Chapter 4

Modeling the interactions of polyamines with the DNA base-pairs and the influence of solvation on such interactions
4.1 Interactions of DNA base-pairs with mono/di-protonated linear/cyclic diamines

4.1.1 Introduction

Deoxyribonucleic acid (DNA) is one of the fundamental units of the living organisms containing the genetic instructions and used in the development and functioning of the living organisms. It is a pharmacological target molecule to various antitumor drugs and newer drugs are designed to interact with DNA for over than 3-4 decades for different reasons like cell replications, controlling neurological and other diseases etc. The interaction of a drug or a binding agent to the DNA may be by alkylation, intercalation, minor/major groove binding, outside binding to DNA or by dependent enzymes. Binding of low molecular weight ligands like polyamines show good interaction with the polyanionic DNA molecule to stabilize the double stranded DNA and such interactions causes a variety of significant biological responses.

Natural polyamines such as putrescine, spermidine, and spermine are omnipresent in cells which have important role in cell replication, modulating gene expression and enzyme activities, activation of DNA synthesis and facilitating protein-DNA interactions. The polyamines are essential in many aspects - in neurological diseases, anticancer and anti AIDS drugs. Polyamines show its vast ability mostly due to its good interaction with the polyanionic DNA molecule. They are known to stabilize the double stranded DNA. Polyamines are also known to induce DNA-conformational transitions, B to Z-DNA and B to A-DNA. Polyamines like spermine are found universally in the mammalian cells and it has been observed that the decrease of spermine inhibits cell growth. Spermine is an effective groove binder of DNA and is known to stabilize DNA duplexes and triplexes. It has a significant role in the regulations of normal and malignant cell proliferation.

However, binding between polyamines and nucleic acids is not properly understood till now. It is known from $^{23}$Na NMR studies that the amines interacts to the DNA only through the electrostatic interactions. Some interaction models have been developed, which are sometimes even contrary to one another. According to Liquori et al. the spermine interacts to the minor groove of the DNA molecule, but later Drew and Dickerson and afterwards Feuerstein et al. observed that spermine interacts with the major groove of the DNA molecule. Feuerstein et al. concluded that the spermine binds to the DNA electrostatcially involving the N7 atom of the GC base-pair (Scheme 1) and the phosphate oxygen. Recently, MD studies performed by Korolev et al. suggested the presence of more spermine molecule in the minor groove than in the major groove through Grand Canonical Monte Carlo simulations.
Ouameur et al. reported on the basis of infrared data that the favourable binding of spermine with the DNA occurs in the major groove through the purine N7 atom and the outer primary amino groups of spermine could bind with PO$_4^{2-}$ groups of the DNA. Recent Raman studies proposed the interactions along and across the major grooves involving contacts between the inner amino groups and purine-N7 and thymine-O4 atoms, which also permit hydrophobic contact between CH$_2$ group of thymine and methylene group of spermine. Alteration of polyamine levels in the cells have been explored as a potential antiparasitic and anticancer strategy used by the researchers, either by the inhibition of enzymes or by direct replacement of natural polyamines by artificial analogues. The mechanistic differences in DNA binding mode between natural and synthetic polyamines have also not been explored.

Polyamine analogues can imitate some of the functions of biogenic polyamines like spermine, spermidine etc., but are not good enough to imitate the supportive nature in the cell growth by the natural polyamines. Binding affinity of natural polyamines with the DNA is higher than different synthesized polyamines. Thus these polyamines have the ability to stop the strand breaks, however, low DNA binding affinity of the synthetic analogues and their ability to induce DNA aggregation and precipitation at a rapid rate make them markedly different from the natural ones. The search of amines with higher binding affinity with the DNA is very important due to the fact that no metal ion is involved resulting less toxicity. The cyclic analogues of linear amines have been employed for better association with DNA molecules and to control the cytotoxicity of such ligands.

In this regard, branched or dendritic polyamines show better interactions with the DNA. Nagamani et al. synthesized conformationally restricted chiral analogues of spermine with pyrrolidine nucleus acting as DNA stabilizing agents. Recently, Kostiainen et al. developed dendritic spermine moieties with higher binding affinity in even large concentrations of NaCl. But molecular level studies of the interactions of DNA base-pairs with spermine type systems and different cyclic diamines which may be units of different synthetic polyamines have not been performed.

![Scheme 1](image-url)
In this section we have explored the role of cyclic analogues of linear amines towards their ability to associate with the DNA molecule and the influence of spatial separation of such amine groups. The study has been performed with model DNA AT and GC base-pairs (Scheme 1) with higher level quantum chemical calculations. Spermine was modeled with linear diamines and small cyclic analogue have been chosen for the study. Cyclic diamines are rigid structures and can induce chirality in the system than the corresponding linear diamines. Further, these cyclic diamines can also have different positional isomers. The constrained diamines i.e., Cyclobutadiamine (CBDA); Cyclopentadiamine (CPDA) and Cyclohexadiamine (CHDA) and their corresponding positional isomers have been examined for the interaction with DNA base-pairs (Scheme 2). The Cyclohexadiamine (CHDA) has been exploited as a ligand to prepare [Dichloro(1,2-diaminocyclohexane)platinum(II)], oxaliplatin--important anticancer drugs to treat the patients.\textsuperscript{25} Recently, improved anticancer drugs have also been synthesized from 1,2-CHDA using metal centres.\textsuperscript{26(b,c)} The chiral constraints induced in ligands have been suggested for better anti-cancer activity than the linear polyamines with the chiral DNA.\textsuperscript{26} The ring size of polyamine analogues suggested to be important while binding with DNA.\textsuperscript{26} We have performed a systematic study with different ring size of protonated amines and their nature of binding with the DNA base-pairs. The higher level DFT calculations would reveal the mode of binding of such cyclic diamines compared to the acyclic linear amines, which can provide insights to researchers to design efficient analogues of biogenic polyamines. The interactions of mono- and di-protonated diamines with DNA base-pairs have been examined (Scheme 2). The strong ionic hydrogen bonds between the ligands and DNA base-pairs revealed that di-protonated amines are more appropriate for better binding with such base-pairs.
DNA-Solvation and Interaction

Chapter 4.1

1,2-CBDA

R S
S
S
1,3-CBDA

achiral
achiral

1,2-CPDA

1,3-CPDA

1,2-CHDA

1,3-CHDA
Molecular Dynamics simulations have been performed using explicit water molecules for the interaction of representative ligands such as linear and 1,2-ee CHDA with the Guanidine base of the DNA employing periodic boundary conditions to examine the interference of solvent molecules with such interactions. The solvent molecules do not affect the binding affinity of these ligands with the base of DNA.

4.1.2. Computational Methods

4.1.2.1. Quantum Chemical calculations

All the reactants and the complexes are fully optimized at B3LYP/6-31G(d)\(^2\) level of theory at solvent phase with the PCM continuum model.\(^2\) The global minimum structures are confirmed with the all positive frequencies of the optimized geometries. Water is used as a solvent with dielectric constant (\(\varepsilon = 78.36\)).

Single point calculations have been performed at MP2/aug-cc-pvdz\(^2\)\(^9\)\(^\text{30}\) for the conformational analysis of CBDA, CPDA and CHDA as observed in the previous reports.\(^3\) MP2/aug-cc-pvdz calculations are computationally expensive and, therefore, we have performed the dispersion corrected DFT calculations\(^3\)\(^2\) [B3LYP-D1/6-31G(d)], which is known to be reasonably accurate for biomolecules.\(^3\) All the calculations are performed with the Gaussian 09 suite of programs.\(^3\)\(^4\) The ChelpG charges have been calculated in B3LYP/6-31G(d) level of theory in solvent.\(^3\)

Binding energies are calculated at the B3LYP-D1/6-31G(d) level of theory using the equation:

\[
\text{Binding energy (\(\Delta E\)) = } E_{\text{complex}} - E_{\text{reactant}} \quad \text{------------------------ (1)}
\]

In the DFT-D method the dispersion corrected total energy is given by

\[
E_{\text{DFT-D}} = E_{\text{KS-DFT}} + E_{\text{disp}} \quad \text{------------------------ (2)}
\]

Where, \(E_{\text{KS-DFT}}\) is the normal self-consistent Kohn–Sham energy, and \(E_{\text{disp}}\) is an empirical term involving pair-wise dispersive interactions.

\[
E_{\text{disp}} = -s_i \sum_j j > i (C_{ij}^{(ij)} | R_{ij} | f_{\text{amp}} (R_{ij})) \quad \text{------------------------ (3)}
\]
Here, the summation is over all atom pairs, $C_{ij}^6$ is the dispersion coefficient for the pair of atoms $i$ and $j$ (calculated from the atomic $C_6$ coefficients), $S_6$ is a scaling factor that depends on the density functional used and $R_{ij}$ is the interatomic distance between atoms $i$ and $j$. A damping function is used in order to avoid near singularities for small distances. This function is given by

$$f_{\text{damp}}(R_{ij}) = \frac{1}{1 + \exp \left\{ -\alpha \left( R_{ij}/R_0 - 1 \right) \right\}}$$ \hspace{1cm} (4)

where $R_0$ is the sum of atomic van der Waals radii and $\alpha$ is a parameter determining the steepness of the damping function. In order to obtain the composite dispersion coefficients $C_{ij}^6$, a simple average of the form is used.

$$C_{ij}^6 = \frac{2C_i^6C_j^6}{C_i^6 + C_j^6}$$ \hspace{1cm} (5)

The IR spectra for free GC base-pair and diamine-GC adducts have been calculated and plotted against absorbance. Specific IR spectral data (Guanine C=O str.; symmetric str. of PO$_2^-$ and H-bond str. of interacted diamine) have been observed to understand the better interacted diamines with the GC base-pairs following the equation:

$$E = \hbar c \nu$$ \hspace{1cm} (6)

where ‘$\hbar$’ is Plank’s constant; ‘$c$’ is the velocity of light in vacuum and ‘$\nu$’ is the wave number in cm$^{-1}$.

### 4.1.2.2. Molecular Dynamics Simulations

The Molecular Dynamics calculations are performed with DMol3 software in Material Studio (version 4.1) of Accelrys Inc$^{36}$ with local spin density approximation with Perdew-Wang correlational (LDA/PWC).$^{37}$ We used DNP double numerical basis sets which is comparable to the 6-31G** basis set. All MD simulations for the interactions of linear and 1,2-ee CHDA with the Guanidine base in presence of explicit water molecules are performed in periodic boundary condition with a cubic box of 20 Å size, with canonical NVT ensemble and the system temperature is kept at around 300 K using Nosé-Hoover chain thermostat.$^{38}$ Six solvent molecules (H$_2$O) were placed inside the periodic box interacting with the Guanidine base as well as the ligand molecule. The simulations were carried out for 1 ps with a time step of 1 fs.

### 4.1.3. Results and Discussion

#### 4.1.3.1. Mono-protonated Linear Diamine Interaction

The mono- and diprotonated amines can be achieved theoretically and their nature of interaction with DNA base-pairs could be different. However, the mono-protonation of
polyamines can be difficult to achieve experimentally, hence the calculations have been performed with diprotonated amines. Nonetheless, the mono-protonated form of amine has also been considered and computed.

The interaction of the mono-protonated linear diamine (A) with the N7 site of the DNA-GC base pair and the \( \text{PO}_4^{3-} \) is given in Table 1. The mono-protonated site of A has been shown to interact to both the N7 site and the \( \text{PO}_4^{3-} \) site of the GC base-pair (Table 1). The binding energy calculated for the interactions suggest that interaction of the protonated site with the \( \text{PO}_4^{3-} \) is \(~10\) kcal/mol stronger than the other one where the protonated site interacts with the N7 (Table 1). The internal hydrogen bonding interactions between the Guanine and Cytosine bases are not much affected by the alteration of the protonated site interactions.

Table 1. B3LYP-D1/6-31G(d)//B3LYP/6-31G(d) calculated geometries of mono- and di-protonated linear diamine interactions with the N7 site of DNA base-pair (GC) and their relative energies in kcal/mol. Distances are given in Å.

<table>
<thead>
<tr>
<th>Mono-protonated Linear diamine</th>
<th>Mono-protonated Linear diamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

4.1.3.2. Di-protonated Linear Diamine Interaction

The interactions of diprotonated linear diamine with the different sites of the AT and GC, DNA base-pairs are given in table 2. The binding energies are calculated using Eq 1. The diprotonated linear diamine (B) prefers to interact with the N7 (Scheme 1) site and the \( \text{PO}_4^{3-} \) group attached to GC base-pair as observed in biogenic polyamines like spermine.\(^{10,14(a-d)}\) The calculated binding energy of B with the GC base-pair is \(-65.2\) kcal/mol (Table 2). Similar binding mode of diprotonated linear diamine with AT base-pair binds \(9.0\) kcal/mol weaker than the corresponding GC base-pair. The hydrogen bonding of B with the carbonyl group of the Guanine base leads to the additional stability in the case of GC base-pair (Table 2).\(^{39}\) The negatively charged \( \text{PO}_4^{3-} \) group abstracts the proton from the \(\text{-NH}_3^+\) group of the diamine (B) (Table 2). The binding ability of A with other major and minor groove sites of the AT and GC
base-pairs have also been examined systematically. The higher binding affinity has been
achieved when the phosphate group is involved in the binding with B (Table 2). Therefore,
these calculated results corroborate the preferential binding of biogenic polyamines to the major
groove of DNA compared to the minor groove site in general.10,14(a-d)

**Table 2.** B3LYP-D1/6-31G(d)//B3LYP/6-31G(d) calculated geometries of di-protonated linear
diamine interactions with different sites of DNA base-pairs (AT and GC) and their relative
energies in kcal/mol. Distances are given in Å.

<table>
<thead>
<tr>
<th>Di-protonated Linear diamine</th>
<th>Di-protonated Linear diamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine-Thymine</td>
<td>Cytosine-Guanine</td>
</tr>
<tr>
<td><strong>Major groove</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>N7\textsubscript{AT}</th>
<th>N7\textsubscript{GC}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{NH}_2-a\textsubscript{AT}</td>
<td>B.E = -56.8 kcal/mol</td>
<td>B.E = -53.0 kcal/mol</td>
</tr>
<tr>
<td>B.E</td>
<td>-56.9 kcal/mol</td>
<td>-65.2 kcal/mol</td>
</tr>
<tr>
<td>O-b\textsubscript{AT}</td>
<td>B.E = -20.8 kcal/mol</td>
<td></td>
</tr>
<tr>
<td>B.E</td>
<td>-20.8 kcal/mol</td>
<td></td>
</tr>
<tr>
<td>O-b\textsubscript{GC}</td>
<td>B.E = -57.2 kcal/mol</td>
<td></td>
</tr>
</tbody>
</table>
The nucleic acids are chiral in nature; introducing chirality to the polyamines was beneficial to the activity of the polyamines.\textsuperscript{23} The polyamines were synthesized with restricted chain flexibility with formation of cyclic geometries and with chirality to interact with the DNA effectively. However, the information is limited towards the influence of constrained ring size and their positional isomers for binding with the DNA. Cyclic rings (CBDA, CPDA and CHDA) with their positional isomers have been considered to examine the binding with DNA base-pairs. Some of these cyclic isomers possess intrinsic chirality and hence can give a fair comparison with their
achiral analogues. A conformational analysis was performed for these cyclic protonated-diamines to predict the most stable conformers in each case, before considering them for their interactions with the DNA base-pairs.

4.1.3.3. Conformational analysis of cyclic diamines

Conformational searches have been performed with the diprotonated Cyclobutadiamine; Cyclopentadiamine and Cyclohexadiamine at MP2/aug-ccpvdz level of theory with the PCM solvation model in aqueous phase. In the case of CBDA, 1,2 and 1,3-positional isomers have been considered. The trans form of 1,2-isomer was found to be relatively stable compared to the cis form, whereas, in 1,3 isomer, cis form was predicted to be stable than the trans form (Table 3). The 1,2-isomer of CBDA possess chirality, whereas, the 1,3-isomer is achiral in nature. Going from cyclobutyl ring to the cyclopentyl ring, the stability of 1,2 and 1,3-positional isomers of CPDA also showed similar trend as observed with CBDA isomers (Table 3). All 1,2 and 1,3-CPDA isomers possess intrinsic chirality in the systems.

The conformational analysis extended with diprotonated CHDA showed that the di-equatorial (ee) conformers of 1,2-, 1,3- and 1,4-isomers is energetically more stable than their corresponding axial-equatorial (ae) and di-axial (aa) conformers (Table 4). This conformational analysis of CHDA isomers is in good agreement with the earlier report in aqueous medium.28 1,2- and 1,3-isomers of CHDA are chiral, whereas 1,4- positional isomers do not possess any chirality.

Table 3. MP2/aug-ccpvdz//B3LYP/6-31G(d) calculated conformational geometries of (1,2- and 1,3-positional) buta- and penta- cyclic diamine rings and their relative energies in kcal/mol are given.

<table>
<thead>
<tr>
<th></th>
<th>1,2-position</th>
<th>1,3-position</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis</td>
<td>6.4</td>
<td>0.0</td>
</tr>
<tr>
<td>trans</td>
<td>0.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 4. MP2/aug-cc-pvdz//B3LYP/6-31G(d) calculated conformational geometries (ee, ae and aa) of 1,2-, 1,3- and 1,4- cyclic diamine rings and their relative energies are given in kcal/mol.

<table>
<thead>
<tr>
<th></th>
<th>1,2-CHDA</th>
<th>1,3-CHDA</th>
<th>1,4-CHDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ee</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>ae</td>
<td>2.0</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>aa</td>
<td>1.4</td>
<td>9.0</td>
<td>4.8</td>
</tr>
</tbody>
</table>

4.1.3.4. Cyclic Diamine interactions with major groove sites

The recent studies and our computational results on the interaction of linear diamines with DNA base-pairs suggest that the major groove is the preferred site for such interactions, hence the
calculations of CBDA, CPDA and CHDA have been performed with the major groove N7 site of the AT and GC base-pairs.

The DFT-D calculated results show that the interaction of 1,2-trans CBDA with the N7 site and the PO₄³⁻ group of the GC base-pair is energetically preferred than its corresponding 1,3-cis isomer (Table 5). The diprotonated amine nitrogen's reside at a distance of 3.57 Å in 1,2-trans isomer of CBDA, which matches the distance between the N7 and PO₄³⁻ group of the optimized GC base-pair (3.84 Å) than the N-N distance of the 1,3-cis isomer (4.53 Å) (Figure 1 and Figure 2). Hence, the appropriate fit of 1,2-trans CBDA with the DNA GC base-pair seems to important for stronger hydrogen binding in this case. We have also examined the interactions of 1,2-trans and 1,3-cis conformers of CBDA with the N7 site at the major groove sites of the AT base-pair. The interaction energy is lower in this case compared to the GC-base-pair as the ‘C=O…H-N’ hydrogen bonding interaction is not possible in AT base-pair (Table 6). The preference for the trans isomer of synthetic cyclobutyl amine analogues to bind with DNA has also been observed experimentally, which corroborates the calculated results. These DFT calculated results show that the chiral 1,2-trans isomer of CBDA is better in term of binding with DNA base-pairs than the achiral 1,3-cis isomer. The ChelpG charges calculated for the linear and the 1,2-trans CBDA suggests that the electrostatic charges on the amine hydrogens are higher in 1,2-trans CBDA than linear diamine. (Figure 3) The ring carbons bear more ‘s’ character than the corresponding linear systems, which consequently enhance the polarization in the bonds. Such polarization in the substituted amine groups can cause a better binding ability for cyclic systems than their corresponding linear amines.

To examine the influence of ring strain on the binding with DNA base-pairs, Cyclopentadiamine (CPDA) was studied. The strain energy in cyclopentane ring is lower than the cyclobutane ring. The interaction energy of the 1,2-trans isomer of CPDA predicted with the GC base-pairs of DNA is comparable to the interaction energy computed for 1,2-trans isomer of CBDA (Table 5). Furthermore, the binding energy computed for 1,3-cis CPDA with GC base-pair is ~5kcal/mol lower than the 1,2-trans isomer (Table 4). The interaction energy for the 1,3-cis isomer of CPDA is even lower than the 1,3-cis isomer of CBDA (Table 5). The weaker interaction of 1,3-cis isomer of CPDA is due to the larger N…N distance of diamine groups (4.82 Å) compared to the distance between the N7 and PO₄³⁻ group of the GC base-pair (3.84 Å) (Figure 1 and Figure 4). Cyclopentyl amine have been studied previously as ligands for the binding studies with DNA. Such ligands were higher homologue of the CPDA examined here and found to be rather inactive in the inhibitory effects on the human prostate cancer cells. Our calculated results suggest that the 1,2-trans isomer of CPDA ligand employed in the present
study can be a better candidate for effective binding with the DNA base-pairs as they show similar binding energies computed for CBDA. The experimental observations reported with the cyclobutyl groups are rather promising, though not much is explored.23(a,c)

Expectedly, the interactions of CPDA with the N7 site of the AT base-pair at the major groove showed much lower interaction energy due to the absence of ‘C=O…H-N’ hydrogen bonding interactions (Table 7).

\[
\begin{array}{cccc}
1.93 & 1.88 & a & b \\
1.87 & & &
\end{array}
\]

**Figure 1.** The N7-PO₄³⁻ distances (in Å) of the B3LYP/6-31G(d) optimised geometries of AT and GC DNA base-pairs.

\[
\begin{array}{cccc}
3.57 & 4.53 & & \\
& & &
\end{array}
\]

**Figure 2.** The N-N distances (in Å) between the B3LYP/6-31G(d) optimised geometries of di-protonated 1,2-trans and 1,3-cis isomer of CBDA.

\[
\begin{array}{cccc}
0.362 & 0.362 & 0.369 & 0.369 \\
0.330 & 0.351 & 0.351 &
\end{array}
\]

\[
\begin{array}{cccc}
0.405 & 0.421 & 0.419 & 0.419 \\
0.421 & 0.405 &
\end{array}
\]

**Figure 3.** The calculated ChelpG charge of the linear and the 1,2-trans CBDA.
3.55 Å between the B3LYP/6-31G(d) optimised geometries of di-protonated 1,2-trans and 1,3-cis isomer of CPDA.

1,2-ee isomer of CHDA showed better interaction with the N7 site of the GC base-pairs than CPDA and CBDA. The N-N distance of amine groups in 1,2-ee isomer of CHDA is 3.11 Å, which is slightly less than the N7-PO₄³⁻ distance (3.84 Å) of the GC base-pair (Figure 1 and Figure 5), however, the larger flexibility in the cyclohexyl ring enables it to interact strongly with the base-pair (Figure 5). The calculated binding energy of 1,2-ee isomer of CHDA with GC base-pair has been found to 72.5 kcal/mol, which is much higher than the CBDA and CPDA cyclic systems (Table 7). The binding energy of 1,3-ee isomer of CHDA was predicted to much lower than the 1,2-ee isomer of CHDA (Table 8). 1,4-ee isomer of CHDA showed the lowest affinity for binding with the GC base-pair. The geometric mis-match between the 1,4-ee of CHDA with the N7 and PO₄³⁻ of GC base-pair is maximum and hence no amine-PO₄³⁻ interaction is observed resulting in a weaker binding energy of 29.8 kcal/mol.

Table 5. B3LYP-D1/6-31G(d)/B3LYP/6-31G(d) calculated geometries of di-protonated 1,2-trans and 1,3-cis isomers of CBDA and CPDA with the N7 and PO₄³⁻ site of the GC base-pair and their relative energies in kcal/mol. Distances are in Å.
Table 6: B3LYP-D1/6-31G(d)/B3LYP/6-31G(d) calculated geometries of di-protonated 1,2-trans and 1,3-cis isomers of CBDA with the N7 and PO₄³⁻ site of the AT base-pair and their relative energies in kcal/mol. Distances are in Å.

<table>
<thead>
<tr>
<th>Adenosine-Thymine</th>
<th>1,2-trans</th>
<th>1,3-cis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-7_AT</td>
<td>N-7_GC</td>
<td></td>
</tr>
</tbody>
</table>
Table 7. B3LYP-D1/6-31G(d)//B3LYP/6-31G(d) calculated geometries of di- protonated 1,2-; 1,3- and 1,4- ee isomers of CHDA with the N7 and PO₄³⁻ site of the GC base-pair and their relative energies in kcal/mol. Distances are in Å.

<table>
<thead>
<tr>
<th></th>
<th>1,2-CHDA (ee)</th>
<th>1,3-CHDA (ee)</th>
<th>1,4-CHDA (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N7_{GC} B.E</td>
<td>-72.5 kcal/mol</td>
<td>-62.4 kcal/mol</td>
<td>-62.4 kcal/mol</td>
</tr>
</tbody>
</table>
AT base-pair of major groove showed remarkably higher interaction energy with 1,2-ee isomer of CHDA (Table 8). The DFT-D calculated interaction energies suggest that the 1,2-ee isomer of CHDA prefers to bind ~2 kcal/mol more strongly than GC base-pair of major groove (Table 7 & 8). Two N-H hydrogens of an amine group in 1,2-ee isomer of CHDA interacts with two oxygen atoms of the PO$_4^{3-}$ and the other amine –N-H hydrogen interacts with the N7 site of AT base-pair (Table 8). The additional interaction between the PO$_4^{3-}$ and amine groups augments the interaction energy in this case compared to the corresponding GC base-pair (Table 7 & 8). The 1,3- and 1,4-CHDA however showed much weaker interactions with the AT base-pair (Table 8). The chiral 1,2-ee and 1,3-ee isomers of CHDA are interacting with the DNA base-pairs strongly compared to the achiral 1,4-ee isomer of CHDA. These representative calculations with the chiral ligands though show a better binding with base-pairs compared to the achiral ones, however, the major difference lies in their geometrical fit. The calculations support the selection of chiral analogues of biogenic polyamines in earlier reports, however, should be considered in qualitative sense.$^{23}$

**Table 8.** B3LYP-D1/6-31G(d)//B3LYP/6-31G(d) calculated geometries of di-protonated 1,2-, 1,3- and 1,4- ee isomers of CHDA interacting with the N7 and PO$_4^{3-}$ site of the AT base-pair and their relative energies in kcal/mol. Distances are in Å.

<table>
<thead>
<tr>
<th>1,2-CHDA (ee)</th>
<th>1,3-CHDA (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.E = -29.8 kcal/mol</td>
<td></td>
</tr>
</tbody>
</table>
4.1.3.5. Di-amine base-pair interactions studied by theoretical IR spectra

In an earlier report, the interaction between calf thymus DNA and polyamine analogues have been studied using Fourier transform infrared, circular dichroism and UV-vis methods. The evidence for binding and stability of analogues-DNA complexation has been observed from these studies. Therefore, the calculated infrared spectral results for the interaction of linear diamines (B) and the cyclic diprotonated diamines are provided in Table 9. We have compared the spectral changes of the free GC base-pair with the diamines bound to this base-pair. The Guanine ‘C=O’ stretching frequency, symmetric stretching of the PO$_2^-$ group and the hydrogen bonded stretching frequency of the ligand –N-H hydrogens with the GC base-pair during the
interactions show some interesting trends in the IR data. In the case of AT base-pair, similar stretching frequencies have also been given in Table 9.

The changes in the spectral pattern of the Guanine ‘C=O’ stretching from the free GC base-pair confirms the interaction between the ligands and the base pair (Table 9). The PO$_2^-$ symmetric stretching showed increased value for the bounded GC base-pairs in all cases, except 1,4-ee isomer of CHDA, as there is no interaction between the phosphate and the ligand in this case (Table 7). The larger shift in the symmetric stretching of PO$_2^-$ (1082.44 cm$^{-1}$) is observed for the 1,2-ee isomer of CHDA, suggests a much better interaction with the base-pair (Table 8). The shift in the -N-H stretching frequencies between the N7···H-N (diamine) and PO$_4^{3-}$···H-N (diamine) of the GC base-pair is also observed compared to the free base-pair. The larger shift in the stretching frequency is observed for the PO$_4^{3-}$···H-N (diamine) of 1,2-ee isomer of CHDA (3308.09 cm$^{-1}$), which further corroborates the much stronger interaction for this cyclic diamine. Interactions of 1,4-ee isomer of CHDA is weaker due to absence of any PO$_4^{3-}$···H-N (diamine) stretching (Table 7 and Table 9). In the case of AT base-pairs, the strong interaction observed for 1,2-ee isomer of CHDA is correlated well with the shift in the IR frequency date of N7···H-N (diamine) (3159.87 cm$^{-1}$) and PO$_4^{3-}$···H-N (diamine) (2600.20 cm$^{-1}$; 3027.75 cm$^{-1}$). The calculated IR results will be valuable in the experimental studies to examine the binding affinity of these cyclic diamines and their potential role for the stability, aggregation, precipitation and conformation of DNA.

**Table 9.** B3LYP/6-31G(d) calculated specific IR spectral bands for free AT and GC base-pair and the base-pair interacted with different diamines at aqueous medium in cm$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>Guanine C=O str.</th>
<th>PO$_2^-$ symmetric str.</th>
<th>H-bond str. in the interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N7···H-N str.</td>
</tr>
<tr>
<td>Free GC base-pair</td>
<td>1736.51</td>
<td>935.43</td>
<td>-----</td>
</tr>
<tr>
<td>Linear diamine GC</td>
<td>1678.43</td>
<td>1075.80</td>
<td>2848.61</td>
</tr>
<tr>
<td>1,2-CBDA(trans) GC</td>
<td>1676.85</td>
<td>1074.68</td>
<td>2915.00</td>
</tr>
<tr>
<td>1,3-CBDA(cis) GC</td>
<td>1677.70</td>
<td>1073.40</td>
<td>3001.63</td>
</tr>
<tr>
<td>1,2-CPDA(trans) GC</td>
<td>1677.00</td>
<td>1073.81</td>
<td>3012.68</td>
</tr>
</tbody>
</table>
4.1.3.6. Molecular Dynamics Simulation

The interaction of diprotonated ligands with DNA base-pair was further examined in presence of explicit water molecules. Ab initio molecular dynamics simulations have been employed using six explicit water molecules with the ligands (linear and 1,2-ee CHDA) and the Guanine base of the DNA employing periodic boundary conditions. The optimized geometries of the ligands with the guanidine base have been taken as the initial geometry for the MD simulations. The water molecules were placed in such a way that they can interact both with the base and the ligand molecules. The interactions (**N_1-O_1** and **N_2-N_7**_{GC}) during the simulations are shown in scheme 3. The intermolecular hydrogen bonding interactions between the ligands and the guanine base of the DNA (**N_1-O_1** and **N_2-N_7**_{GC}) is largely unperturbed with time (Figure 6). The plots generated for such interatomic distance vs. time steps suggests that the explicit water molecules are not influencing the binding of ligands with DNA base-pair significantly (Figure 6).
Scheme 3. Interactions of ligands (1) B and (2) 1,2-CHDA, with Guanine base.

Figure 6. The distance vs. time plot for the Interactions of ligands (1) B and (2) 1,2-CHDA, with Guanine base.

The snapshots taken at different intervals for the interaction of linear diamine (B) and 1,2-CHDA with the DNA Guanine base pair are given in figure 7 and 8. The water molecules are primarily
interacting with the ligand molecules (linear diamine, B and 1,2-CHDA) and are away from the intermolecular hydrogen bonding sites.

Figure 7. Snapshots at different time steps for the interaction of linear diamine (B) with the Guanine base-pair in periodic boundary condition in presence of explicit water molecules. The hydrogen bonding interactions between the base, diamine and the water molecules are given in Å.
Figure 8. Snapshots at different time steps for the interaction of 1,2-ee CHDA with the Guanine base-pair in periodic boundary condition in presence of explicit water molecules. The hydrogen bonding interactions between the base, diamine and the water molecules are given in Å.

4.1.4. Conclusion

In this section, we have examined the binding affinity of protonated linear and cyclic diamines with DNA base-pairs using *ab initio* and DFT levels of theory. The DFT-D calculations predicted the better binding of linear diamine with N7 and PO₄³⁻ at the major site of the GC base-pair, which is in good agreement to the earlier studies performed with biogenic polyamines like spermine.¹⁰,¹⁴(a,d) The mono-protonated ligands showed similar trends like the diprotonated ligands but interaction energies are much lower. The interactions of rigid cyclic diamines with
the N7-site of the AT and GC base-pairs have been examined. The cyclic diamines showed stronger binding with the DNA base-pairs compared to the linear diamine by ~5-9 kcal/mol. The cyclic rings induce the change in the hybridization of carbon centers, which influence the bond polarization and results better binding with the DNA base-pairs. The larger flexibility in the Cyclohexadiamine (CHDA) allows the protonated amines to interact much more strongly compared to the smaller cyclic diamines. It has been observed that chiral analogues of CBDA and CHDA bind better with the DNA base-pairs than their corresponding achiral isomers. Furthermore, the calculated results suggest that the binding affinity of ligands with the DNA base-pairs can be efficient with the geometric match of the binding sties of ligands with such base-pairs. In general, the cyclic diamines experience the additional -C=O…H-N interaction with GC base-pair and hence becomes the energetically preferred than the AT base-pair. The orientation of the diprotonated amino groups in 1,2-ee isomer of CHDA allows the additional interaction with the PO_4^{3-} of AT base-pair, which leads to a stronger interaction compared to the GC base-pair. The calculated IR spectral data corroborate well with the binding energies calculated for such cyclic systems. The MD simulations suggest that the strong ionic hydrogen bonding interactions between the ligands and the base of DNA are not influenced by the explicit solvent molecules. This study will shed light to design more efficient synthetic polyamines analogues with antiproliferative effects.
References


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DNA-Solvation and Interaction

Chapter 4.1


29. Møller C.; Plesset, M. S. Phys Rev. 1934, 46, 618.
4.2. Interaction of different polyamines with DNA moiety and the effect of bulk solvent on these systems: A Classical Molecular Dynamics Study

4.2.1. Introduction

Hydration of DNA plays an important role in its structure, conformation, and function of DNA.\(^1\) X-ray crystallography, NMR, dielectric relaxation, and molecular dynamics simulation studies have shown that a significant amount of water molecules are bound to DNA. Water also plays a central role in the thermodynamics of the macromolecules.\(^2\) The biological activity of DNA, generally occurs within a relatively narrow range of temperature, solvent, chemical potential, and ionic concentration. Most cellular functions are driven not by temperature gradients but by changes in solvent environments including varying pH and ionic activities as well as different solute concentrations between cellular and subcellular compartments. It is thus of both practical and fundamental interest to understand the relation of the aqueous solvent to these important macromolecules.\(^3\) The surface water molecules can be different from bulk water present around DNA.\(^4,5\) The thermodynamic properties of these surface water molecules are known to play an important role in biological processes such as recognition, intercalation, etc.\(^6\) Recently, a detailed study on the nature of molecular interactions between DNA fragments and solvent media expected to cause detection of single nucleotide polymorphisms (SNPs) had been performed.\(^7\) They mainly focused on the process of sequence separation resulted from electrostatic and van der Waals (VDW) interactions between the solute and solvent molecules.\(^7\) Molecular dynamics simulations also have previously shown that the diffusivity of DNA fragments is actually influenced by solvent molecules that form hydrogen bonds with dissociated base molecules.\(^8\) Hydration effects on DNAs and the molecular structures were well investigated in terms of experiments and computations.\(^9\) The dynamics of the water molecules near the DNA surfaces are considered to affect an interesting transition: B–DNA ↔ A–DNA.\(^10\) Pettit et al. stated the ability of the three dimensional integral equations (3D-IEs) to predict the solvent and ion distributions specific to DNA structures and their influence on the corresponding solvent thermodynamics.\(^11\)

Molecular dynamics (MD) studies of nucleic acids have shown considerable progress in the recent years owing to new methods and force fields that made possible molecular simulations with the explicit treatment of water and counter ions.\(^12\) These calculations were performed within periodic boundary conditions in bulk solvent. The box must be large enough to reduce interactions between periodic images and also to provide reasonable concentrations of all ingredients, namely, DNA, water, and the counter ions. Such calculations are very demanding, and a delicate balance has to be found between the accuracy, the system size, and the
reasonable duration of trajectories. The more economical implicit solvent models also are considered to examine different problems of DNA in solvent conditions. Recent approach using a more physical screening function derived from the generalized Born solvation model are considered for the implicit solvation calculations which are much cheaper than the explicit solvation calculations.

The role of solvation effect on DNA, while interacting with oppositely charged ligands is also an area of interest during the last few decades. Efforts have been made on studies of the polyelectrolyte properties of DNA in the presence of simple metal ions with both the experimental and theoretical methods. Moreover, attention has also been paid to the interactions between DNA and polycationic polyamines like spermine\(^4^+\) (Spm\(^4^+)\), spermidine\(^3^+\) (Spd\(^3^+)\) and putrescine\(^2^+\) (Put\(^2^+)\) in dilute solutions to understand the influence of the charged ligands on DNA condensation or DNA double helix-single coil transitions. However, informations on the descriptions on any structural aspects of polyamine binding to DNA are scarce in these studies.

Polyamines are present in millimolar concentrations in all eukaryotic cells and have a significant role in biological reactions involving DNA or RNA. Recently, it has been observed that the condensation/decondensation in the solutions of DNA and nucleosomes are influenced by polyamines. Polyamines are also known to be used as potential radio-protective agents and anticancer drugs. Studies performed on the interaction of the polycationic spermine (Scheme 1) (a natural polyamine) with DNA showed groove binding and major groove was observed to be the better site. Polyamine analogues can imitate some of the functions of biogenic polyamines like spermine, but are not good enough to imitate the supportive nature in the cell growth by the natural polyamines. Binding affinity of natural polyamines with DNA is higher than different synthesized polyamines. These synthesized polyamines have the ability to stop the strand breaks, however, low DNA binding affinity of the synthetic analogues and their ability to induce DNA aggregation and precipitation at a rapid rate make them markedly different from the natural ones.

Scheme 1. Tetra-protonated Spermine

Recently, Peña et al. synthesized new cyclic-chiral polyamine analogues and proposed that it may bind to DNA phosphate chain to one or two strands of DNA depending on the spacer of the ligands (Scheme 2). The –CH\(_2\) group used as spacers with n = 1 – 4, increases the distance
between the consecutive cyclohexanediamine moieties and thus between the amine groups. This change in the distances between the amine groups varies the interaction of the polyamine with DNA single/dual strands. The interaction of cyclic polyamines with DNA base-pairs has been described in the previous section; however, the larger polyamines have not been examined. Here, we have examined the interactions of larger polyamines with single and dual strands of DNA in implicit aqueous medium and extended the study with molecular dynamics simulation for the interactions of the polyamines at the major and minor groove of DNA in explicit solvent molecule.

**Scheme 2.** Tri-protonated cyclic polyamine with spacer n (n = 1 – 4).

### 4.2.2. Computational Details

#### 4.2.2.1. Conformational Search of the cyclic polyamines

The following protocol was used to generate conformations of the spermine and the cyclic polyamines. The spermine was tetra-protonated and the cyclic polyamines were tri-protonated.

(i) An exhaustive conformational search of the spermine and the cyclic polyamines with different spacers was performed with the AMBER* force field along with the GB/SA solvation model\(^{28}\) using the molecular modeling program Macromodel.\(^{29}\) GB/SA treats the solvent as an analytical dielectric continuum that starts near the van der Waals surface of the solute and extends to infinity. The model includes both generalized Born based (GB) solvent polarization terms and surface area-based (SA)\(^{30}\) solvent displacement terms. All non-bonded cutoffs were set to infinity for all calculations. Energy minimizations were performed with the Polak-Ribiere conjugate gradient (PR-CG) method,\(^{31}\) which involves the use Polak-Ribiere first derivative method with restarts every 3N iterations; the derivative convergence criterion was set to 0.002 kJ Å\(^{-1}\) mol\(^{-1}\). Conformational search was performed by the Monte Carlo method\(^{32}\) for the random variation of all rotatable bonds combined with the Monte Carlo multiple minimum (MCMM) conformational search algorithm.\(^{32,33}\) For each calculation 5000 Monte Carlo steps were carried out.
(ii) All found conformations were sorted according to energy.
(iii) Only conformations whose molecular mechanics energy differences to the calculated global energy minimum (GEM) of a compound are within 20 kJ mol\(^{-1}\) were stored. The resulted conformations were clustered based on torsional root mean square difference (RMSD) after rigid-body superposition using XCluster.\(^3^4\) The best conformers of the ligands were chosen based on energy and geometry criterion, which can easily bound to DNA structure.

### 4.2.2.2. Implicit solvent model Calculation

The implicit solvent phase Molecular dynamics calculations for DNA interacting with the polycationic linear polyamine (spermine) and the cyclic polyamines was carried out using the AMBER\(^\ast\) force field with the GB/SA solvation model. A total of 10 base pairs have been taken for our calculations. To nullify the charge of the polyanionic DNA, 18 Na\(^+\) atoms were fixed at a distance of \(\sim 2.5 \text{ Å}\) from each of the anionic phosphates and with an P-O-Na angle of 106°. The distances and angles were fixed with force constants of 24 kcal/mol.\(^3^5\) Periodic Boundary Condition was not employed during the Molecular Dynamics calculations with the implicit solvation, the Na\(^+\) ions were kept constraint at their positions. The PR-CG method was used with the convergence threshold of 0.002 kJ Å\(^{-1}\) mol\(^{-1}\). The simulation temperature was kept at 300 K. The Molecular dynamics simulations were performed for a total of 2 ns, with a time step of 1 fs. The equilibration time was maintained 1 ps before the MD run.

### 4.2.2.3. Explicit solvent model Calculations

The gromacs file and the topology files of the spermine and the cyclic polyamines were performed with the help of the PRO-DRG software\(^3^6\) using the pdb file of the respective polyamines obtained from the conformational search. The gas phase and the explicit solvent phase molecular dynamics simulations with DNA and the polyamines were performed with the AMBER 03 force field\(^3^7\) implemented with GROMACS 4.5.5.\(^3^8\) The initial cubic simulation boxes for DNA and with the ligands were generated with a linear dimension of 58.991 Å for gas as well as solvent phase. Approximately, 6700 water molecules were placed around DNA initially and when interacted with the polyamine ligands. The water–water interaction is described by the TIP4P solvation model\(^3^9\) provided by GROMACS 4.5.5.\(^3^8\) The simulation protocol comprises three major steps: (a) energy minimization (b) equilibration run at a desired temperature with harmonic restraints (c) the full molecular dynamics run or production run.

(a) In all cases an energy minimization of 1000 ps was performed initially.
(b) The equilibration step for the calculations was performed with a protocol and divided into no of steps with 500 ps simulations of each step. For the first step, the equilibration was performed for the water molecules while a harmonic restraint was used on DNA, Na\(^+\) ions and/or the
ligands, while in the second step the equilibrations was performed on the ligand and Harmonic restraints was used on DNA, Na\(^+\) ions and solvent molecules. In the last step, the equilibration of DNA and the Na\(^+\) ions were performed and harmonic restraints were used on the water molecules and/or the ligands. For the first equilibration step an NPT ensemble is used. The temperature was set to 300K and at pressure of 1 atm. Temperature and pressure kept constant with the coupling constants of 0.1 and 0.5 ps, respectively, by the v-rescale\(^40\) and Berendsen scheme.\(^41\) For the other steps, NVT ensemble was used with constant temperature (300K) using the Berensden scheme. A periodic boundary condition was applied in all cases. The time step used in the simulations was 0.5 fs, and the total time for the production run was 2.0 ns. For electrostatic interactions, we used the particle mesh Ewald method.\(^42\)

### 4.2.3. Results and Discussions

#### 4.2.3.1. Conformational search of protonated polyamines

The conformational search performed for the polyamines with Monte Carlo search method employing AMBER\(^*\) force field in the GB/SA implicit solvent phase predicted the best conformers in each case which were filtered using Xcluster process. Figure 1 shows the conformations of different polyamines taken for further calculations.

![Spermine\(^4+\) [A] cyclic polyamine\(^3+\) (n=1) [B] cyclic polyamine\(^3+\) (n=2) [C]]
4.2.3.2. Molecular Dynamics Simulations in implicit solvent

We have performed molecular dynamics calculations to examine the interactions of A (spermine) and the cyclic polyamines (B-E) with DNA (Figure 2) employing implicit solvent model. The interaction of ligands was primarily focused with the major site of phosphate. It has been observed that polyamines like spermine interact preferentially with the major groove. Thus, the polyamines were placed to the major groove for interactions with the PO$_4^{3-}$ group of a single and a dual strand of DNA.
**Figure 2.** The geometry of DNA taken for the MD simulation. The different bases [DG5: terminal Guanine base; DC3: terminal Cytosine base; DA: Adenine base and DT: Thymine base] are shown {Carbon: grey; Nitrogen: blue; Oxygen: red; Phosphorus: orange; Hydrogen: white and Sodium: violet}.

Snapshots of interactions of the spermine [A] with DNA single and dual strands at regular intervals are shown in figure 3 and figure 4, respectively. In the case of single strand interactions of A, it has been observed that spermine (A) interacts with the N7 atom of the GC base-pair and with the phosphate in the major groove site. This result corroborates the previous studies on the interactions of A with DNA.\textsuperscript{43} The interaction of A with the dual strand of DNA is different compared to the case of single strand DNA. Ligand A moves away from DNA and only two of the amine groups were observed to interact with DNA-phosphate at ~2000 ps time scale.
Figure 3. The interactions of spermine with the single strand of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions at 2000ps between spermine and DNA are shown.
Figure 4. The interactions of spermine with dual strands of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.

The interaction of the cyclic polyamine with n=1 [B] and the single strand of DNA showed similar interaction as observed with spermine during the simulation (Figure 3 and Figure 5). The interaction of B with the dual strand of DNA showed that the ligand interacted with the phosphate group and only two of the phosphate groups were involved (Figure 6). The better interaction of B with the single strand of DNA than the double strand was observed similar to the report by Peña et al., however, the nature of interactions is different than the reported results. The MD simulation results suggest the binding of ligand B near the major groove of DNA, whereas, Peña et al. speculated the binding along the phosphate chain.27 The cyclic polyamine B showed analogous interaction with DNA as observed using the model system of this compound 1,2-CHDA with selected base-pairs in the previous section.
Figure 5. The interactions of cyclic polyamine (B) with single strand of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between ligand B and DNA are shown at 2000 ps.
Figure 6. The interactions of cyclic polyamine (B) with dual strands of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.

The interaction geometries of the cyclic amine with n=2 [C], with the single and dual strands of DNA at regular time intervals are given in figure 7 and figure 8, respectively. The MD calculations suggest that the ligand C interacts weakly with DNA strands compared to the case...
of cyclic amine with $n=1$ [B]. The ligand C is feebly connected with one of the phosphate groups of DNA during the simulation process.

**Figure 7.** The interactions of cyclic polyamine (C) with single strand of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.
Figure 8. The interactions of cyclic polyamine (C) with dual strands of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.

The cyclic amine with n=3 [D] showed better interactions with the dual strand of DNA compared to single strand DNA (Figure 9 and Figure 10). The ligand is firmly associated with the dual strand of DNA throughout the simulation process.
Figure 9. The interactions of cyclic polyamine (D) with single strand of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.
Figure 10. The interactions of cyclic polyamine (D) with dual strands of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.

The interactions of the cyclic polyamine (E) with single and dual strands of DNA at regular time intervals in implicit solvation are given in figure 11 and figure 12, respectively. The ligand (E) binds favorably with both the strands of DNA over the simulation period of 2 ns. The preliminary DNA binding studies using UV-measurements of melting temperatures (Tm) indicated that the
dual strand of DNA would be the favored binding site. The MD simulations also suggest a favorable binding of ligand E with the dual strand of DNA.

\[
\begin{align*}
\text{PO}_4^{3-} & \quad \text{PO}_4^{3-} \\
0ps & \quad 500ps \\
1000ps & \quad 2000ps
\end{align*}
\]

**Figure 11.** The interactions of cyclic polyamine (E) with single strand of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.
The implicit solvation model deoids the physical interaction of solvent molecule with the solute systems. Therefore, it is important to examine the interactions of solvent molecules with DNA and ligand during the simulation process. We have performed molecular dynamics simulations with explicit water molecules involving periodic boundary conditions.

**Figure 12.** The interactions of cyclic polyamine (E) with dual strands of DNA at regular intervals (0, 500, 1000, 2000 ps). The specific interactions between spermine and DNA are shown.
4.2.3.2. Molecular Dynamics Simulations in the gas phase
The gas phase molecular dynamics simulations for the un-interacted DNA as well as the polyamine interacted DNA were performed with the AMBER 03 force field. The MD runs show that the geometries are fully ruptured during the process and DNA system does not survive in vacuo conditions. Figure 13 shows the un-interacted DNA moiety in gas phase at 0 and 2 ns.

![Figure 13. Snapshots of the un-interacted DNA in gas phase at time scales 0 and 2000 ps (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; Na⁺ ions: blue).](image)

4.2.3.2. Molecular Dynamics Simulations in explicit solvent molecules
The molecular dynamics simulation of DNA neutralized with Na⁺ ions in bulk water is largely unperturbed employing periodic boundary condition during the 2 ns run. Some of the Na⁺ ions present in the system to maintain the neutrality of the polyanionic DNA, moved away in the simulation process (Figure 14).

![Figure 14.](image)
Figure 14. Snapshots of the un-interacted DNA with explicit solvent molecules at time scales 0 and 2000 ps (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; Na⁺ ions: blue; Solvent: black dots).

4.2.3.2.a. Interaction of the cyclic polyamine with n=1 [B] in the major and minor groove of DNA

The cyclic polyamines are flexible in nature with increasing spacers hence it is important to explore the binding affinity of such polyamines with both major and minor grooves of DNA. We have performed molecular dynamics simulation studies on the interactions of these ligands with the major and minor groove of DNA in explicit solvent molecules. Snapshots of the orientations and the interactions of the cyclic polyamine B, in the major and minor grooves of DNA are given in figure 15 and figure 16, respectively. The MD simulation results suggest that the ligand B prefers to interact to the major groove site of DNA. As observed with model systems in previous section and the implicit solvation model the 1,2-CHDA moiety of the ligand B prefers to interact to the phosphate group and the N7 site of the GC base-pair in explicit solvent molecules (Figure 15). The explicit water molecules do not perturb the effective binding of DNA with ligand B via ionic hydrogen bonds. The Hydrogen bonding interactions between DNA and B was preserved throughout the simulation process (Figure 17). The simulation results suggest that B is less interactive with the minor groove site of DNA. The Hydrogen bonding interactions plotted for the association of DNA with B provides the evidence of less binding in the minor groove of DNA system (Figure 17).
Figure 15. Interactions of the ligand B with the major groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; ligand D: magenta; Na⁺ ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.
Figure 16. Interactions of the ligand B at the minor groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green;
ligand D: magenta; Na\(^+\) ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.

![Diagram of DNA solvation and interaction](image)

**Figure 17.** Hydrogen bonding interaction plots during the MD simulations for the interaction of B towards the (a) major and (b) minor groove of DNA.

### 4.2.3.2.b. Interaction of the cyclic polyamine with n=2 [C] in the major and minor groove of DNA

The interactions of cyclic polyamine C with the major and minor grooves of DNA at different time scales are given in figure 18 and figure 19, respectively. The larger spacer in the ligand C showed a better interaction with the minor groove of DNA compared to the major groove site. The observed hydrogen bonding plots suggest that the ligand C plot is more closely associated with the minor groove site of DNA (Figure 20).

![Diagram showing ligand C](image)
Figure 18. Interactions of the ligand C at the major groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; ligand D: magenta; Na\\textsuperscript{+} ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.
Figure 19. Interactions of the ligand C at the minor groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; ligand D: magenta; Na⁺ ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.
4.2.3.2.c. Interaction of the cyclic polyamine with n=3 [D] in the major and minor groove of DNA

Increasing further the spacer size for such cyclic polyamines, it appears that the binding of ligands get preferred in the minor groove of DNA in comparison to the generally observed major groove site (Figure 21 and Figure 22). The Hydrogen bonding interactions plot given in figure 23, showed clearly that interaction of D is better towards the minor groove site of DNA.

![Hydrogen bonding interaction plots during the MD simulations for the interaction of C towards the (a) major and (b) minor groove of DNA.](image)
Figure 21. Interactions of the ligand D at the major groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; ligand D: magenta; Na\(^+\) ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.
Figure 22. Interactions of the ligand D at the minor groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green;
lignand D: magenta; Na\(^+\) ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.

**Figure 23.** Hydrogen bonding interaction plots during the MD simulations for the interaction of D towards the (a) major and (b) minor groove of DNA.

4.2.3.2.d. Interaction of the cyclic polyamine with n=4 [E] in the major and minor groove of DNA

Further, increase in the spacer chain length in cyclic polyamine, E also showed a preference for the interaction with the minor groove of DNA molecule (Figure 24 and Figure 25). The snapshots showed that E prefers to associate to the AT base pairs in the minor groove more strongly than the major groove site. The Hydrogen bonding interactions during the simulation process also clearly shows that interaction of E is better towards the minor groove site than the major groove site (Figure 26).
Figure 24. Interactions of the ligand E at the major groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; ligand D: magenta; Na⁺ ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.
Figure 25. Interactions of the ligand E at the minor groove of DNA at time scales 0, 250, 1000 and 2000 ps are given (DNA: yellowish ribbon; DG5: orange; DC3: gray; DA: silver; DT: green; ligand D: magenta; Na⁺ ions: blue; Solvent: black dots). Solvent molecules are sometimes omitted for a better view.
Figure 26. Hydrogen bonding interaction plots during the MD simulations for the interaction of E towards the (a) major and (b) minor groove of DNA.

4.2.4. Conclusions

In this section, we have examined the interaction of cyclic polyamines with DNA in implicit solvent phase and in bulk explicit solvent molecules. The MD simulations suggest that DNA is unstable in the gas phase suggesting that gas phase calculations are not suitable for the studies performed on DNA.

In implicit solvent model, the interactions of the polyamines with DNA were performed through the phosphate group binding at the major groove site as examined in earlier report. The simulation results corroborate the previous reports towards the interaction of spermine with the phosphate group and the N7 site of the GC base pair at the major groove site. The cyclic polyamine ligand B was also observed to have similar interactions like the spermine. The interaction of the 1,2-CHDA moiety of the ligand B with the single strand of DNA showed similar interactions as observed for a simple protonated 1,2-CHDA with the DNA base pairs in the previous section. The interaction of cyclic polyamines with different spacers (C - E) showed no definite pattern of association with the single and dual strands of DNA in implicit solvent model.

The MD simulations have been performed for the interaction of cyclic polyamines with both the major and minor groove of DNA using explicit solvent molecules in periodic boundary conditions. The observed interaction of cyclic polyamines of smaller spacer units [n=1 and n=2] prefers to bind the major groove site, whereas, the larger spacer units [n=3 and n=4] showed better preference for interaction on the minor groove site. The explicit water molecules does not interfere the ionic hydrogen bonding interaction of ligands with the DNA and hence calculations
performed in implicit solvent model can also be considered as a suitable model in examining the interaction of protonated ligands with DNA. Moreover, the MD simulation results suggest that the sites of interaction can vary with the chain length of cyclic polyamines and hence a generality for the interaction of ligands is not appropriate in such case.
References


