Premelting Base Pair Opening Probability and Drug Binding Constant of a Daunomycin-Poly d(GCAT)·Poly d(ATGC) Complex

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ABSTRACT We calculate room temperature thermal fluctuational base pair opening probability of a daunomycin-poly d(GCAT)·poly d(ATGC) complex. This system is constructed at an atomic level of detail based on x-ray analysis of a crystal structure. The base pair opening probabilities are calculated from a modified self-consistent phonon approach of anharmonic lattice dynamics theory. We find that daunomycin binding substantially enhances the thermal stability of one of the base pairs adjacent the drug because of strong hydrogen bonding between the drug and the base. The possible effect of this enhanced stability on the drug inhibition of DNA transcription and replication is discussed. We also calculate the probability of drug dissociation from the helix based on the selfconsistent calculation of the probability of the disruption of drug-base H-bonds and the unstacking probability of the drug. The calculations can be used to determine the equilibrium drug binding constant which is found to be in good agreement with observations on similar daunomycin-DNA systems.

INTRODUCTION

Daunomycin is an anticancer drug used for the treatment of such diseases as acute leukemia. It is known to inhibit both DNA replication and transcription through binding and intercalation into DNA base pairs (DiMarco et al., 1974). Since both replication and transcription in DNA involves base pair separation, it is expected that binding of daunomycin inhibits the unwinding process through both direct blocking and enhancing the stability of the base pairs at the binding site. The increase in the stability of the base pairs at a daunomycin binding site is evidenced by the observation of the significant increase in the melting temperature of a number of daunomycin-bound DNAs (Remeta et al., 1993). Because of the significant change in the observed melting temperature, one can speculate that this increase in the stability of the drug-bound base pairs may play an important role in effectively inhibiting the base pair separation process at the binding site.

To examine to what extent binding of daunomycin affects the thermal stability of the base pairs at the binding site, we carry out a calculation to determine the thermal fluctuational base pair opening probabilities of a daunomycin-DNA complex at room temperature (293 K). We will show that the opening probability of the base pair to which the drug forms hydrogen bonds is substantially decreased, and this decrease may be sufficient to slow down or block the unwinding process at the base pair. We will also estimate the drug-base dissociation probability and show that it agrees with the observed equilibrium daunomycin binding constants.

The method used in our calculation is the modified self-consistent phonon approach (MSPA) of anharmonic lattice dynamics theory which is a microscopic theory that analyzes temperature-dependent thermal vibrational dynamics of macromolecules such as DNA (Prohofsky, 1985). The theory can be used to calculate thermal fluctuational disruption probability of individual bonds. Our calculated interbase H-bond disruption probabilities and base pair opening probabilities (Chen et al., 1991; Chen and Prohofsky, 1992) are in fair agreement with observations for various DNA polymers.

The base pairs and the daunomycin drug are stabilized by a number of H-bonds and stacking interactions. As shown below the stacking interaction in a base pair in a drug-free helix is disrupted along with the disruption of the H-bonds. Therefore the opening probability of a base pair can be determined by the disruption probabilities of the individual H-bonds. On the other hand the role of stacking interactions between the daunomycin and the helix is quite different from the drug-free base-base stacking interactions. Therefore, in determining the drug dissociation probability, one has to take into consideration the drug-base stacking interactions as well as the drug-base H-bonds as discussed below.

CONTRIBUTION OF STACKING INTERACTION TO THE DYNAMICAL STABILITY OF BASES AND DAUNOMYCIN

The individual nonbonded interactions are weak compared to both valence bonds and H-bonds. If these interactions were operating independently they would be above the critical temperature at which they would dissociate long before the observed melting temperature of DNA. They are principally stabilized by the H-bonds against dissociation by motion transverse to the helix axis and are stabilized against dissociation by motion along the axis by cooperative reinforcement. The bases are planar structures that have large overlap when helically stacked, each atom of the planer structure is near several atoms of neighbor bases. For motion parallel to the helix axis that would tend to separate the base pairs, all the nonbonded interactions contribute coherently to oppose

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separation. The combined effect of the nonbonded interaction contributes significantly to the energy of the helical structure.

The same cumulative effects that stiffens the system against z displacements softens it against displacements transverse to z. As a base is slid transversely between neighbor bases the distance between one atom and an interacting neighbor atom may increase, but, at the same time, it is brought into closer proximity to a different neighbor base atom. For any transverse motion the forces for all possible interactions tend to cancel. That effect is illustrated in Fig. 1 as the displacement in that figure is for transverse motion, a rotation of the base pivoted about the sugar ring. The base is a guanine base in a stack of drug-free CGTA bases H-bonded to complimentary bases in B conformation. Positive motion is rotation toward the major groove and negative is toward the minor groove. The stacking energies are calculated by using the AMBER van der Waals parameters (Weiner et al., 1986) and the interbase H-bond energy is calculated using a Morse potential (Chen and Prohofsky, 1993a). Similar behavior would be observed for other displacements transverse to the helix axis.

The total nonbonded potential is seen to show relatively small variation with displacement of the center of mass of the base for distances of several angstroms. When interbase H-bond energy is included there appears a narrow deep well around the equilibrium position. This indicates the predominant role of interbase H-bonds in dynamically stabilizing the bases. More informative curves are shown in Fig. 2. That figure shows the force constant or second derivative of the potential of Fig. 1. The principal restoring force for transverse motion arises from the H-bonds. It is dominant for distances of tenths of angstroms displacement from the equilibrium position. In the absence of drugs the helix can

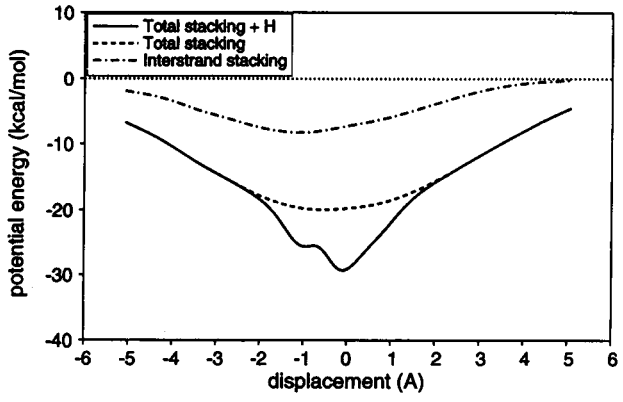

FIGURE 1 Different potential energies of the guanine base in a B-form CGTA double helix as a function of its center of mass transverse displacement with respect to its observed equilibrium position. The transverse motion is a rotation of the base pivoted about the sugar ring. The positive displacement is rotation towards the major groove and the negative is rotation towards the minor groove. The solid line corresponds to the total stacking energy plus the total interbase H-bond energy. The dashed line corresponds to the total stacking energy. The chain-dot line corresponds to the total inter-strand stacking energy.

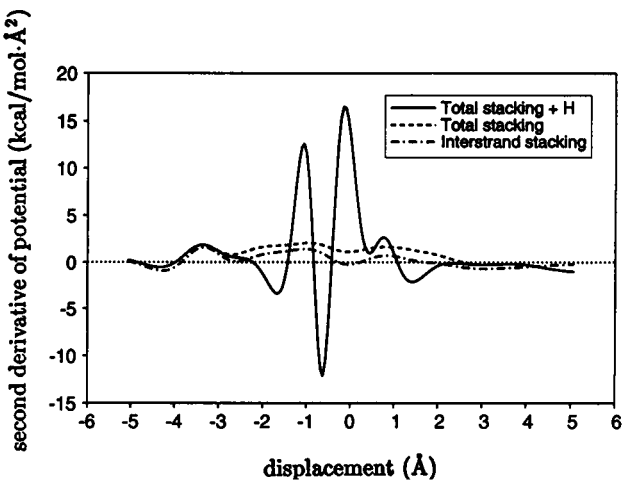

FIGURE 2 Second derivatives of the potential energies described in Fig. 1. The lines refer to the same potential energies given in Fig. 1.

separate into single strands for transverse displacements of tenths of angstroms. The intra-base pair interactions can sever and the individual strands remain mostly stacked one above another.

The principal element retarding base pair dissociation is then the H-bonds. The nonbonded interactions retard transverse base pair separation by their secondary role in enhancing H-bond stability. The bulk of the nonbonded interactions don't directly contribute to base pair stability as base pairs can separate and the bases on each strand remain stacked. Only the interstrand nonbonded interactions contribute directly to base pair stability. From Fig. 2 one can see that the contribution from these terms to the force constant is very small. When all the H-bonds are disrupted the bases will separate as the interstrand nonbonded interactions are not strong enough to maintain stability at a level of excitation that is already great enough to be capable of disrupting the H-bonds. The proper order parameter for determining melting of the helix in the absence of drugs is therefore $(1 - P^{op})$, where P^{op} is the probability that the H-bonds are dissociated (Chen and Prohofsky, 1993c).

The role of nonbonded interactions between the daunomycin and helix is quite different. For the drug to dissociate from the helix the nonbonded interactions have to be completely overcome. If the drug remains stacked it is not fully dissociated from the helix. The dissociation then requires that all H-bonds between drug and helix be dissociated and the large number of nonbonded interactions act cooperatively. The drug dissociation probability then has terms from the H-bond dissociation probability and from the nonbonded dissociation probability. Since the nonbonded dissociation probability enters as an independent factor, it should be determined independently of the stabilizing effect of the H-bonds. The proper calculation for the nonbonded dissociation probability should be calculated in a model where the H-bonds to the helix are disrupted, i.e., where the H-bond force constants are set to zero.

The daunomycin drug is composed of two groups. One is the aglycon chromophore (AC) group consisting of four fused rings and the other is the amino sugar (AS) group. The AC group is intercalated into the space between base pairs and is oriented at the right angles to the long dimension of the base pairs. Since the main four ring structure is completely intercalated into the base pairs, there is strong van der Waals interaction between the group and the bases above and below it. The AS group on the other hand rests in the minor groove. Since the minor groove is wide the stacking interaction between this group and the atoms on the floor of the minor groove is much weaker.

Our calculation indicates that the stacking interaction between the daunomycin AC group and the bases are much stronger than base-base stacking interaction. Fig. 3 and Fig. 4 displays the calculated stacking potential energy and the stacking plus drug-base H-bond potential energy and their second derivatives as a function of the center of mass displacement from its observed position along the orientation of the ring system of the AC group. The positive displacement corresponds to the dissociation of the drug from the bases and the negative displacement corresponds to the movement of the drug further into the bases. The calculation is carried out on a daunomycin-bound CGTA double helix. The details of this calculation will be described below. As shown in Figs. 3 and 4 both the stacking energy and its second derivative without the drug-base H-bonds are comparable with that in which the H-bond potential is included over all drug displacement. This indicates that stacking interaction plays an important role in dynamical stability of the daunomycin AC group. The contribution from this stacking interaction has to be included in determining the drug dissociation probability or the drug binding constant.

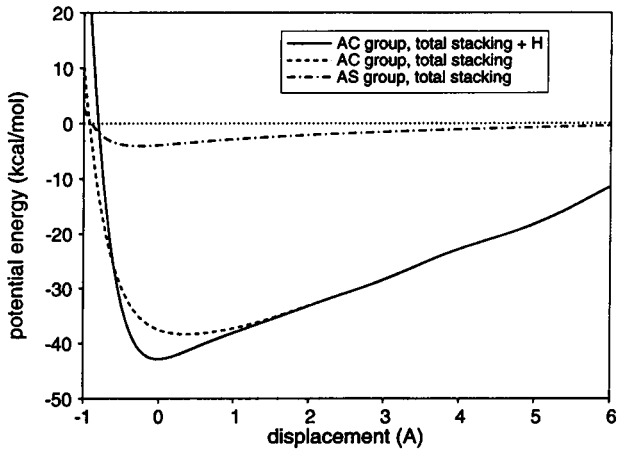

FIGURE 3 Potential energies of the aglycon chromophore (*AC*) group and the amino sugar (*AS*) group of a daunomycin bound to the GC pairs of a CGTA double helix as a function of their center of mass displacements. The displacements are from their observed equilibrium position along the orientation of the ring system of the AC group. The positive displacement is the dissociation of the drug from the bases and the negative is the movement of the drug further into the bases. The solid line corresponds to the total stacking energy plus the total drug-base H-bond energy for the AC group. The dashed line corresponds to the total stacking energy for the AC group. The chain-dot line corresponds to the total stacking energy for the AS group.

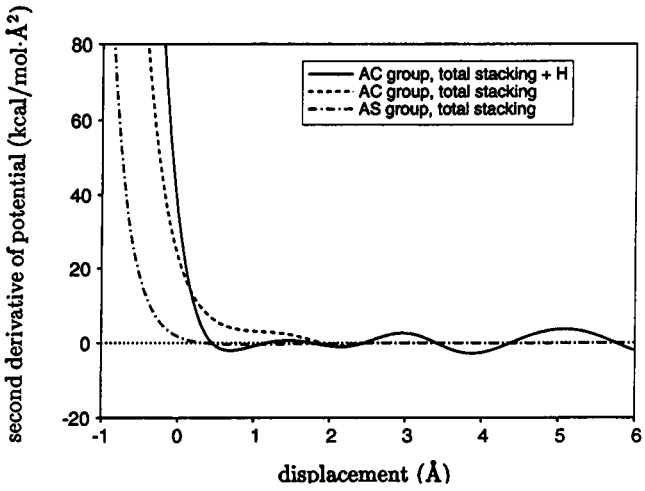

FIGURE 4 Second derivatives of the potential energies described in Fig. 3. The lines refer to the same potential energies given in Fig. 3.

As shown in Figs. 3 and 4 the stacking interaction between the daunomycin AS group and the base atoms on the floor of the minor groove is very small. The second derivative is negligibly small over the region from ~ −0.5 Å to large separation, and it becomes significant only when the AS group approaches the floor of the minor groove. Therefore the stacking interaction should play a less significant role in the dynamical stability of the AS group. Experimental measurement (Wang et al., 1987) has revealed that the AS group in a DNA-daunomycin crystal is connected to the bases through a number of water bridged H-bonds. These H-bonds in turn stabilize the stacking between the AS group and the bases. This stacking is disrupted along with the disruption of these water bridged H-bonds.

CALCULATION DETAILS

The complex considered here is an infinitely long repeating DNA sequence poly·d(GCAT)·poly d(ATGC) with one daunomycin bound to each GC segment. Our system therefore has a drug-base pair ratio of 4 bp/drug. The coordinates of this system are generated directly from the x-ray crystal structure of a daunomycin-d(CGTACG) complex (Wang et al., 1987). This system is described, at the atomic level of detail, by an MSPA harmonic Hamiltonian. The force constants of this Hamiltonian are determined as follows: The valence force constants are assumed to be independent of temperature. The valence force constants for the DNA bases are from a DNA spectra study (Tsuboi et al., 1973) and those of the DNA backbones are from our own refinement analysis (Lu et al., 1977). The valence force constants for daunomycin are from the AMBER force fields (Weiner et al., 1986; Cieplak et al., 1990). The interbase and drug-base effective H-bond force constants are determined selfconsistently by an integration over the second derivative of a Morse potential (Prohofsky, 1985). These H-bond force constants are further scaled by $1 - P_{li}$ to take into consideration the effects of disrupted bonds in a mean field theory (Chen and Prohofsky,

1993a). Here P_{li} is the bond disruption probability of the ith bond in the lth base pair or drug-base connection. The Morse parameters for both AT and GC pairs are those used in our cooperative MSPA calculations (Chen and Prohofsky, 1993a). The Morse parameters for the drug-base H-bonds are derived by using the x-ray determined bond length as well as the force constant and dissociation energy derived from the Lippincott-Schroeder model (Schroeder and Lippincott, 1957). The maximum stretch lengths of the drug-base H-bonds are assumed to be the same as that of the similar type of bonds in DNA. These H-bond parameters are given in Table 1.

In addition to the interbase H-bonds and drug-base H bonds, the crystal measurements revealed the existence of a number of water bridged H-bonds between the drug and the bases (Wang et al., 1987). Our own analysis indicates that the bonding between these water molecules and the drug-DNA complex is very weak. Unlike the water molecules in the well organized minor groove spine of hydration in some DNA sequences, most of these water molecules can not be considered as frozen to the complex. Our earlier study (Chen and Prohofsky, 1993b) showed that water molecules that dissociate from the helix play an insignificant role in stabilizing the helix dynamically, although these molecules play an important role in stabilizing the helix statically. The static role of these water molecules is implicitly incorporated into our theory as our theory deals with the vibrational deviation from a given conformation which is stabilized by balancing the static forces. Since the dynamic contribution from these water bridged H-bonds is small we only consider the two most prominent bridges in our dynamic calculation. One water molecule (W1) bridges the O13 atom of the drug AC group to the O2 atom of the C8 base. Another water molecule (W2) bridges the N3′ atom of the drug AS group to the N3 atom of the A5 base. The force constants of these water bridged H-bonds are calculated by using the same formalism as that used in our study of the spine of hydration (Chen and Prohofsky, 1993b). The parameters for these H-bonds are the same as those for the spine of hydration and they are given in Table 1.

Apart from the valance force constants and H-bond force constants, we also incorporate base stacking and long range Coulomb force-induced interactions into our dynamic force field. These force constants are necessary to reproduce the observed acoustic modes (Mei et al., 1981) and are formulated in our earlier works (Mei et al., 1981; Prabhu et al., 1990). The cooperative effect of nearest-neighbors can be introduced into our base stacking force constants by scaling these force constants by a factor of $1 - \sqrt{P_l^{op} P_{l'}^{op}}$ (Chen and Prohofsky, 1993a). Here P_l^{op} and $P_{l'}^{op}$ are base pair opening probabilities of the two neighboring pairs. For base pairs adjacent to the drug AC group one of the P_l^{op} terms is replaced by the drug dissociation probability. In calculating the Coulomb force constants the effective charges of the atoms in daunomycin are from Newlin et al. (1984). The x-ray crystal measurement showed that for every drug there is a sodium ion coordinating with the base as well as daunomycin molecule (Wang et al., 1987). The interaction between this hydrated sodium ion and the drug-DNA complex is largely Coulomb in origin. The dynamic contribution from this interaction is incorporated into our force constant matrix. These force constants are calculated by using the same formula as that for other Coulomb force constants except a bulk water dielectric constant is used in this case.

In dealing with an ideal infinite repeating sequence helix we exploit helical symmetry to reduce the calculation to a number of calculations each of which has the dimensionality of a unit cell. For our daunomycin-bound poly d(GCAT)·poly d(ATGC) system a unit cell contains a four base pair GCAT sequence plus the drug. The number of heavy atoms in a unit cell of our system is 205, and the dimensionality of our calculation is therefore 615 × 615. The bases in a unit cell are labeled such that the bases on one strand are G1, C2, A3, and T4, and the complementary bases on the opposite strand are C8, G7, T6, and A5, respectively. The daunomycin molecule in the unit cell is denoted as D.

TABLE 1 Morse parameters (a, r^0, and V^0) and maximum stretch length L_{max} of the drug-DNA H bonds in daunomycin-bound poly d(GCAT)·poly d(ATGC)

Bond	a	r^0	V^0	L_{max}
	(Å^{-1})	(Å)	(kcal/mol)	(Å)
O9—H—N3	3.024	2.644	4.332	3.069
O9—H—N2	2.458	2.685	2.022	3.119
W1—O13	2.005	2.799	2.362	2.999
W1—O2	2.005	2.799	2.362	2.999
W2—N3′	2.316	2.731	2.405	3.004
W2—N3	2.316	2.731	2.405	3.004

PREMELTING BASE PAIR OPENING PROBABILITY

Using the selfconsistently determined eigenfrequencies we calculate individual H-bond disruption probability and base pair opening probability. The detailed formulation can be found in our earlier works (Chen et al., 1991; Chen and Prohofsky, 1993a). We have defined a premelting base pair open state as a state in which all the interbase H-bonds as well as the stacking interactions are disrupted (Chen et al., 1991; Chen and Prohofsky, 1993a). In all of our calculations of the drug-free helix the stacking interactions are disrupted along with the disruption of the interbase H-bonds as described earlier in the paper. The base pair opening probability P_l^{op} for a drug-free base pair is then

$$P_l^{op} = \prod_i P_{li} \tag{1}$$

where l is the index of base pairs in a unit cell, i is the index of the interbase H bonds in the lth base pair or drug-base pair and P_{li} is the individual H-bond disruption probability.

In the daunomycin-bound structure there are two distinct hydrogen bonds between the O9 atom of the drug AC group and the N2 and N3 atoms of the G7 base. These two additional H-bonds can restrict the motion of the G7 base and

block the separation of the C2–G7 base pair. Therefore the open state of the C2–G7 base pair in the presence of the drug should be a state in which not only the interbase H-bonds but also the drug-base H-bonds are simultaneously disrupted. The associated opening probability then becomes

$$P^{op}_{C2\text{–}G7} = P_{O9\text{–}H\text{–}N2} \times P_{O9\text{–}H\text{–}N3} \times \prod_i P_{C2\text{–}G7,i}. \quad (2)$$

In addition there are also two distinct water bridged H-bond connections between the drug and the bases. One connection is between the O13 atom of the drug AC group and the O2 atom of the C8 base. The O13 atom is connected to the O2 atom through two H-bonds and this connection is disrupted if any one of the H-bonds is disrupted. Therefore the opening probability of the G1–C8 base pair in the presence of the drug is

$$P^{op}_{G1\text{–}C8} = (P_{W1\text{–}O13} + P_{W1\text{–}O2} - P_{W1\text{–}O13} \times P_{W1\text{–}O2}) \times \prod_i P_{G1\text{–}C8,i}. \quad (3)$$

The second connection is between the N3′ atom of the drug AS group and the N3 atom the A5 base. The opening probability of the T4–A5 base pair in the presence of the drug is then

$$P^{op}_{T4\text{–}A5} = (P_{W2\text{–}N3'} + P_{W2\text{–}N3} - P_{W2\text{–}N3'} \times P_{W2\text{–}N3}) \times \prod_i P_{T4\text{–}A5,i}. \quad (4)$$

Table 2 gives the calculated disruption probability and equilibrium bond length of the individual interbase H-bonds and drug-base H-bonds of our daunomycin-bound poly d(GCAT)·poly d(ATGC) system. We find that the disruption probabilities for the water bridged H-bonds are all very large. They are of the order of 0.5. This is in contrast with the water bridged H-bonds in the minor groove spine of hydration in poly(dA)·poly(dT). We have shown (Chen and Prohofsky, 1993b) that the hydration spine is stabilized by the synergistic effects of the strong stacking which arises from the narrow minor groove of poly(dA)·poly(dT). This spine would be disrupted in a wider minor groove where the stacking is weaker in agreement with observations and simulation studies (Chuprina, 1987; Chuprina et al., 1991; Quintana et al., 1992). Since the minor groove is not narrow in our daunomycin-bound poly d(GCAT)·poly d(ATGC) system, the stacking between the water molecules and the bases and the drug is much weaker than that in the narrow minor groove of poly(dA)·poly(dT). Therefore the water bridged H-bonds here are much softer resulting in large disruption probabilities.

For comparison we also include a calculation on the drug-free standard B-form poly d(GCAT)·poly d(ATGC). The coordinates of the drug-free polymer are generated from the fiber structures (Chandrasekaran and Arnott, 1989). The calculated P^{op} terms for both daunomycin-bound and drug-free poly d(GCAT)·poly d(ATGC) are given in Table 3. We find from Table 2 and Table 3 that both individual interbase H bond disruption probabilities and the base pair opening probabilities are changed. These changes however are small except for the P^{op} of the C2–G7 base pair. The P^{op} of the C2–G7 base pair decreases three orders of magnitude from the value of 4.64×10^{-6} in the drug free case to 2.84×10^{-9} in the drug-bound case. This significant change arises because of the strong hydrogen bonding between the O9 atom of daunomycin and the N3 atom of the G7 base. A similar change in P^{op} occurs when water molecules in a hydration spine form H-bonds across base pairs in poly(dA)·poly(dT) (Chen and Prohofsky, 1992).

A three orders of magnitude decrease in the P^{op} of a base pair adjacent the drug can have a significant impact on the base unwinding processes in DNA transcription and replication. Assuming that base separation in these processes is facilitated by enhanced thermal fluctuational base pair disruption, such a significant decrease in P^{op} of a drug-bond base pair may be sufficient to slow down or block the unwinding process at the base pair. Our earlier study (Chen et al., 1992) indicated that the life time for a base pair at the junction of a replicating fork is in the range of 0.01–0.1 ms at physiological temperatures. Since base pair life time is proportional to the inverse of P^{op}, a three order magnitude decrease in P^{op} is equivalent to a three order magnitude increase in the base pair life time. This would give rise to a base

TABLE 2 Disruption probability P_i and bond length R_i of the interbase H-bond and drug-base H-bond in daunomycin-bound and drug-free poly d(GCAT)·poly d(ATGC) at 293 K

		P_i		R_i(Å)	
System	Bond	Drug-bound	Drug-free	Drug-bound	Drug-free
G1–C8	O6—H—N4	0.0047	0.0050	2.8065	2.8074
	N1—H—N3	0.0357	0.0372	2.8706	2.8714
	N2—H—O2	0.0235	0.0256	2.8180	2.8201
C2–G7	N4—H—O6	0.0050	0.0049	2.8075	2.8073
	N3—H—N1	0.0385	0.0371	2.8721	2.8713
	O2—H—N2	0.0210	0.0253	2.8157	2.8198
A3-T6	N6—H—O4	0.0429	0.0397	2.8895	2.8865
	N1—H—N3	0.0843	0.0823	2.9064	2.9054
T4–A5	O4—H—N6	0.0364	0.0406	2.8833	2.8875
	N3—H—N1	0.0669	0.0831	2.8973	2.9058
D–G7	O9—H—N3	0.0020		2.7415	
	O9—H—N2	0.3507		3.0199	
W1–D	W1—O13	0.5108		3.0036	
W1–C8	W1—O2	0.5095		3.0029	
W2–D	W2—N3	0.5430		3.0210	
W2–A5	W2—N3	0.5050		3.0051	

TABLE 3 Opening probability P^{op} of the base pairs in daunomycin-bound and drug-free poly d(GCAT)·poly d(ATGC) at 293 K

	P^{op}	
Base pair	Drug-bound	Drug-free
G1–C8	3.00×10^{-6}	4.74×10^{-6}
C2–G7	2.84×10^{-9}	4.64×10^{-6}
A3–T6	3.62×10^{-3}	3.26×10^{-3}
T4–A5	1.88×10^{-3}	3.38×10^{-3}

pair life time of 10–100 ms for the drug-bound base pair at the junction of a replicating fork. This is much larger than the 1 ms time scale associated with the helicase unwinding process. It is therefore likely that the probability of a helicase encountering a disrupted base pair will be diminished at the binding site.

Our calculated P^{op} values for the AT pairs in both daunomycin-bound and drug-free poly d(GCAT)·poly d(ATGC) are in the order of 10^{-3}. This differs from the value of $\sim 10^{-5}$ given by the nearest neighbor helix-coil transition theory (Wilcoxon et al., 1984) and by experimental estimates for some AT sequences but agrees with other experimental estimates. We have shown (Chen and Prohofsky, 1992) that the two order magnitude discrepancy between observed AT pair opening probability arises because of differences in the hydration status in different sequences. The hydration spine significantly enhances the thermal stability of the base pairs to which it is attached. Simulations as well as experiments (Chuprina, 1987; Chuprina et al., 1991; Quintana et al., 1992) have revealed the existence of a well defined spine of hydration in some AT sequences with a narrow minor groove. This spine of hydration is missing in TA steps which has a wider minor groove. The AT pairs with lower opening probability are those with the spine attached to their minor groove and the AT pairs with higher opening probability are those without the spine. Since the AT sequences in poly d(GCAT)·poly d(ATGC) are formed by TA steps, it is highly unlikely a well defined spine of hydration could form in the minor groove of these AT pairs. Therefore the opening probability of these AT pairs is expected to be in the order of 10^{-3} rather than 10^{-5}.

DAUNOMYCIN DISSOCIATION PROBABILITY

Many thermodynamic measurements of daunomycin-DNA interactions have determined the equilibrium daunomycin binding constants in different DNA polymers and in various solvent conditions (Remeta et al., 1993). The equilibrium drug binding constant can be found from the drug-base dissociation probability. The dissociation of daunomycin requires drug-base unstacking as well as the disruption of all the drug-base H-bonds. The drug dissociation probability is then

$$P_D = P_{O9\text{–}H\text{–}N2} \times P_{O2\text{–}H\text{–}N3} \times P_{\text{water-bridge}} \times P_{\text{unstacking}} \quad (5)$$

where

$$P_{\text{water-bridge}} = (P_{W1\text{–}O13} + P_{W1\text{–}O2} - P_{W1\text{–}O13} \times P_{W1\text{–}O2}) \times (P_{W2\text{–}N3'} + P_{W2\text{–}N3} - P_{W2\text{–}N3'} \times P_{W2\text{–}N3})$$

is the probability of the disruption of all the water bridged drug-base H-bonds. $P_{\text{unstacking}}$ is the drug-base unstacking probability which can be further divided as: $P_{\text{unstacking}} = P^{ac}_{\text{unstacking}} \times P^{as}_{\text{unstacking}}$, where $P^{ac}_{\text{unstacking}}$ and $P^{as}_{\text{unstacking}}$ is the probability for the drug AC group and AS group, respectively. Since the stacking between the drug AS group and the bases is weak $P^{as}_{\text{unstacking}} \approx 1$ at levels of excitations where the other opening probabilities are appreciably dominant.

The $P^{ac}_{\text{unstacking}}$ can be estimated by assuming that, after the disruption of the drug-base H-bonds, the drug can oscillate along the orientation of the ring system of the AC group in the potential well described in Fig. 3. Such an oscillating motion can then be described by an effective one-dimensional MSPA harmonic Hamiltonian

$$H_0 = \tfrac{1}{2}M\dot{u}^2 + \tfrac{1}{2}\phi u^2 \quad (6)$$

where M is the total mass and u is the displacement around an equilibrium position R. $R = R_0 + dR$, where R_0 is the x-ray observed position and dR is the mean displacement of the drug after the disruption of the drug-base H-bonds, and it is determined selfconsistently as shown below. In accordance with Fig. 3 we set R_0 as zero. ϕ is the effective force constant determined by minimizing the variational free energy $F = F_0 + \langle H - H_0 \rangle$. F_0 is the free energy of the effective harmonic system Eq. (6): $F_0 = -k_B T/\ln[\exp(-H_0/k_B T)]$. T is the temperature and k_B is Boltzmann's constant.

The stationary condition $\partial F/\partial D = 0$ ($D = \langle u^2 \rangle$) then gives

$$\phi = (1 - P^{ac}_{\text{unstacking}}) \frac{\int_{u_m}^{\infty} du e^{-u^2/2D} (d^2/du^2) V(dR + u)}{\int_{u_m}^{\infty} du e^{-u^2/2D}} \quad (7)$$

where V is the stacking potential described in Fig. 3 and u_m is the inner bound of the hard core of the potential chosen as -3 Å. $1 - P^{ac}_{\text{unstacking}}$ is used to scale the force constant to take into consideration the effect of dissociated drugs in our mean field theory. The mean square vibrational displacement D can be given from another stationary condition $\partial F/\partial \phi = 0$, which gives

$$D = \frac{\hbar}{2M\omega} \coth\left[\frac{\hbar\omega}{2k_B T}\right] \quad (8)$$

where $\hbar$ is the Planck's constant and ω is the frequency obtained from

$$M\omega^2 = \phi. \quad (9)$$

The mean displacement dR of the drug is determined by the classical condition that at the classical turnaround point all the energy of oscillator is stored in potential energy

$$V(dR + \mu) = V(dR - \mu). \quad (10)$$

As in our earlier studies we define μ as the full width at half maximum of the distribution function $\exp(-u^2/2D)$ which gives $\mu = 2\sqrt{2D \ln 2}$. Finally the $P^{ac}_{\text{unstacking}}$ can be determined by the distribution function $\exp(-u^{-2}/2D)$ as

$$P^{ac}_{\text{unstacking}} = \int_{L_{max}}^{\infty} du \exp[-(u - dR)^2/2D] \quad (11)$$

where L_{max} is the maximum displacement before dissociation. It is chosen as the first inflection point of the potential. From Fig. 4 (the *dashed line*) we find L_{max} to be 1.95 Å.

Eqs. 6 to 11 can be self-consistently solved together with the equations for DNA and drug-DNA H-bonds to give a selfconsistent $P^{ac}_{\text{unstacking}}$. The calculated $P^{ac}_{\text{unstacking}}$, D, and dR are found to be 4.6×10^{-5}, 0.1 Å^2, and 0.7 Å, respectively.

Using the calculated $P^{ac}_{unstacking}$ and data in Tables 2 and 3 we find P_D to be 1.88×10^{-8}.

The binding constant B in terms of dissociation probability is

$$B = \frac{1 - P_D}{P_D} \approx \frac{1}{P_D} \tag{12}$$

in the premelting region. The binding constant is predicted to be 5.31×10^7. We have not found a reported binding constant for the daunomycin-bound poly d(GCAT)·poly d(ATGC) system which we considered here because of availability of structural information. We can however compare to the available experimental estimates on some daunomycin-bound DNA polymers in which the drugs intercalate between alternating GC pairs. A recent experiment (Remeta et al., 1993) gave a drug binding constant of 2.27×10^7 for poly d(GC)·poly d(GC) (3.7 bp/drug). Our calculated value is in fair agreement with the observed value for poly d(GC)·poly d(GC) and it is in better agreement with the observed value of 4.84×10^7 for a salmon testes DNA (3.7 bp/drug) (Remeta et al., 1993).

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