COMPREHENSIVE ASSESSMENT OF THE MODE OF DNA BINDING BY FEW ANTICANCER DRUGS

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Abstract

DNA-interacting anticancer drugs are able to affect the propensity of DNA to interact with proteins through either reversible binding or covalent bond formation. The effect of the drugs on transcription factor interactions with DNA is reviewed. These effects can be classified as *(i) competition between a drug and regulatory protein for target sequences; (ii) weakening of this interaction; (iii) enhancement of this interaction by chemical modification of the DNA and the creation of non-natural binding sites; and (iv) a 'suicide' mechanism, which is observed when a transcription factor induces changes in DNA structure, allowing a drug to bind to a target sequence.* Several new strategies – the antigene approach with oligonucleotides, peptide nucleic acids or locked nucleic acids, and sequence-specific polyamides – are also reviewed. *Most anticancer drugs affect nucleic acid synthesis, structure or function in cells*. As pointed out by Kohn et al.,with few exceptions, they exhibit at least one of the following interactions with DNA: *(i) covalent modification, (ii) blocking or poisoning of a DNA topoisomerase (DNA breakage), (iii) inhibition of DNA and/or RNA polymerases, (iv) mimicking nucleic acid precursors, (v) decreasing the precursor concentration, or (vi) blocking the mitotic spindle. Other DNA-related activities relevant to the mechanisms of anticancer drug actions have also been proposed or identified*. These include altering the acetylation state of histones and transcription factors, affecting the actions of DNA helicase, or altering DNA–transcription factor interactions, targeting telomerase and DNA structures like G-quadruples in telomeric DNA and the AT islands that probably function as matrix attachment. *There are also anticancer drugs which affect transcription processes less directly by inhibition of protein kinases, growth factors or proteasome function, in the latter case sparing proteins which inactivate transcription factors*. These multiple mechanisms of action are in principle related to drug structure and its effective concentration, and may result in either a cytotoxic effect or an inhibition of cellular proliferation and induction of cell differentiation.

The authors review the existing anticancer drugs for which there are experimental data demonstrating that they affect the interactions of transcription factors with their target sequences in DNA. The authors also outline approaches for the design of new compounds with sufficient selectivity to compete with DNA-dependent RNA polymerase and other elements of the transcriptional machinery, and new strategies which are targeted to a specific interaction with the transcription process or transcriptional regulation. These strategies focus on either the ability of transcription factors to interact with DNA, or engage in protein–protein interactions in transcript some structure. Anticancer drugs like tamoxifen, which are targeted to nuclear receptors (i.e., ligand-dependent specific transcription factors), also remain outside the scope of this review.

INTRODUCTION

The approach of rational design of anticancer drugs targeted towards DNA is based on the thorough understanding of DNA binding of drugs. The drugs may either intercalate within sequences of DNA or bind within the grooves to acquire unique DNA sequence specificity. So, it is rather essential to explore the stacking interactions between drug and base pairs of DNA, as well as the donor-acceptor interactions (hydrogen bonding) within the grooves. The anticancer drugs can damage normal double helix DNA resulting in various effects, which are related to biological and clinical properties. At the same time, there are highly toxic drugs that give adverse therapeutic values. The anticancer drugs may bind or interact with DNA in a sequence specific manner. The non-covalent interactions of some promising anticancer drugs (tricyclic and tetracyclic drugs) are found related to therapeutic values. Much interest has been shown to understand the recognition of certain sequences of DNA by these aromatic molecules. In this context, sequence specific interaction of drugs and base pairs of DNA may be noted, in which the planar aromatic molecules may contribute to the stabilization of drug between base pairs. The extent of stabilization of some aromatic drugs has been demonstrated by theoretical studies. It is rather important to understand the concrete binding mode of anticancer drugs for correlation with medicinal property as well as biologically related topics. In order to understand insight into the stabilization of drug through %-n interactions, it is rather essential to estimate the stacking interactions of small aromatic molecules .

The n-n and non-bonded types of interactions can be compared from the stacking interactions of benzene-benzene and pyridine-pyridine molecules. The knowledge of such interactions is essential for comparing the extent of n-% or non-boned stacking interactions of small molecules to that of nucleobases in DNA . The prediction of such stacking and hydrogen bonded interactions are the interest of quantum-chemical studies. However, the inclusion of large basis set in the accurate quantum-chemical calculations is limited, and hence the force field calculations are also extensively used in biomolecular systems . The binding of drug within base pair sequences of DNA might depend on the %- % interaction as well as lone pair interaction. Here, the stacking interaction of two benzene rings can be initially taken for assuring the primary role of %-% type stacking interaction, and then the other larger aromatic molecules may be studied to quantify the extent of stacking interaction with the size of the aromatic rings as well as other lone pair-lone pair interaction. In addition, the use of high level ab initio calculations is must for studying such systems, but it is limited only to small- size molecules. Hence, the ab initio calculations with the inclusion of correlation effects at least the MP2 levels are necessary. So the choice of large basis set in the MP2 calculation is possible for small molecules because large basis set

may be difficult for large molecules due to large computational cost. The comparison of stacked geometries and energies obtained from MP2 calculations with different basis set can be tested with small molecules like benzene and pyridine. Systematic analysis of these calculations may be essential to choose the appropriate basis set in the MP2 calculations of large molecules.

In addition to all these information, the binding of acridine-4-carboxamides within DNA sequences may be analyzed with respect to the types of drug-DNA binding. The drug may intercalate between the sequences of DNA or it may bind within the grooves of DNA. As shown in many experimental studies, the binding of drug with DNA depend on the chemical properties of chromophore, which acts as an intercalator within DNA sequences. The sequence selectivity of various intercalators, as distinguished by the presence of several aromatic rings, can be analyzed with the force field calculations. In this study, the role of various molecular part of drugs, which serve as typical intercalator as well as groove binder are chosen to analyze the DNA sequence selectivity. The model so obtained has been taken for CHARMm force field calculations, and the electrostatic and van der Waals interaction energies are used to understand the sequence selectivity in DNA binding. As we know, the double helix DNA consists of long chain base pair combinations, but the interaction of certain chromophores may be either GC or AT specific. However, there are abundant GC specific drugs, but the AT specific drugs are rarely available. Sometimes the sequence specific intercalations are more important than the side chain binding or vice versa. The well-known anticancer drugs, acridine-4-carboxamides bind within GC rich sequences; however some are highly AT specific. In such situation the dominance of chromosphere intercalation or side chain binding cannot be concisely known. Based on the electrostatic and van der Waals interaction energies, it is essential to identify the effectiveness of chromophore as well as side chain binding. So the molecular models of all types of conformation may be collected for analyzing such drug-DNA interaction.

A typical groove binding drugs, diphenyl diamine (DAPI) are found to be highly AT specific, and usually these drugs bind within the minor groove covering three to four base pair sequences. The molecules are found to possess sufficient torsional flexibility, and also the cationic amidine group can bind with other donor sites of DNA sequence. The molecules can form hydrogen bond with thymine (at 02) and adenine (at N3) in the minor groove of DNA. In such situation, it is not very clear whether the minor groove binding is entirely contributed from the compatible molecular sizes and shapes of drugs with that of minor groove or on the hydrogen bonding ability of amidine groups. The stability of the different types of groove binding drugs is an essential topic in drug discovery. There are various reasons for minor groove selectivity of drugs. Also, the bulky group present in CG base pair in sequences of DNA might prevent the drugs to enter within the minor groove or the conformational accessibility of this drug could be another factor. It has been known that the minor groove of DNA acquires more electron density than the major groove, and any charged drug can be attracted towards this region. Some of the diphenyl diamine drugs bind within mixed sequences, and also very few diphenyl diamine drugs are found intercalated between the base pairs of DNA. So the basis of drug binding within DNA is not very conclusive. Hence the nature of DAPI and DNA binding can be taken up for investigation.

Daunomycin is a promising anthracycline drug, which intercalates within GC rich sequences of DNA. The sugar ring ofthis drug may bind within the minor groove of DNA. The sequence selectivity of this drug can also be analyzed by constructing various intercalated models of this drug. There are other tricyclic intercalating drugs such as Amsa and Acridine-4-carboxamide. Researchers have found that the base pair selectivity of different intercalating drugs is expected to depend on the conformational disposition of rings at the intercalation site. Depending on the steric hindrance and electrostatic interaction of these drugs, it is possible to analyze the selectivity of daunomycin for a base pair sequence on the basis that the strong binders should have better selectivity of certain base pairs. There should be certain advantages oftetracyclic chromophore than the tricyclic chromophore or vice versa. So it is necessary to estimate the interaction ability of tricyclic and tetracyclic drugs. The mode of DNA binding may be related to the therapeutic value as well as other cytotoxic effects of drugs. Most anticancer drugs and many carcinogens bind with DNA through covalent bond. Such analogy of these types of compounds could be explained from the nature of drug binding with DNA. The common features of the carcinogens and anticancer drugs are the sequence selective intercalation with nucleic acid. There are many aromatic carcinogens that contribute similar intercalation with DNA. From the closely related behaviors of these two types of molecules i.e. carcinogens and anticancer drugs, it is extremely important to note that the aromatic anticancer drugs (acridine and daunomycin) can bind with DNA in a similar manner as far as the extent of DNA sequence selectivity is concerned. The stacking of various larger molecules may be studied to explain the different intercalated structures of aromatic drugs within base pair sequences of DNA. There may be additional contribution from the charge of aromatic molecules of certain drugs that exist as cationic or anionic form. Some acridine analogues can exist as cationic and anionic form. The possibility of enhancing stacking interaction due to the charged molecular form is evidenced from the intercalation of acridine analogues within DNA sequences. The contributions of electrostatic interaction of charged species are entirely different from that of neutral molecules. So the origin of the stacking stabilization ofsome selected chromophores may be taken up to distinguish the electrostatic (dispersion type) as well as hydrogen bonding type of interactions. So the study on the tricyclic aromatic compound as well as anthracyclic drugs might indicate some distinguishable features ofsequence selectivity in DNA binding. The understanding of chemical phenomena in different solvent environments has been an important area of research. The fundamental chemical properties, which are well known under certain solution environment may not exactly remain the same under different solution environments. Such physiochemical properties are significantly related to the acid-base equilibria of molecules, which in fact play central role in many chemical reactions when the stability of a molecular form is concerned. The most well known double helical DNA structure can be distorted as a result of the change in the concentration ofions, and water molecules present in solution. In this regard the molecule may undergo prototropic reactions to form various types of base pairs (non Watson Crick), which is usually known as mismatch pairing of the nucleobases. Following this, there might result various consequences that are related to the genetically related diseases such as cancer. There are many contexts about the proton translocation within the helix leading to the tautomerization of nucleobases which is also suggested as another cause of DNA mutation. Again, one of the fundamental issues that contribute important role for the

stabilization of base pairs within the helix is the proton transfer processes. The proton transfer process within double helix DNA may be incorporated with different reactions. Here, the effect of counter molecules present outside the helix can catalyze the prototropic reaction (proton transfer) within AT and GC base pair. The existence of proton transfer mechanism has been shown in many contexts. There are several accurate ab initio studies on this topic. The knowledge of physiochemical behavior within DNA is quite important for analyzing the recognition of this biomolecule by anticancer drugs. On the other hand, there may be other secondary effect on the DNA conformation which may lead to DNA cleavage leading to side effect in the process of mutagenesis. The conformation of DNA may be drastically changed due to strong binding of drugs that might lead to free radical generation.

The mechanistic studies how the stacking interaction of drugs with base pair can affect the proton transfer reactions within double helix DNA are important. The study focuses on the strategies of proton transfer within base pair using different level of theories, and comparison may be made when the drug molecule is incorporated in stacked manner. We aim to compare the extent of stabilization of base pair when the proton is shifted within counter nucleobases. It is believed that the proton transfer phenomenon is a dynamical motion that facilitates the movement of proton between counter nucleobases. The stacking interaction between drug and base pair might disturb the equilibrium proton transfer reaction, and it is expected that the proton may localize at a particular region of drug-intercalated sequence, thereby fail to maintain equilibrium proton motion within base pairs. One such feature that may be exploited in the stacked model of proton transfer is the consistency or deviation of this proton kinetics compared to that of normal base pair. The disturbances on the equilibrium proton transfer kinetics may lead to various forms of tautomers of nucleobases as well as free radical intermediates.

The kinetics of proton transfer reaction consists of certain rate determining steps where the electron and hole transfer processes might take place. So the mechanism of proton coupled electron transfer has been highlighted in both experimental and theoretical studies. The existence of GC anion and cation radicals, and the radicals in the nucleobases along with the tautomeric forms has been evidenced in many contexts. If the formation of such ions is essential during proton transfer kinetics, it is not surprising that the other ions present in physiological environment (the surrounding medium) should compete with the formation of such molecular forms. Moreover the proton transfer reaction is usually assisted by the surrounding ions and hydrogen bonded molecules. There are lots ofinterests in understanding the chemical phenomena in aqueous solution. Usually the fundamental chemical properties ofmolecule depend on the thermodynamically stable forms existed at that environmental condition. As an example, the ion solvation and acid-base equilibria, chemical reactions and also phase equilibra are closely related properties. There are certain challenges in the experimental measurement of acid-base properties of molecules. Determination of relative pKa values of molecules is extremely necessary, because in certain systems one cannot definitely specify the exact molecular form in a particular solvent environment. In such situation the experimentally determined pKa values cannot match with the actual values, while dealing with weak acids and strong bases, hence certain measures should be adopted for predicting pKa of such molecules. Although the change in acid base behavior of molecules in aqueous solution usually affect most reactions, but little analysis has been shown how the pH or pKa can be understood from the Bronsted concept of proton dissociation or association from acid or base.

The dissociation of H2O to H^+ and OH^- is not so commonly accepted whereas the other equation is usually known to all. However the situation is not so simple, as we can see for HCI molecule. In some reactions, the proton may not involve at all, and the acid-base eqilibria would depend on the donor-acceptor type ofreactions in solution. On the other hand, electron donor ability of molecules may not be so easy in aggregated state. Similarly, for the weak acids, the proton may not be completely dissociated, and the relative change of acidity of such molecule relative to H2O may not be accurately calculated as given in the above equation. Again, it might be possible to change the molecular forms after the release of proton, and in that case the use of direct approach for estimating pKa or pH of molecules may not be appropriate. Hence, it is necessary to construct characteristic models for describing the solvation of ions as well as molecules. Here, the quantum mechanical calculations can be used for explaining the complex physiochemical properties of molecule. The prediction of pKa values of drug molecules is very important, since the physiological compatibility of drugs indirectly related to the therapeutic efficiency.

The basic ideas of the nature of the molecules i.e. the drug as well as the nucleobase that exist under different solutions can be primarily understood from the acid-base characteristic of the molecules. So, the theoretical model for estimating the pKa of molecules may be another aspect, herein the dissociation of hydrogen atom from an acid can be considered for predicting the pKa of molecules. There are certain limitations of predicting pKa from basic principles. For handling the complicated molecular properties, the basic concepts of Bronsted and Lewis acid-base theories may be applied for predicting pKa of drugs as well as other relevant drug binding sites. It is apparent that the drug and the target molecule should have comparable pKa values so that the secondary polar effect due to the differences of acid-base characteristics can be eliminated. Depending on the types of molecules and their behavior in solution, the pKa values may be predicted by theoretical methods. The knowledge of such physio-chemical properties ofmolecules are very important for explaining the drug and DNA binding.

The acridine-4-carboxamides are well known anticancer drugs that can intercalate within the sequences of DNA, and also the side chain can bind within the grooves. The prediction of pKa values of these compounds are of interest in relation to drug transport and biological activities. The existence of different molecular forms of aromatic tricyclic ring as well as the side chain can lead to different mechanism of drug action. Although, the pKa of few molecules of this family are well established, but there are substantial debates in literatures to explain why the sudden change of pKa of certain molecules at different solution environment take place. The experimentally reported pKa values are not in concurrent with the expected pKa values. There are many reasons for such unexpected results, but in general the existence of molecules as monomeric or higher aggregated forms are also one ofthe factors. On the other hand, the pKa values ofside chain and chromophore may not be exactly equal, since the dissociation ability of proton

from the donor sites of the chromophore and side chain are found different. Moreover, the conformational change of such large molecule and the formation of certain tautomers should not be neglected in constructing the energy change used in pKa determination. It has been shown that ab initio, DFT and MP2 methods are useful for rationalizing the intrinsic pKa values of molecules. There are other methods like molecular dynamics simulation and QSPR which are found important for better understanding of pKa. Consequently, the application of quantum mechanical methods may be useful for analyzing the pKa properties of certain drugs.

DNA Drug Interaction

Deoxyribonucleic acid, DNA, is a molecule of great biological significance. The total DNA content of a cell is termed the 'Genome'. The 'Genome' is unique to an organism, and is the information bank governing all life processes of the organism, DNA being the form in which this information is stored. Stretches of DNA called 'genes' have the extremely important function of coding for proteins. The function of the rest of the genome, loosely termed as 'non-gene' regions, is not very clearly known.

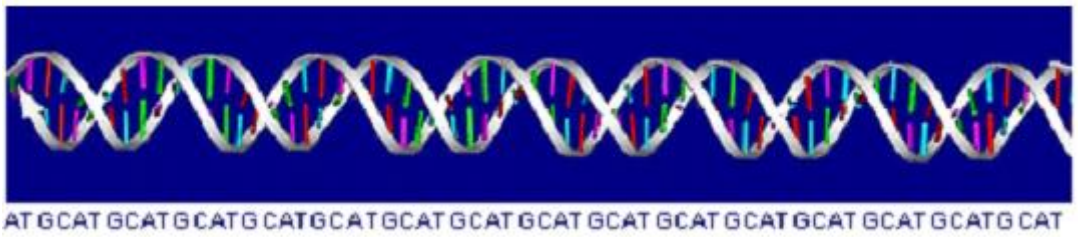


Fig.1 The DNA molecule

DNA has two main functions,

1**. Transcription**: Information is retrieved from the DNA by ribonucleic acid, RNA, and utilized to synthesize proteins in the body. Proteins are involved in all body processes and play many roles. e.g. as hormones, enzymes, carriers, structural proteins, receptors, regulators etc.

2. Replication: DNA is responsible for its own regeneration, i.e., DNA self-replicates.

DNA is present in the body in the form of a double helix, where each strand is composed of a combination of four nucleotides, adenine (A), thymine (T), guanine (G) and cytosine (C). Within a strand these nucleotides are connected via phosphodiester linkages. The two strands are held together primarily via Watson Crick hydrogen bonds where A forms two hydrogen bonds with T and C forms three hydrogen bonds with G (Figure2).

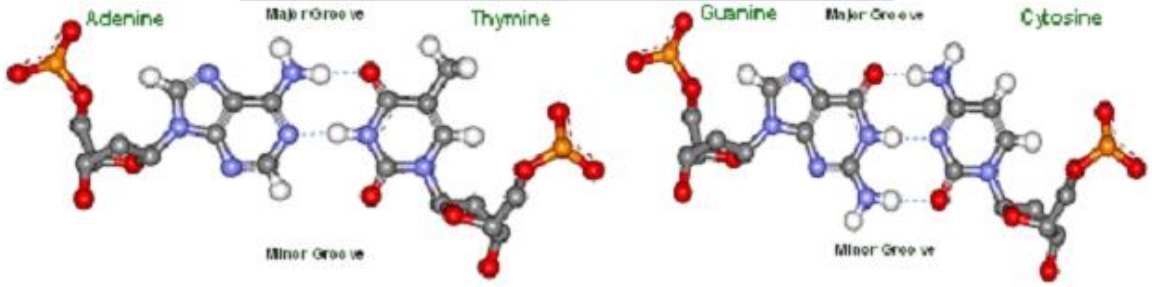


Fig.2 Watson Crick Base pairing, A-T and G-C base pairing

Specific recognition of DNA sequences by proteins/ small molecules is achieved via the combination of hydrogen bond acceptor/donor sites available on the major groove or minor groove. e.g. the A-T base pair offers a hydrogen bond acceptor, N7, a donor N6, and an acceptor, O4 on the major groove side.

DNA-Drug Interaction

Transcription and replication are vital to cell survival and proliferation as well as for smooth functioning of all body processes. DNA starts transcribing or replicating only when it receives a signal, which is often in the form of a regulatory protein binding to a particular region of the DNA. Thus, if the binding specificity and strength of this regulatory protein can be mimicked by a small molecule, then DNA function can be artificially modulated, inhibited or activated by binding this molecule instead of the protein. Thus, this synthetic/natural small molecule can act as a drug when activation or inhibition of DNA function is required to cure or control a disease (Table 1).

Table 1. Drug, action and mode of binding for some DNA binding drugs

SNo	Drug	Action	Mode of Binding	PDB
1	Hoechst 33258	Antitumor	Minor groove binding	264D
2	Netropsin	Antitumor, Antiviral	Minor groove binding	121D
3	Pentamidine	Active against P. carinii	Minor groove binding	1D64
4	Berenil	Antitrypanosomal	Minor groove binding	1D63
5	Guanyl bisfuramidine	Active against P. carinii	Minor groove binding	227D
6	Netropsin	Antitumor, Antiviral	Minor groove binding	121D
7	Distamycin	Antitumor, Antiviral	Minor groove binding	2DND
8	SN7167	Antitumor, Antiviral	Minor groove binding	328D
9	SN6999	Active against P. falciparum	Minor groove binding	144D
10	Nogalamycin	Antitumor	Intercalation	182D
11	Menogaril	Antitumor- Topoisomerase II poison	Intercalation	202D
12	Mithramycin	Anticancer antibiotic	Minor groove binding	146D
13	Plicamycin	Anticancer antibiotic	Minor groove binding	1BP8
14	Chromomycin A3	Anticancer antibiotic	Minor groove binding	1EKH
15	cis -Platin	Anticancer antibiotic	Covalent cross-linking	1AU5

DNA activation would produce more quantities of the required protein, or could induce DNA replication; depending on which site the drug is targeted. DNA inhibition would restrict protein synthesis, or replication, and could induce cell death. Though both these actions are possible, mostly DNA is targeted in an inhibitory mode, to destroy cells for antitumor and antibiotic action.

Drugs bind to DNA both covalently as well as non-covalently. Covalent binding in DNA is irreversible and invariably leads to complete inhibition of DNA processes and subsequent cell death. Cis-platin (cisdiamminedichloroplatinum) is a famous covalent binder used as an anticancer drug, and makes an intra/interstrand cross-link via the chloro groups with the nitrogens on the DNA bases.

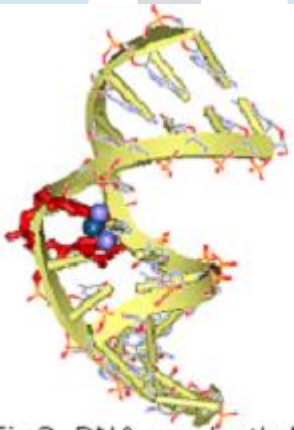


Fig.3. DNA covalently bound to cisplatin.(PDBID: 1AU5)

Non-covalently bound drugs mostly fall under the following two classes:

1. Minor groove binders- Minor groove binding drugs are usually crescent shaped, which complements the shape of the groove and facilitates binding by promoting van der Waals interactions. Additionally, these drugs can form hydrogen bonds to bases, typically to N3 of adenine and O2 of thymine. Most minor groove binding drugs bind to A/T rich sequences. This preference in addition to the designed propensity for the electronegative pockets of AT sequences is probably due to better van der Waals contacts between the ligand and groove walls in this region, since A/T regions are narrower than G/C groove regions and also because of the steric hindrance in the latter, presented by the C2 amino group of the guanine base. However, a few synthetic polyamides like lexitropsins and imidazole-pyrrole polyamides have been designed which have specificity for G-C and C-G regions in the grooves.

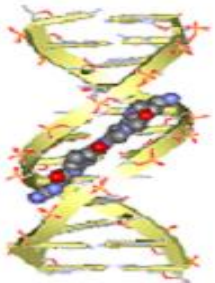


Fig.4 DNA complexed with netropsin, a minor groove binder. (PDB ID: 121D)

2. Intercalators- These contain planar heterocyclic groups which stack between adjacent DNA base pairs. The complex, among other factors, is thought to be stabilized by π-π stacking interactions between the drug and DNA bases. Intercalators introduce strong structural perturbations in DNA.

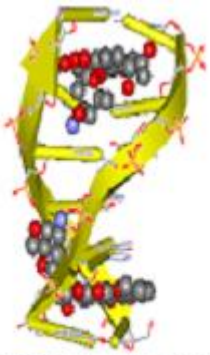


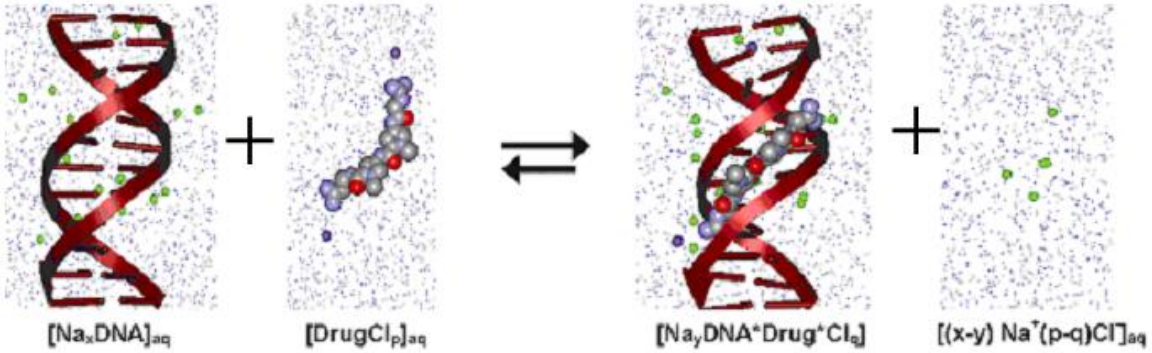
Fig.5 DNA complexed with actinomycin D, an intercalator. (PDB ID: DSC)

Non-covalent binding is reversible and is typically preferred over covalent adduct formation keeping the drug metabolism and toxic side effects in mind. However, the high binding strength of covalent binders is a major advantage. Proteins are large molecules and bind quite strongly to the DNA, with binding constants in the nanomolar range. It has been difficult to achieve similar specificity and affinity using small non-covalent binders, and remains a major challenge to the design of drugs for DNA.

Forces involved in DNA-drug recognition:

Understanding the forces involved in the binding of proteins or small molecules to DNA is of prime importance due to two major reasons. Firstly, the design of sequence specific drugs having requisite affinity for DNA requires a knowledge how the structure of the drug is related to the specificity/affinity of binding and what structural modifications could result in a drug with desired qualities. Secondly, identifying the forces/energetics involved in such processes is fundamental to unraveling the mystery of molecular recognition in general and DNA binding in particular. Some of the forces that are known to contribute to biomolecular recognition and also to DNA-drug binding are direct electrostatic interactions, direct van der Waals/packing interactions, complex hydration/dehydration contributions composed of hydrophobic component, solvation electrostatics, solvation van der Waals, ion effects and entropy terms.

DNA-drug binding may be described in the following manner,



Consider DNA-drug binding in an aqueous environment. DNA is polyanionic in nature and the drug molecule is also often charged. The associated counterions lie near the charged groups and are also partially solvated. When binding occurs, it results in a displacement of solvent from the binding site on both the DNA and drug. Also, since there would be partial compensation of charges as the DNA and drug are oppositely charged, some counter-ions would be released into the bulk solvent and are solvated fully. Also, the binding process would be associated with some structural deformation/adaptation of the DNA as well as the drug molecule in order to accommodate each other. All these events are associated with some energetic gains/losses, the comprehensive estimation of which is a major challenge. We are attempting to understand the energetics of DNA-drug interaction by theoretically estimating the above contributions employing classical and statistical mechanical methods. Developing a theoretical protocol for detailed quantitative analysis of DNA ligand binding in solution is a daunting task due to some major challenges. Simulations of DNA with solvent and the attendant counterion atmosphere require careful consideration to ensure system stability. Also, evolving a computationally efficient technique using statistical mechanical principles for quantitative estimates of binding free energies in large biomolecular systems is an equally challenging task. Our study is aimed at providing such a theoretical protocol for complementing experimental techniques and facilitating a minute study of the structure-energy relationships in DNA drug complexes.

Structural and conformational changes in the DNA and drug on binding in solution are associated with enthalpic and entropic contributions to the binding free energy, which can be theoretically estimated from ensembles of structures generated via simulations. The only drawback of this approach is the long time taken for the simulations. The other terms, namely, electrostatics, van der Waals, hydrophobic component, rotational and translational entropy can be estimated from single structures. The web tool, PreDDICTA, estimates the components of DNA-drug binding free energy which can be calculated from a single structure, and correlates it with experimental binding free energy and ΔTm, thus providing a swift method for evaluation of potential lead candidates for researchers pursuing structure based drug design for DNA.

CONCLUSION

The present study has enabled us to understand some of the features of drug binding with DNA. Analysis of the factors involved in the sequence specific intercalation of drug within sequences of DNA has understood from the stacking interactions. The stacking energies of small aromatic molecules computed with different level of theories could demonstrate the appropriate method for the computation of stacking interactions of large molecules. The studies on the stacking interaction of benzene and pyridine molecules have been found useful to assess the level of theoretical calculations. The extent of dispersion forces included in various calculations from the relative variation of stacking interactions of aromatic molecules is well explained. The results ofMP2 calculations on the stacking of benzene rings are found similar to that of the reported CCSD(T) calculations. The MP2/6-31G+(d,p) and MP2/6-31+G(df,p) are found feasible for explaining the n-% type of stacking interactions, and these methods can be applied to the computation of larger stacked molecules instead of using other high level expensive techniques.

The sequence specific binding of acridine-4-carboxamide has been explained by force field studies. It has been found that the carboxamide side chain do not directly bind within dGpC and dCpG regions, and the presence of AT base pair in the d(ApCpGpT) is found necessary for side chain binding. The interactions of chromophore with certain atomic sites are also found prominent besides the contribution of side chain. Although the intercalation of acridine ring plays important role in DNA sequence specific binding ofthis drug, the observed information may be useful to analyze overall assessment of DNA binding. The structures of base pairs within the intercalation sites are drastically changed after intercalation, and some of the Watson Crick hydrogen bonds are found completely broken. The preference for major or minor groove, in different sequence combinations is well established in this study. We also report here the computation of another type of drug, diphenyl diamine (DB). The structural aspects and molecularsizes ofthese drugs are the important reason for effective binding of these drugs within minor groove of DNA. The interaction between cationic amidine group and thymine nucleobase may be one of the factors for stabilizing drug-DNA complex. However, the formation of other hydrogen bonds is also found in some DB drug-DNA complexes. Apparently, the stabilization of drug within DNA might depend on the nature of the hydrogen bonds formed within minor groove. The AT sequence selectivity of DB drugs is found to be due to the interaction of amidine group and thymine nucleobase.

Among the anthracyclines, daunomycin is a powerful anti cancer agent used in chemotherapy. Intercalation of ehromophore (tetracyclic ring) within the double helical DNA is an important feature that may be relevant to therapeutic value. Also the intercalation of oxidized (Chrom-A) and reduced (Chrom-C) forms of daunomycin shows distinguished features ofDNA sequence specificity. It has been observed that the ehromophore preferentially intercalates within the inner region (SEQ-III) of DNA than that of terminal region (SEQ-I and SEQ-II) of DNA nucleobase which was found from electrostatic and van der Waals interaction energies. The result is contradictory to the experimental findings, which may be due to the lack of calculating stacking interaction between ehromophore and base pair with CHARMm.

The existence ofproton transfer in double helical DNA is highlighted in studies. Such equilibrium proton transfer mechanism may be changed due to drug intercalation, where the stacking interaction between aromatic drug and base pair usually occur. The model study on the proton transfer mechanism in base pair in stacked situation has been slight deviation from that of normal condition is found. Also, there observed significant variations of activation energies of proton transfer (PT1 and PT2) in GC base pair. The proton transfer mechanism, PT2 occurs at slightly higher energy level than the other mechanism, PT1. The model calculation could predict the equilibration between two structures, (AHa)T and A(HaT) for maintaining continuous proton transfer reaction within AT base pair. The equilibrium potential energy plots (EQ) of proton transfer in GC and AT base pairs are the indirect interpretation of solvent effect, proton and other cation interaction that could participate in the compensation of acid-base behavior of counter nucleobases during proton transfer reaction. The proton transfer reaction for PT1 and PT2 in the stacked structure pass through higher energy barriers compared to the non stacked situations. Hence, the stacking interaction of aromatic molecules with base pair would inhibit the proton transfer phenomena within GC and AT base pairs. The acid-base characteristics of drugs are also essential

to understand insight into the nature of DNA binding. Here, we have demonstrated the differences of pKa values of various donor sites in acridine-4-carboxamides. The electron donating ability of donor sites of substituted aeridine-4-carboxamide in chromophore [N(l)] and side chain [N(2) and N(3)J have been estimated from the proton affinity values. The changes ofpKa values for the substituted acridine-4-carboxamides are quite small. The variation of pKa values with respect to different types of substituent in the chromophore is found little dependant on the electron donating or withdrawing group.