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The intercalation of DNA double helices with doxorubicin and nagalomycin

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Abstract

A variety of molecules bind to DNA in its major and minor grooves, and some, like the anthraquinoids, are known to form intercalates in which these molecules are inserted directly into the double helix, between the bases. Several researchers have pointed to an electron transfer mechanism (leading to ion pairing) as one of the factors that could hold the intercalated entities like doxorubicin in place, but the bulky anthraquinone nogalamycin did not seem to become engaged in electron transfer. The molecular modeling program STR3DI32 was used to investigate the stabilities of these intercalated anthraquinone before any possible electron transfer has occurred.

Keywords: DNA; Double helix; Intercalation; Stereo-electronic effects; Hydrogen bonding; Molecular mechanics; STR3DI32

1. Introduction

The DNA double helix, and its Watson–Crick (W–C) hydrogen bonding features, have been known for more than 50 years. The DNA double helix binds with a wide variety of molecules in its major and minor grooves. Some of these associations seem to be due to classical hydrogen bonding interactions between the guest molecule, like spermine, and compatible sites on the surface of the double helix. These associations have stimulated many elegant research efforts that have generated interesting, and obviously very important, molecules that show site specificity in their interactions with DNA in its major grooves [1]. Other researchers have focused attention on flat aromatic molecules that intercalate directly into the double helix, between the bases, in investigations of the ability of the double helix to delocalize a ''hole'' (a positive charge) that has been injected into one of the bases [2]. A careful review of structures available from X-ray crystallographic investigations has established that the DNA bases are aromatic when they are in the Watson–Crick structures double helix and has confirmed the fact that some intercalated anthraquinoids, like doxorubicin, do engage in electron transfer with the DNA double helix in the solid phase [3].

These important studies have generated interesting data, but somehow did not carefully examine all of the possible stereo-electronic effects that could contribute to the binding of the intercalated aromatic entity, except the obvious ion pairing due to electron transfer. These studies also focused very narrowly on the aromatic entities involved, and paid almost no attention to the roles of the sugar-phosphate ''backbone'' in the intercalation processes, except to acknowledge this as the scaffolding of the DNA system.

The fact that the intercalation of flat aromatic molecules into DNA double helices is a dynamic process obviously indicates that any electron transfer process must be reversible, but does leave open the question of exactly when electron transfer occurs. Does electron transfer occur after the aromatic entity is intercalated, so generating favourable coulombic interactions that lead to intercalation, or does it occur before intercalation, thus generating the intimate ion pair that ''snuggles'' together for greater stability?

It seemed important to evaluate the stereo-electronic stability of the intercalated cluster before electron transfer occurs, because if this cluster was unstable (with respect to the separated materials) then this would be evidence for the electron transfer ion pair, generated before intercalation, being the critical factor driving intercalation.

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However, if the intercalated cluster was quite stable before electron transfer occured, then we could speculate that electron transfer was not critical before intercalation had occurred. This validation of an electron transfer process occurring after intercalation would also reinforce the notion that the electron transfer process must be rapidly reversible. After all, Mother Nature does not like separated charges.

There is some indication of the direction the discussion is likely to take. This arose from a careful analysis of the solid phase X-ray crystallographic studies of some DNA intercalated clusters [3] which showed that the relatively flat anthraquinoid molecules, like daunomycin, form crystalline intercalated clusters with DNA in which there is clear evidence of electron transfer. However, this study also showed that the sterically bulky anthraquinoid molecule nogalamycin, which also forms a crystalline intercalated cluster with DNA, did not demonstrate clear evidence of electron transfer in the solid phase [3].

The answers to the questions and speculations posed above can be obtained most easily through theoretical studies. Unfortunately, the quantum mechanical (QM) methods are not suited for the simulations of large entities like the intercalated DNA clusters of interest. We have seen that some of the well known molecular mechanics (MM) methods are also not well suited for this task since they cannot handle the intermolecular interactions present in these intercalated DNA clusters. Any program that will be used successfully to perform these calculations must be able to minimize the structure energy of molecular clusters.

Recently, the DNA double helix was successfully simulated using the molecular modeling program STR3DI32 [4]. Not only did the simulation reproduce the known classical hydrogen bonding features of the double helix, but it also revealed the considerable importance of C–H hydrogen bonding in these polymers. The simulation revealed hitherto undocumented stereo-electronic interactions in the double helix and uncovered some of the critically important roles played by the 2-deoxyribosyl units in these molecules. Thus, we decided to re-examine the anthraquinoid intercalated DNA double helix, using STR3DI32, and once again use X-ray crystallographic coordinate data as the molecular starting point in order to embrace the available experimentally obtained data, in this area.

Since STR3DI32 automatically generates its own connectivity list from the X-ray crystallographic structural coordinate data, and then proceeds to identify all existing hydrogen bonds and dipolar interactions, the success of a study of a densely hydrogen bonded entity, like a polynucleotide, will be independent of human errors and omissions. If there are dipolar stereo-electronic interactions present in the cluster being studied that have not, hitherto, been recognized, then a study using STR3DI32 will most likely recognize these interactions.

Indeed, one usually does not pay a lot of attention to the possibility of encountering unusual stereo-electronic interactions, especially as organic chemistry is regarded as a relatively ''mature'' science in which most, if not all stereo-electronic effects have been identified. However, unusual stereo-electronic interactions do occasionally intrude into our research, like the recognition that there is no π–π electronic delocalization between the nitro group and the aromatic ring of nitrobenzene [5], and that aromaticity can be turned on and off in some simple heterocyclic molecules [6]. Every theoretical investigation of biomolecular clusters must make every allowance for new stereo-electronic effects to be recognized and assessed, as we shall see below.

2. STR3DI32 and the QVBMM force field

The QVBMM (quantized valence bonds' molecular mechanics) force field can be regarded as a quantitative version of the well-known VSEPR (valence shell electron pair repulsion) model for predicting the structures of covalently bonded molecules, and is further enhanced by several sophisticated structure-energy algorithms [7]. As in the VSEPR model, the roles of every entity in the molecule, including the lone pairs of electrons, are intimately involved in the calculations performed using the QVBMM force field. The modeling program STR3DI32 couples the QVBMM force field with several sophisticated, extensively tested, molecular structure recognition algorithms.

STR3DI32 requires only the input of *X*-, *Y*-, and *Z*-coordinates, and the type of entity (atom or lone pair), for each moiety in the structure being examined. STR3DI32 then assembles the molecule, with all of the implied structural information, and presents the molecule, or cluster, to the user. No connectivity lists are needed from the user, no hydrogen bonds need be specified, nor is it necessary for the user to specify dipolar interaction, hybridization types, or delocalization data, since all of this information is embedded in the coordinate data and is automatically recognized by STR3DI32. Thus, STR3DI32 is ideally suited for the simulations of any type of molecule, especially when looking for new stereo-electronic interactions that might be embedded in simple, or complex, molecules, or molecular clusters.

It is this ability of STR3DI32 to recognize intermolecular interactions that allows the user to create clusters of molecules, which can be treated as one ''logical'' molecule, and perform a normal structure-energy minimization of these clusters. This ability to handle molecular clusters will be most important in any examination of the DNA double helix, which possesses extensive arrays of intermolecular and intramolecular hydrogen bonds. Further, since the user does not have to ''inform'' STR3DI32 of dipolar interactions, or hydrogen bonds of any type, in order for these effects to be included by the program into its calculations, because the program detects these automatically, the calculations performed by STR3DI32 on these DNA clusters will be more reliable. Thus, STR3DI32 will, using its sophisticated structure analytical capabilities, enable the user to identify routine, or unusual, dipolar stereo-electronic interactions in any molecule, or molecular cluster, being studied [8–14].

3. The DNA project

We intentionally started this study by using experimentally obtained the X-ray crystallographic structural coordinate data

of the intercalated DNA clusters of interest. In this way, our study will embrace a significant experimental base of data and will not be entirely theoretical.

Given the significant amount of effort involved in refining the X-ray crystallographic structural coordinate data, an examination of the structural and stereo-electronic features of two relatively simple intercalated double helices, PDB2DES and PDB224D, each of which had two intercalated molecules, was undertaken. The cluster PDB2DES was intercalated with 2,3′-desamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin (hereafter called doxorubicin), while PDB224D was intercalated with ''nogalamycin''. Curiously, although the ENT file identified the intercalate of PDB224D as ''nogalamycin'', the two intercalated units were different, one molecules was the C-7 methyl ester and the other the C-7 dimethyl acetal of the corresponding aldehyde. Nogalamycin is the methyl ester [15] and so the ''acetal'' was converted into the methyl ester.

The coordinate data and atom types were extracted from the Brookhaven ENT files and were converted into a format used by STR3DI32. The structures were then reliably elaborated with hydrogens and lone pairs of electrons. It was then necessary to correct many bond length–bond type inconsistencies that were found in these structures. This was especially true in the data for the bases, in which some bonds in some of the hetero-aromatic rings were much too long, and occasionally, much too short. The phosphorous–oxygen bonding also presented extensive bond length–bond type inconsistencies. This aspect of these projects continues to be particularly time-consuming and will remain so until crystallographers pay more attention to the details of the structures they generate.

As was mentioned above, the X-ray crystallographic data show that the anthraquinoid molecules, like daunomycin and doxorubicin, form crystalline intercalated clusters with DNA in which there is clear evidence of electron transfer. Thus, the central ''quinoid'' ring of the intercalated structure clearly possessed the geometrical features of a phenol, showing that these anthraquinoid molecules had accepted an electron from the DNA [3].

Since the goal of this study is to assess the stability of the cluster of the DNA double helix and the intercalated anthraquinone, then STR3DI32 was used to adjust the geometrical features of the central ''quinoid'' ring of the intercalated entity back to those typical of an anthraquinone.

This exercise was not necessary for the nogalamycin intercalates since the X-ray crystallographic data for PDB224D show that this sterically bulky anthraquinoid molecule did not demonstrate evidence of electron transfer (acceptance) from DNA in the solid phase. Thus the structural features of the central ring of the nogalamycin entities were consistent with those of anthraquinones [3].

Having prepared the structures of the intercalated double helices, they were then subjected to structure-energy minimization using STR3DI32. For each intercalated DNA cluster, STR3DI32 was then used to:

- remove one intercalated entity from the cluster and then evaluate the stereo-electronic interaction energy of the ''separated cluster'' (in which the two entities no longer share intermolecular interactions).
- remove the other intercalated entity from the double helix and evaluate the interaction energy of the ''separated cluster'', in which the double helix is now ''empty''.
- minimize the structure energy of (relax) each of the separated component of the cluster in order to assess whether these components were in, or close to, their minimum energy conformations.
- The coordinate data for the STR3DI32 structure energy minimized molecular models of PDB2DES and PDB224D are available as Supplementary Information.

4. Solvation effects

The normal milieu of DNA is water, and solvation by water must play an extremely important role (via the entropy of solvation and other cluster effects [14]) in determining the total free energy data for these molecules. However, any careful examination of the structure of water solvated DNA structures, like PDB224D and PDB2DES, using the experimentally generated X-ray coordinate data, shows that the water molecules detected are all located on the outer surface of the structure. This information does not provide us with any reliable estimate of the number, and locations, of water molecules inside the region of the Watson–Crick (W–C) hydrogen bonded core. Thus, it seemed reasonable to assume that while solvation effects due to water will be strongly encountered on the outer surface of the DNA double helix, these solvation effects could be ignored when stereo-electronic effects found within the W–C hydrogen bonded core are to be examined.

Further, given the fact that polar solvation effects normally will attenuate stereo-electronic effects due to dipolar interactions (especially intermolecular dipolar interactions), the use of STR3DI32 to investigate and simulate these clusters from which the external (surface) water molecules had been excluded, seemed justified. Indeed, the focus will be on the stereo-electronic interactions within the intercalated double helix and not on the surfaces of these entities.

Thus, STR3DI32 will be used to closely examine and identify the stereo-electronic effects native to the unsolvated core of the polynucleotide system, and the interactions between the two helices and their intercalated entities.

5. The data

5.1. Intercalation of the glycosides

Once the experimental (X-ray crystallographic) structures had been corrected for their bond length and bond angle inconsistencies, the structures of the energy minimized (hereafter referred to as relaxed) intercalated double helices were essentially unchanged from those derived directly from the X-ray crystallographic coordinate data. Thus, the QVBMM force field did provide excellent simulations of these clusters.

Table 1
Interaction energy data (in kcal/mol) for PDB224D

5′-TpGpApTpCpA-3′	Stereo-electronic interaction energy (kcal/mol)				
	Intercalated entity	Double helical entity	Total strain interaction energy	Change due to intercalation	Change due to relaxation
Intercalated nogalamycin					
Intercalated double helix			3603.7		
Intercalate A removed	353.7	3279.5	3633.2	−29.5	
Intercalate A relaxed	335.0				−18.7
Intercalate B removed	334.1	2985.5	3319.6	−40.1	
Intercalate B relaxed	334.1				0.0
Separated components	687.8	2985.5	3672.3	−68.6	

The sequence of removal of the intercalated anthraquinoids from the double helical cluster did not affect the associated stereo-electronic interaction energy change. Thus, no matter which intercalated entity was removed first, the energy change associated with its removal was the same, and the overall energy change when both intercalated entities were removed, in any sequence, was the same. The stereo-electronic (strain) interaction energy data (in kcal/mol), generated by STR3DI32, for the molecules studied are shown in Tables 1 and 2.

The data clearly indicate, for both intercalated DNA clusters, that the intercalated cluster is more stable than the separated components, before any relaxation of the separated entities involved was allowed to occur. Table 1 shows that for the two nogalamycin units in PDB224D, removal of the anthraquinoid entities raises the interaction energy of the system significantly. Relaxation of these nogalamycin units (structure energy minimization) after they were removed from the cluster caused the interaction energy of one to decrease significantly, while that of the other remained constant, showing that it was already in an energy well. The conformation of the higher energied nogalamycin unit, while intercalated, was significantly distorted from its nearest local minimum energy structure and this distortion must have occurred in order to maximize the hydrogen bonding interactions between the DNA double helix and the intercalated ester, and otherwise to ensure a ‘‘best fit’’ between the intercalated entity and the double helix.

As expected, the double helix was also able to relax significantly after the intercalated entities were removed. The resulting partially relaxed structure (definitely caught at some local minimum, rather than at the global minimum energy) still showed evidence of the huge ‘‘slots’’ that were once occupied by the intercalated molecules, and this partial molecular relaxation achieved by STR3DI32 was mostly due to a change in the conformation of the 2-deoxyribosyl phosphate backbone that had occurred in order to create the needed ‘‘slots’’.

This situation is also interesting in the context of thought that the DNA double helix normally has a ‘‘breathing’’ mode of vibration, when it stretches (like a ‘‘slinky’’?) and one or more ‘‘slots’’ move rhythmically through the structure [16]. The energy involved in this ‘‘breathing’’ mode of vibration would need to be added to the totally energy of the double helix, and the energy changes might be small enough to be compensated for by solvation (a widened slot could hold more water molecules).

Thus, intercalation might well be a coupled process which is preceded by the interaction of the nogalamycin’s sugar with the surface of the double helix, and then, when the double helix ‘‘breathes’’ at that point, the anthraquinone slips into the ‘‘slot’’ [16].

Table 1 shows that the total interaction energy of the three entities, obtained from the separation of the intercalated PDB224D, was 68.6 kcal/mol higher than that of the intercalated PDB224D. Table 2 also shows that the total interaction energy of the three entities, obtained from the separation of the intercalated PDB2DES, was 43.1 kcal/mol higher than that of the intercalated PDB2DES. These significant interaction energy differences might also be compounded by the significant increase in entropy that would occur during desolvation of the anthraquinone units as the intercalations proceeded. In that case, the intercalation process might well be much more energetically favourable.

Table 2
Interaction energy data (in kcal/mol) for PDB2DES

5′-CpGpTpApCpG-3′	Interaction energy (kcal/mol)				
	Intercalated entity	Double helical entity	Total strain interaction energy	Change due to intercalation	Change due to relaxation
Intercalated doxorubicin					
Intercalated double helix			3532.0		
Intercalate A removed	283.4	3271.6	3555.1	−23.0	
Intercalate A relaxed	270.0				−13.4
Intercalate B removed	273.8	3017.9	3291.7	−20.1	
Intercalate B relaxed	266.0				−7.8
Separated components	557.2	3017.9	3575.1	−43.1	

It is also clear that the intercalated molecules, in both PDB224D and PDB2DES, have different conformations while they are in the intercalated cluster, one being higher in energy than the other. In each case, the intercalated entity relaxed to a different local energy minimum. The interaction energy data in Tables 1 and 2 also indicate that the conformations of the intercalated entities were not identical.

5.2. The electron transfer process

It is widely held that nogalamycin and the other ''threading'' intercalators [17] first bind to the double helix using their sugar units, and then the aglycone slips into the double helix during one of its ''breathing'' modes of vibration—at that site. It is also widely known that the bioactivities of these ''threading'' intercalators can be manipulated by changing the structures of their sugar units. Thus, one cannot ignore the roles of the sugar units of the intercalators in these studies.

In the discussion on solvation, above, the case was made to ignore solvation effects on the outer surface of the intercalated DNA double helix, even though the sugar units do bind onto the outer surface. The effects of solvation on these interactions have therefore not been assessed in this work. Similarly, any solvation interactions between the intercalators and water molecules on the surface of the DNA have not been accounted for. Thus, it is possible that interactions between the intercalators and the DNA double helix, that occur on the surface of the double helix, and are mediated by solvent water molecules, have not been considered in this work. It must also be remembered that since the positions and numbers of the water molecules that might be involved in any such interactions are not known from the X-ray crystallographic data, it will be a very difficult task to recreate these interactions.

It is known that the intercalation process is reversible [16–18] and this demands that the electron transfer process – from the guanosine to the anthraquinone – must also be reversible. The stability of the intercalated cluster, before electron transfer has occurred, suggest that electron transfer is not a prerequirement for intercalation, and that electron transfer occurs after intercalation. Thus, it seems most likely that after the intercalation occurs, the anthraquinone snuggles up to the guanosine unit, and then reversible electron transfer occurs.

The X-ray crystallographic coordinate data for PDB2DES clearly show that, in the solid state, the doxorubicin anthraquinone moiety has been reduced and is a phenolic radical anion. Further, the electron transfer process must be quite favourable in the PDB2DES cluster since the X-ray data clearly suggests that the electron spend most of its time in the ''anthraquinone''. This is what we would have expected from the discussion above.

However, the X-ray crystallographic coordinate data for PDB224D do not show the anthraquinone moiety of the nogalamycin to be reduced. Is this an artifact due to erroneous coordinate data, or does this suggest that the electron transfer process in the PDB224D cluster is less favourable that in PDB2DES, and that the electron spends most of its time in the host guanosine?

The data shown and discussed above suggest that these intercalated double helices could possess significant stability, especially the intercalated PDB2DES involving doxorubicin. This conclusion is obviously arrived at in the absence of entropic and solvation contributions to the intercalation process, but since there must be a very large favourable entropy of desolvation involved, it is likely that the conclusions drawn above are correct. An interesting point of speculation here must concern the entropy changes involved in the breathing mode of vibration of the double helix, since when the double helix ''inhales'', and opens a ''slot'', many water molecules probably rush into the ''slot'', only to be expelled in the ''exhalation'' process.

5.3. The dipolar interactions involved in the intercalation process

STR3DI32 is a molecular mechanics based implementation of the VSEPR theory and so, obviously, does not recognize orbital interactions like π-stacking. The interactions discussed in this work, between the intercalated entities and their double helix host, must therefore be solely dipolar in nature. This fact does not to deny the existence of possible π-stacking and other orbital-based phenomena, but it certainly shows that the contributions of the dipolar phenomema cannot be ignored, and might certainly be as important, if not more important, that those due to orbital-based phenomena.

It would be almost impossible to show, or describe, all of the dipolar interactions present between an intercalated entity and the bases that form the molecule ''slot'' of the host. However, a few points of interest should be noted.

Notwithstanding the inability of STR3DI32 to account for π-stacking, it was apparent that there were instances of stabilizing interactions between the lone pairs of the intercalated entity and favourably oriented dipoles in the π-systems of the bases [19]. Note that the hydrogen bonding between the bases was not disrupted, but there were ''new'' C–H hydrogen bonds between suitably located/oriented lone pairs of the intercalated entities and the C–H bonds of the bases.

It was also quite obvious that the 2-deoxyribosyl groups that held the bases together to form the ''slot'' were also involved in C–H hydrogen bonding interactions with the intercalated entities. These interactions are difficult to describe in the absence of a congruent numbering system and do require the use of a program, like STR3DI32, that can detect, and quantify the interactions.

For example, in the case of the nogalamycin intercalated entity (the nogalamycin in the DNA slot), there is a strong (3.5 kcal/mol) C–H hydrogen bond between the ring oxygen of the 2-deoxyribosyl group and the hydrogen that is alpha to nogalamycin's ester group. The other 2-deoxyribosyl group's ring oxygen is hydrogen bonded to the nogalamycin glycosyl hydrogen (2.0 kcal/mol).

The doxorubicin's aglycone's methoxyl oxygen shows a strong interaction with the p-systems of the DNA cytosine, and there are several small interactions between the doxorubicin's sugar and the the 2-deoxyribosyl groups of the grove.

6. Conclusion

This study has demonstrated that the stability of intercalation of the anthraquinones into suitable DNA double helices involves highly stabilizing dipolar interactions, in addition to any stabilization that arises from the π-stacking of the intercalated entity and the DNA bases. These dipolar interactions can be accounted for through the involvement of extensive hydrogen bonding (O–H, C–H, N–H) and favourable lone pair–dipole interactions [19], especially involving the 2-deoxyribosyl groups.

In a previous paper, some of the important structural/conformational roles of the DNA double helix's 2-deoxyribosyl unit were discussed [4]. This study had revealed that this humble, much neglected and trivialized sugar unit, plays very important roles in the intercalation of anthraquinones like nogalamycin and doxorubicin.

This work also demonstrates the tremendous importance of carefully conducted X-ray crystallographic studies of important molecules, especially if the crystallographers spend more time and effort to remove erroneous coordinate data and to properly refine their final coordinates. This effort would not be wasted, since the resulting data would be much more valuable to theoreticians. Crystallographers ought to enlist the assistance of a program, like STR3DI32, that will enable them to rapidly review a proposed structure for accuracy and credibility.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2006.09.005.

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