967;
E-mail: prabalgiri@yahoo.co.in

#**Present address:** Department of Chemistry, Guskara Mahavidyalaya, Guskara, Burdwan 713128, West Bengal, India; Tel: +91 03452 255105 ext. 27; Fax: +91 03452 257635; E-mail: prabalgiri@yahoo.co.in

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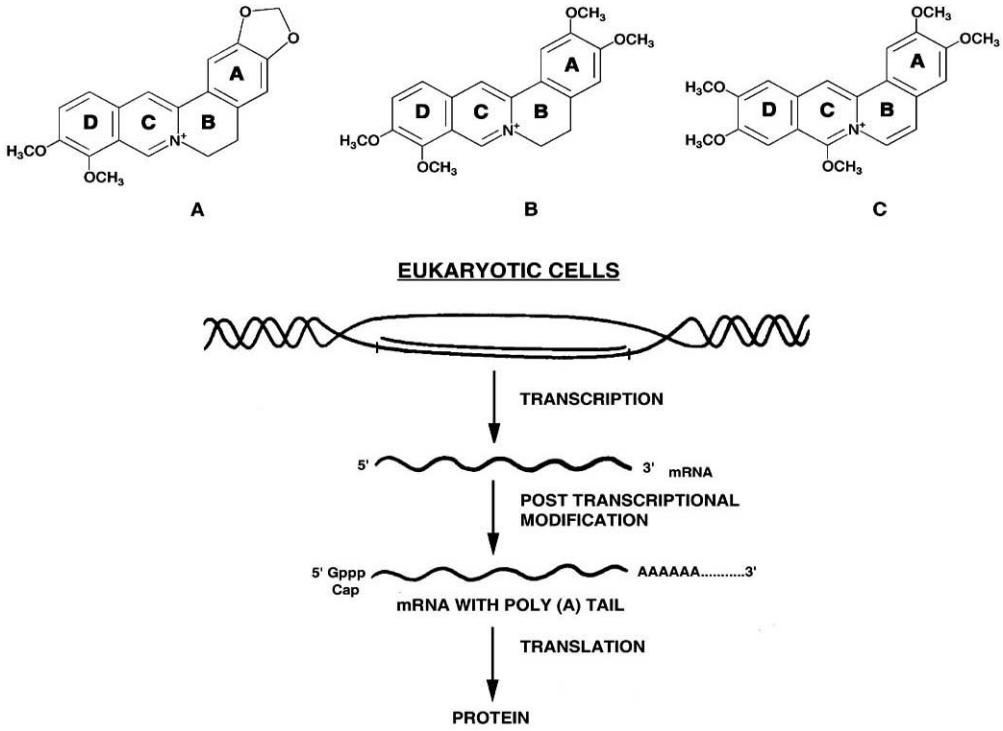

Fig. (1). Top panel: Chemical structures of (**A**) Berberine, (**B**) Palmatine and (**C**) Coralyne. Bottom panel: A schematic diagram for transcription process in eukaryotic cells. (Reprinted from Yadav *et al*. [52] *Bioorg. Med. Chem*., **2005**, *13*, 165-174. © 2005, with permission from Elsevier).

Table 1. Some Physicochemical Properties of Isoquinoline Alkaloids[a]

Properties	Berberine	Palmatine	Coralyne
Empirical formulae	$C_{20}H_{18}O_4N^+$	$C_{21}H_{22}O_4N^+$	$C_{22}H_{22}O_4N^+$
Chemical name	7,8,13,13a-tetradehydro-9,10-dimethoxy-2,3-methylenedioxy berberinium	7,8,13,13a-tetradehydro-9,10-dimethoxy berberinium	5,6,7,8,13,13a-hexadehydro-8-methyl-2,3,10,11-tetramethoxy berberinium
Crystal colour	Yellow	Canary yellow	Dark yellow
Solubility	water	water	ethanol
Molecular weight (ion)	336.36	387.9	364.42
Melting point (°C)	210 (chloride salt)	221 (chloride salt)	250-252 (chloride salt)
Peak position of absorption spectrum (nm)	230, 267, 344 and 420 (in aqueous buffer)	232, 268, 344.5 and 420 (in aqueous buffer)	219, 231, 300, 311, 326, 360, 405 and 424 (in 30% ethanol) 219, 231, 300, 311, 326, 360, 405 and 420 (in aqueous buffer)
Peak position of fluorescence emission spectrum	very weak, at 530 nm	very weak, at 530 nm	Strong, at 475 nm
Optical rotation $[\alpha]_D$ (solvent)	0° (H_2O)	0° (H_2O)	0° (H_2O and 30% ethanol)
IC_{50} (in mice)	27.5 mg/Kg	65 mg/Kg	40 mg/Kg
Molar extinction coefficient (ε) (M^{-1} cm^{-1})	22,500 at 344 nm	25,000 at 344.5 nm	14,500 at 420 nm (in aqueous buffer) 17,500 at 424 nm (30% ethanol)

[a]For references see [86-88].

regions in RNA such as mRNA so that new drugs could be potentially designed for regulation of gene expression. Various serious human diseases like HIV, AIDS, hepatitis C etc. caused by RNA viruses also necessitates immediate research leading to the designing of new RNA binding compounds for therapy.

In eukaryotes, polyadenylic acid [poly(A)] tail consisting of 200-250 adenine bases at the 3'-end of mRNA occupies a significant role for the initiation of translation, maturation and stability of mRNA as well as in the production of alternate forms of protein [19-24]. Recent discovery of the role of 3'-end formation in splicing and transcriptional termination also gives a special recognition to poly(A) [25-28]. A schematic diagram of the transcription process in a eukaryotic cell is given in the bottom panel of Fig. (**1**). Recently, poly(A) has been recognized as a contributor in increasing length and a platform to recruit mRNA export factors [29]. Polyadenylation of mRNA is a critical cellular event in the transcription process for the maturation of all eukaryotic mRNAs. Polyadenylation is catalyzed by the enzyme polyA polymeraze (PAP). Neo-PAP, a recently identified human poly(A) polymerase, is significantly over expressed in human cancer cells in comparison to its expression in normal or virally transformed cells, and may represent a tumor-specific target [30, 31]. Thus, molecules capable of recognizing and binding to the poly(A) tail of mRNA might interfere with the full processing of mRNA by PAP and switch off protein synthesis suggesting poly(A) tail as a potential malignancy specific target [32-35]. Polymorphic conversion of poly(A) to double stranded form from single stranded form depending on pH and salt concentration also makes it a potential target for chemico-biological investigations [36-39]. The parallel double stranded poly(A) conformation was formed by the pairing of adenine bases at the N1 position. It has been suggested that such double helix formation in eukaryotic cell provides the basis of polyadenylation termination of mRNA, autoregulation of cytoplasmic poly(A)-binding protein (PAB I) and stabilization of AU-rich region containing mRNA by embryonic lethal abnormal visual (ELAV)-like proteins [40]. It was shown that the G-rich region of the SV40 L poly(A) signal is likely to be capable of forming G-quadruplexes [41]. Adenine protonation in domain B of the hairpin ribozyme has been shown to result in the stabilization of the loop structure of the RNA enzyme [42]. This review summarizes the biophysical characterization with biological perspectives on the interaction of isoquinoline plant alkaloids with single and double stranded poly(A) in the present context of this RNA based therapeutics .

ISOQUINOLINE ALKALOIDS

Berberine-poly(A) Interaction

Berberine (7,8,13,13a-tetradehydro-9,10-dimethoxy-2,3-methylenedioxy berberinium) (Fig. **1A**) is the most widely known important member of the isoquinoline group of alkaloids. The alkaloid is distributed in several botanical families exhibiting myriad therapeutic applications. It exhibits antisecretory, antiinflammatory, antibacterial, antimalarial as well as anticancer activities with low cytotoxicity [43-45]. Berberine has also been established as a drug in the treatment of dermal leishmaniasis, gastroenteritis in children and cholera disease [46]. The alkaloid induces apoptosis in HL-60 leukemia cells [44]. The DNA binding affinity of this alkaloid was studied extensively and it was reported that it binds DNA by partial intercalation exhibiting adenine-thymine base pair preference [47-50]. Maiti and coworkers first observed a stronger affinity of berberine molecules to single stranded poly(A) over B-DNA and tRNA as revealed from absorption, fluorescence and circular dichroism studies [51]. A detailed study on the interaction with poly(A) was subsequently published by Maiti and colleagues (2005) [52]. The binding process exhibited typical hypochromic and bathochromic effects in the absorption spectrum of berberine (Fig. **2A**), enhancement of fluorescence intensity of berberine (Fig. **2B**), increase of relative specific viscosity (Fig. **2C**) and perturbation of circular dichroic spectrum of poly(A) with generation of strong induced CD bands (Fig. **2D**) [52]. The non-cooperative binding constant determined from absorption spectral study was (1.60± 0.03) $x10^6$ M^{-1} and was shown to decrease with increasing salt concentration of the medium indicating a strong role for electrostatic interaction in the binding process. The UV melting and CD melting patterns of berberine complexed poly(A) was non-cooperative (not shown) studied further from our laboratory indicating no self-structure induction [53]. More recently, Hud's laboratory investigated the self-structure formation by an indirect method namely diluting/concentrating a sample of poly(A) about the critical concentration [54]. Giri & Kumar (2008) investigated the energetics involved in the binding from calorimetry and it was revealed that berberine binds poly(A) in exothermic manner (ΔH^o = -6.219±1.20 kcal mol^{-1}) with a binding free energy (ΔG^o) of -6.666 kcal mol^{-1} [53,55]. There is very negligible contribution of the entropy term ($T\Delta S^o$ = 0.475±0.015 kcal mol^{-1}) indicating that the binding is clearly enthalpy driven. The large negative binding enthalpy is generally typical for intercalative interaction [56-58]. The DSC thermal melting profile of single stranded poly(A) is neither cooperative nor reversible (ΔH_{cal} = 5.82±1.50 kcal mol^{-1}, ΔH_v = 21.51±1.64 kcal mol^{-1}) with a melting temperature of 54.8 ^oC. In presence of the alkaloid the stability of the polymer was enhanced with a melting temperature of 65.2 ^oC. The thermodynamic parameters of the complexation of the plant alkaloids with single and double stranded poly(A) structure is given in Table **2**. The enthalpy of denaturation has also become higher than that for native RNA indicating appreciable stabilization of the complex. The mode of the interaction has been revealed form the viscosity measurements. The findings on the increase in relative specific viscosity of the helical single stranded poly(A) structure resemble its complexation with B-DNA structure indicating that the strong binding of berberine is responsible for helical stability and an elongation of single stranded poly(A) structure on complexation with berberine [59]. The binding mode was proposed to be significantly different from the original intercalation model of Lerman and is in favor of partial intercalation [60].

The interaction process between double stranded poly(A) and berberine on the other hand has revealed marginal changes in the absorption and fluorescence spectra of berberine and marginal changes in the CD spectrum of poly(A) (not shown) [52]. These observations together with marginal changes in the specific viscosity are indicative of weak binding of berberine to double stranded poly(A). This may be

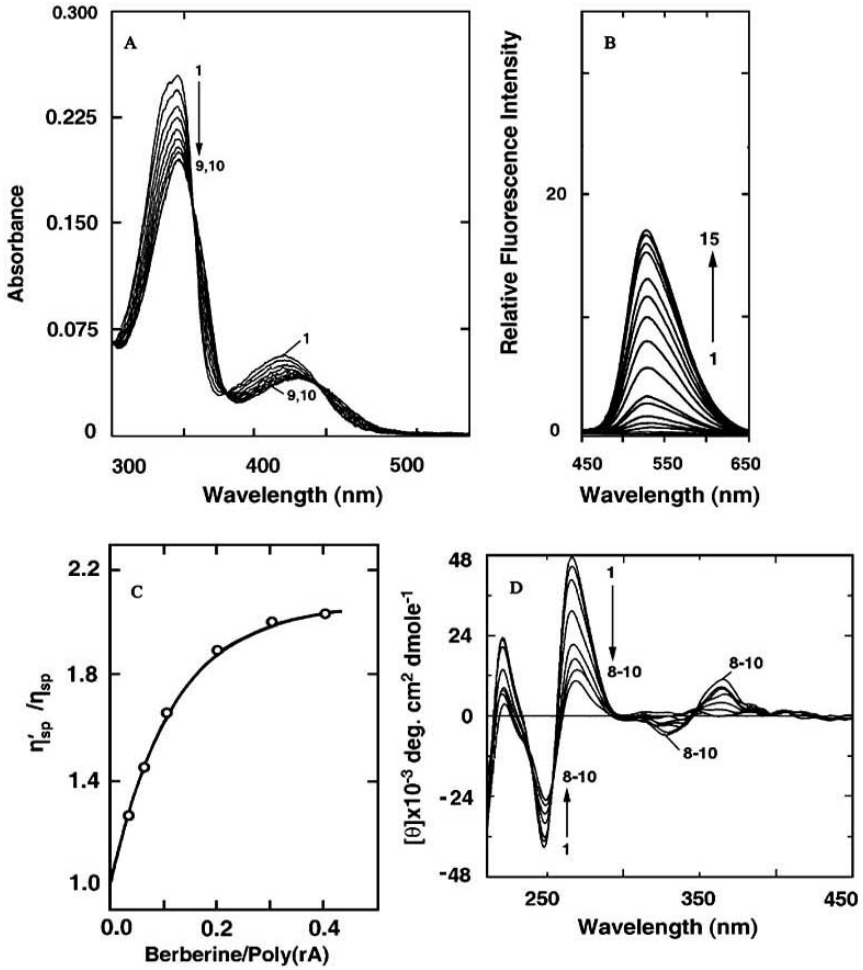

Fig. (2). (**A**) Representative absorption spectral changes of berberine in presence of varying concentrations of single stranded poly(A). (**B**) Representative fluorescence spectral changes of berberine in presence of varying concentrations of single stranded poly(A). (**C**) Plot of change of relative specific viscosity of single stranded poly(A) with increasing concentration of berberine. (**D**) Representative circular dichroic spectral change of single stranded poly(A) with increasing concentration of berberine. (Reprinted from Yadav *et al*. [52] *Bioorg. Med. Chem.*, **2005**, *13*, 165-174. © 2005, with permission from Elsevier).

Table 2. Comparative Thermodynamic Parameters Obtained from ITC and DSC Analysis of Isoquinoline Plant Alkaloids Binding to Single and Double Stranded poly(A)[a]

poly(A) conformation	Alkaloids	K_a (x 10^5 M^{-1})	N	ΔG^o (kcal mol^{-1})	ΔH^o (kcal mol^{-1})	$T\Delta S^o$ (kcal mol^{-1})	T_m (^{o}C)	ΔH_v (kcal mol^{-1})	ΔH_{cal} (kcal mol^{-1})
Single stranded	Berberine	9.79±0.45	0.34	-6.66	-6.219±1.20	0.475±0.015	65.2±1.1	21.31±1.90	18.75±1.45
	Palmatine	8.36±0.26	0.63	-7.99	-8.61±1.40	0.665±0.020	63.4±1.0	22.90±1.78	19.67±2.12
	Coralyne	18.0±3.10	0.24	-8.40	-8.30±0.60	0.10±0.02	57.8±1.1	22.38±1.32	22.30±1.66
Double stranded	Berberine	NA	NA	NA	NA	NA	66±1.0	21.50±1.66	21.78±1.37
	Palmatine	NA	NA	NA	NA	NA	66.5±1.0	22.59±1.43	22.34±1.62
	Coralyne	6.57±0.67	0.25	-7.85	-4.76±0.95	3.09±0.52	70.0±1.2	24.73±1.62	35.87±1.12

NA: Data not available.

[a]For references see [33, 34, 52, 53, 55, 66, 67].

attributed to the non-planar buckled structure of berberine that may be actually resisting complexation with the double stranded structure of poly(A).

Palmatine-poly(A) Interaction

Palmatine (7,8,13,13a-tetradehydro-9,10-dimethoxy berberinium) (Fig. **1B**) is a naturally occurring alkaloid and close analogue of berberine. Palmatine occurs to a lesser amount compared to berberine in plants. This alkaloid has been shown to exhibit significant antitumor activity against HL-60 leukemic cells and has antimicrobial properties [4]. Inhibition of reverse transcriptase has also been suggested to be one of the many reasons for the antitumor activity of palmatine [61]. Pharmacological activities of the alkaloid also include antipyretic, antifungal, hepatoprotective and vasodilatory effects [62-65]. The molecular aspects of the interaction of palmatine with single and double stranded poly(A) have been revealed first time from our laboratory [66,67]. High selectivity of palmatine towards single stranded poly(A) was revealed from the competition dialysis assay, a new, powerful and effective tool for the discovery of ligands with specificity to bind nucleic acids based on the principle of equilibrium dialysis [68,69]. The striking result from this experiment was the selective binding of palmatine to single stranded poly(A) (Fig. **1**). The interaction process was characterized by the hypochromic and bathochromic effects in the absorption spectrum of palmatine (Fig. **3A**), enhancement of fluorescence intensity of palmatine (Fig. **3B**), the increase in relative specific viscosity (Fig. **3C**) and perturbation of circular dichroic spectrum of poly(A) with concomitant generation of induced CD bands (Fig. **3D**) [67, 68]. The binding of the alkaloid to single stranded poly(A) was non-cooperative and the binding constant evaluated by the analysis using excluded site model of McGhee-von Hippel (1974) was in the order of ~10^5 M^{-1} [67,70]. The effect of [Na^+] ion concentration on the binding process revealed the significant role of electrostatic forces in the complexation. The energetics of the binding was studied from thermodynamic estimation from both van't Hoff's analysis of the temperature dependent binding constants and isothermal titration calorimetry and a good correlation was obtained between them. Exothermicty as well as enthalpy predominance of the binding was revealed from these studies (Table **2**). The thermodynamic parameters describing the binding reactions of small molecules to nucleic acids may have contributions from the molecular interactions between the bound ligand and nucleic acid binding site as a result of hydrogen bonding and hydrophobic interactions, arising from the conformational changes in either the nucleic acid or drug upon binding and or from processes like ion release, proton transfer or change in the water structure. A partial intercalative binding mode of the alkaloid was suggested from viscosity measurements [67].

The binding of palmatine to double stranded poly(A) was on the other hand very weak and the binding constant evaluated from the Benesi-Hildebrand analysis of the absorbance titration data was in the order of 10^2 M^{-1} [67,71]. The enhancement of the intrinsic fluorescence of palmatine in presence of double stranded poly(A) was very low compared to single stranded poly(A) (not shown). Marginal changes in the CD spectrum of poly(A) on complexation with the alkaloid revealed no change in the secondary conformation of ds poly(A). The drastic differences of interactions of palmatine with single and double stranded poly(A) may be attributed to its non-planar structure.

Coralyne-poly(A) Interaction

Coralyne [5,6,7,8,13,13a-hexadehydro-8-methyl-2,3,10, 11-tetramethoxy berberinium] (Fig. **1C**) is a synthetic isoquinoline alkaloid and structural analogue of berberine. Unlike berberine with buckled structure, coralyne is planar. The alkaloid is particularly noticed for its excellent antileukemic activity and dual poisoning of topoisomerase I and II [72-75]. Coralyne has a high tendency to aggregate and this property was an inhibition for several years in understanding its nucleic acid binding aspects. The high tendency of aggregation was solved by the use of 30% alcoholic buffer by Maiti and colleagues that enabled detailed investigation on its DNA binding establishing true intercalative binding with guanine-cytosine base pair specificity from a variety of studies [76,77]. Coralyne and its derivatives have been reported to be less toxic and have high antitumor activities compared to other protoberberine alkaloids [32,76,77]. The topoisomerase I poisoning and the DNA interaction properties of coralyne derivatives have been correlated where a model involving intercalation and groove binding depending on the state of saturation of the ring system has been suggested [72]. The alkaloid has been reported to induce self-structure formation in single stranded poly(A) [32,53,54]. The selectivity of this alkaloid to poly(A) was revealed from the competition dialysis assay where pronounced binding of coralyne to poly(A) was observed at the equilibrium [32]. Our laboratory reported that the free coralyne absorption spectrum had a maximum around 420 nm, whereas the bound one had two local maxima at 412 and 435 nm respectively (Fig. **4A**), consistent with the previous results obtained from Xing *et al*. [32,53]. Xing *et al.* have first reported that coralyne binds to poly(A) non-cooperatively with a K_a of $1.8x10^6$ M^{-1} at pH 7. In presence of coralyne, the circular dichroic spectrum of poly(A) underwent significant changes with generation of induced CD (Fig. **4B**). The strong evidence for the self-structure induction was the cooperative CD melting of the complexed poly(A) with a melting temperature of 60 ^oC (Fig. **4C**) reported from the work of Xing *et al.* as well as from our laboratory [32, 53]. The self-structure was proposed to be antiparallel duplex, which is recently supported further from the work of our laboratory [55]. The binding stoichiometry determined from the Job plot analysis was two base pairs per alkaloid indicating a true intercalative mode of binding of the alkaloid to self-structured poly(A) obeying the nearest neighbor exclusion principle. The cooperative UV melting profile of coralyne-poly(A) complexes was favored self-structure induction (not shown). The energetics of the binding was evaluated from isothermal titration calorimetry, which indicated that the binding was enthalpy driven (ΔH^o= -8.3±0.6 kcal mol^{-1}) with binding free energy of -8.40 kcal mol^{-1} (Table **2**). Our laboratory also revealed the cooperative reversible melting from differential scanning calorimetry (Fig. **4D**) where the calorimetric and van't Hoff enthalpy was found to be identical for the complex. It may be noted that coralyne was reported to induce such secondary conformation changes in poly(dA) and homo-adenine polymers

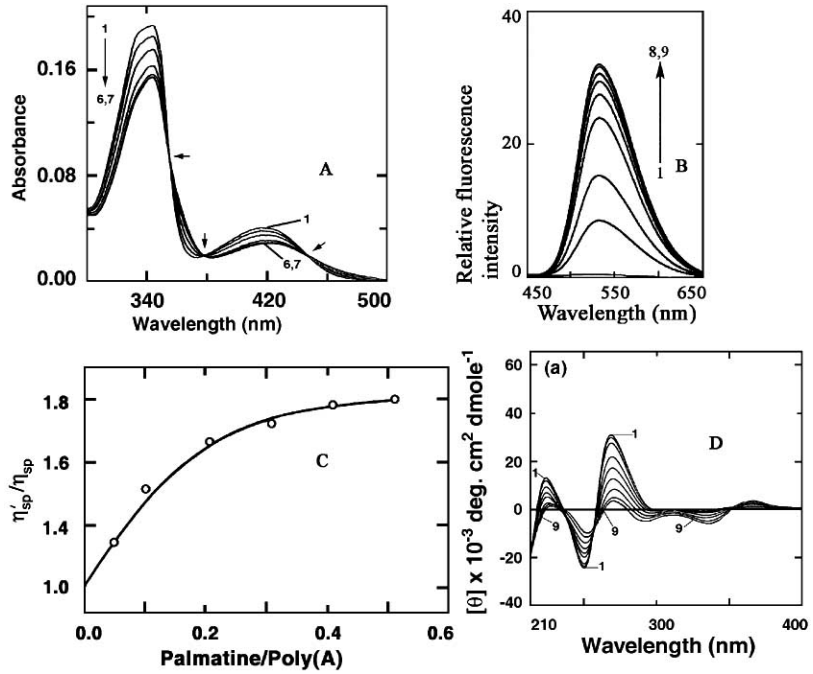

Fig. (3). (**A**) Representative absorption spectral changes of palmatine in presence of varying concentrations of single stranded poly(A). (**B**) Representative fluorescence spectral changes of palmatine in presence of varying concentrations of single stranded poly(A). (**C**) Plot of change of relative specific viscosity of single stranded poly(A) with increasing concentration of palmatine. (**D**) Representative circular dichroic spectral change of single stranded poly(A) with increasing concentration of palmatine. (Reprinted from Giri *et al.* [66] *Bioorg. Med. Chem. Lett.*, **2006a**, *16*, 2364-2368 and Giri *et al.* [67] *Int. J. Biol. Macromol*, **2006b**, *39*, 210-221. © 2006, with permission from Elsevier).

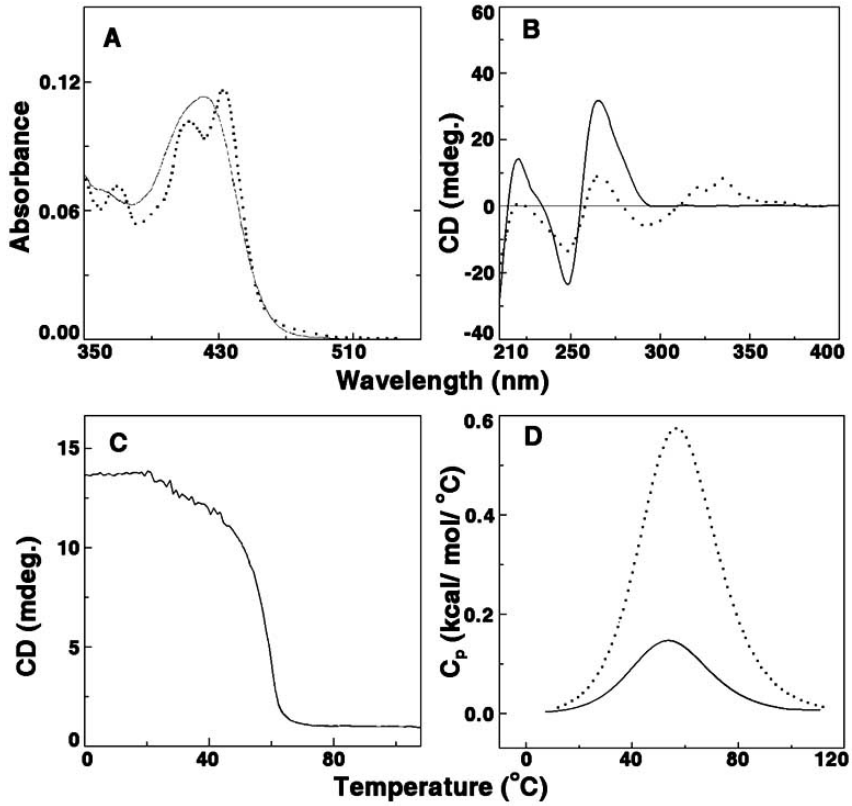

Fig. (4). (**A**) Representative absorption spectral changes of coralyne in absence (dashed line) and in presence (solid line) of single stranded poly(A). (**B**) CD spectra of single stranded poly(A) in absence (solid line) and in presence (dashed line) of coralyne. (**C**) CD melting profile of solution containing complex of poly(A) and coralyne. (**D**) DSC thermogram of poly(A) (—) and complex of poly(A) and coralyne (…) in cacodylate buffer. (Reprinted from Giri and Kumar [53] *Arch. Biochem. Biophys*, **2008**, *474*, 183-192. © 2008, with permission from Elsevier).

also [78,79]. Recently Hud's laboratory has proposed that coralyne induced homo-(dA) duplex structure adopts a Watson-Crick Hoogsteen [*trans*WH] geometry as evidenced from molecular simulation and base substitution experiments [80]. Although the molecular basis underlying self-structure formation in poly(A) by ligands is still unclear, an interplay of size, shape and planarity *inter alia* appears to be critical features for this [34].

Complexation of coralyne with double stranded poly(A) was studied recently in our laboratory using diverse biophysical tools [81]. On binding to poly(A), the absorption spectrum of coralyne underwent hypochromic and bathochromic shifts with an isosbestic point at 438 nm indicating clearly the equilibrium between free and bound alkaloids (Fig. **5A**). The binding constant evaluated from this titration was in the order of ~10^5 M^{-1} with an exclusion parameter of 4 nucleotides (2 base pairs). The fluorescence spectrum of coralyne was characterized by an emission spectrum in the 450-650 nm regions with a maximum around 475 nm when excited at 424 nm. The binding of poly(A), however, remarkably quenched the strong fluorescence of coralyne by about 45% indicating strong association of the alkaloid to poly(A) (Fig. **5B**). The Stern-Volmer quenching constant (K_{sv}) calculated from this data was in the order of ~10^4 L/mol. This high value of the quenching constant indicated a stronger bimolecular binding process between coralyne and poly(A) over other unimolecular photophysical processes. This study again clearly underscored the remarkably high affinity of coralyne to poly(A). Fluorescence quenching studies using anionic quencher, potassium ferrocyanide showed a large decrease in the magnitude of Stern-Volmer constant in presence of coralyne suggesting an intercalative binding of the alkaloid to ds poly(A). Fluorescence polarization experiments provided an effective parameter for investigating dynamic characteristics of coralyne in different microenvironments [82]. In the absence of poly(A), fluorescence of coralyne was weakly polarized (0.014± 0.002) due to the rapid tumbling motion of coralyne in aqueous media. But on binding to poly(A) the fluorescence was significantly polarized (0.320± 0.003) again suggesting intercalation of the alkaloid into the helix. The binding stoichiometry determined from Job plot analysis was four nucleotides bound per coralyne molecule, which was in excellent agreement with the spectrophotometric result. The CD spectrum of poly(A) underwent significant changes in presence of the alkaloid with the generation of induced CD in the 325-400 nm region (Fig. **5C**) for the bound hitherto optically inactive coralyne molecules clearly suggesting the alteration of the poly(A) secondary structure on binding of coralyne on one hand and the strong asymmetric environment of the bound coralyne in the helical organization of poly(A) on the other presumably by intercalative binding. The energetics of the binding was determined from the ITC and DSC studies. ITC profile revealed the binding as exothermic in nature (ΔH^o= -4.76±0.95 kcal mol^{-1}) with a binding affinity of 6.57±0.67 x 10^5 M^{-1}, with a considerable contribution from entropy term ($T\Delta S^o$= 3.09±0.52 kcal mol^{-1}), which was explained in the light of release of solvent molecules in the binding process. The melting of double stranded poly(A) was cooperative and fully reversible (ΔH_v =21.22±1.23 kcal mol^{-1}, ΔH_{cal} = 21.54±1.52 kcal mol^{-1}) with a melting temperature of 64.8±1 ^oC whereas in presence of coralyne, the stability of poly(A) enhanced with a melting temperature of 70±1.2 ^oC and the enthalpy of helix denaturation became several fold higher (Fig. **5D**). The stability enhancement of double stranded poly(A) on complexation with coralyne was also verified by thermal melting studies and was in agreement with the DSC melting studies. Interactions of coralyne with both single and double stranded poly(A) is attributed to its planar structure favoring strong complexation process.

SUMMARY AND SIGNIFICANCE

Over the past decade, herbal medicines have been accepted universally and they have an impact on world health as well as international trade. Hence medicinal plants continues to play an important role in healthcare system of a large number of the world's population. Isoquinoline alkaloids represent an interesting group of the abundant natural products that exhibit remarkably significant and diverse biological activities. In order to exploit these alkaloids as futuristic therapeutic agents, their ability to modulate the nucleic acid structure-function must be understood in details. The rational drug design based on nucleic acids targeting has become an emerging area in medicinal chemistry. Single stranded poly(A) tail is the most important determinant in eukaryotic cells for the mRNA maturation, stability and alternate protein production. The DNA binding of the alkaloids have been studied since several years and more recently their RNA binding potential is getting unraveled. Of the isoquinoline alkaloids discussed in this review coralyne has been reported to bind ss poly(A) strongly over the two other alkaloids which may be attributed to the planarity of coralyne molecule causing a preferable interaction between adenine bases and the ligand molecules. Self-structure formation is very significant with respect to its biological relevance. Coralyne was the first molecule discovered to induce self-structure in single stranded poly(A). The natural analogues, berberine and palmatine could not induce similar effects as tested by circular dichroic melting and other experiments although a different indirect technique involving the dilution/concentration of ligand-poly(A) complexes below or above a critical concentration suggested self-structure formation with berberine also. Although the differences in planarity was thought to be the primary cause to account for this, subsequent studies with a number of molecules have suggested more specific features to be responsible for this. Recently, the mechanism and biological significance of self-structure formation induced by alkaloids in poly(A) was discussed in details in a mini-review published by the same authors [34]. The possibility of the formation of double poly(A) helices in a cell can allow to elucidate the mechanisms of several biological processes that proceed with the participation of poly(A) sequences *viz*. the termination of mRNA polyadenylation, autoregulation of poly(A) binding protein I (PAB I) and stabilization of AU rich region containing mRNA by embryonic lethal abnormal visual (ELAV)- like proteins [40]. The rules governing the molecular recognition of double stranded RNA have drawn increasing attention due to some important biological processes such as RNA interference (RNAi), the interferon-controlled antiviral response and pre mRNA editing [83-85]. The comparative strong binding of coralyne to ds poly(A) over other isoquinoline alkaloids

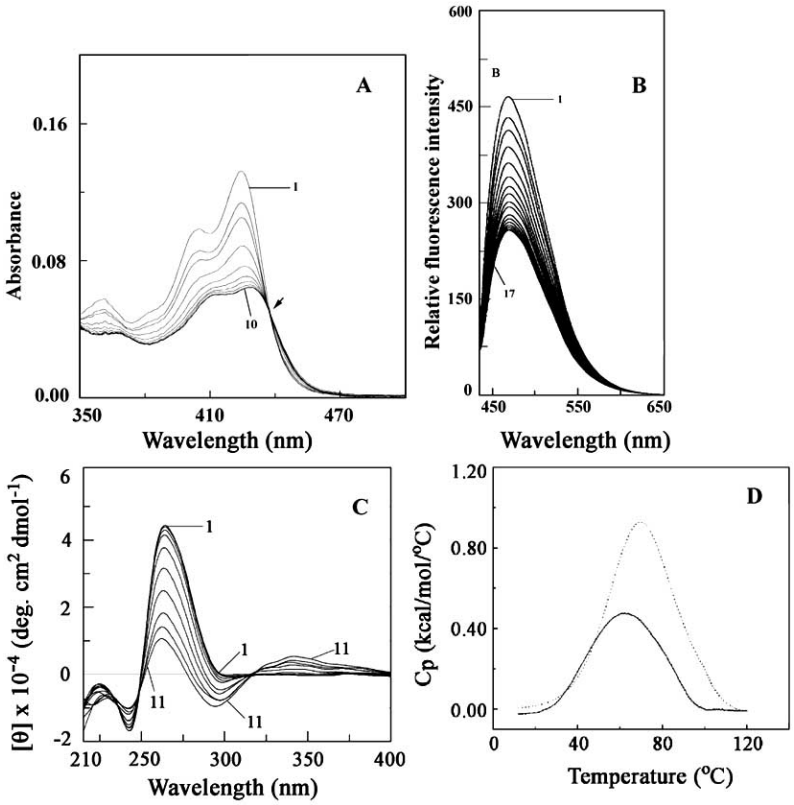

Fig. (5). (**A**) Representative absorption spectral changes of coralyne in presence of varying concentrations of double stranded poly(A). (**B**) Representative fluorescence spectral changes of coralyne in presence of varying concentrations of poly(A). (**C**) Representative circular dichroic spectral change of poly(A) with increasing concentration of coralyne. (**D**) DSC thermogram of poly(A) (—) and complex of poly(A) and coralyne (…)in 10 mM CP buffer, pH 4.5. (Giri and Kumar [80] *Mol. BioSystems*, **2008**, *4*, 341-348. © 2008, Reproduced by permission of The Royal Society of Chemistry).

viz. berberine and palmatine may be explained in terms of the structural difference of the alkaloid. Berberine and palmatine have buckled structure leading to the non-planarity in the molecules whereas their synthetic analogue coralyne is a planar molecule. Thus, there is greater chance of energy transfer from the adenine bases to the alkaloid molecules for coralyne over other two ligands. As a result, the binding is much more prominent for coralyne to poly(A) over other two alkaloids and the planarity in the structure leads to better intercalation of the alkaloid between adenine bases. So the molecules that can specifically target the poly(A) tail can be promising leads for the design of new compounds that might recognize single stranded nucleic acids for the development of therapeutic agents by medicinal chemists. The binding of small molecules to poly(A) is clearly different from the binding of small molecules to a pre-structured nucleic acids like double stranded DNA or tRNA. Thus the differential binding of the plant based alkaloids to poly(A) structures discussed in this review may convey some specific meaning for their regulatory roles in biological processes and opens up a new avenue in anti-cancer as well as anti-viral drug design.

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ABBREVIATIONS

RNA	=	ribonucleic acid
DNA	=	deoxyribonucleic acid
mRNA	=	messenger RNA
tRNA	=	transfer RNA
poly(A)	=	polyadenylic acid
ss	=	single stranded
ds	=	double stranded
PAP	=	the human Poly Adenylic Polymerase
RNAi	=	RNA interference
CD	=	circular dichroism
T_m	=	thermal melting
ITC	=	isothermal titration calorimetry

DSC = differential scanning calorimetry

Cp = molar heat capacity at constant pressure

CP = citrate-phosphate

D/P = alkaloid/RNA nucleotide phosphate molar ratio

P/D = RNA nucleotide phosphate/alkaloid molar ratio

K_{sv} = Stern-Volmer quenching constant.

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