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Melting profile and temperature dependent binding constant of an anticancer drug daunomycin-DNA complex

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Abstract. We calculate thermal fluctuational base pair opening probability and the drug binding constant of a daunomycin-bound Poly d(CGTA) · Poly d(TACG) at temperatures from room temperature to its melting temperature. For comparison we also carry out a calculation on a drug-free DNA with the same sequence. Our calculations are carried out by means of a statistical approach using microscopic structures and established force fields and with cooperative effects incorporated into the algorithm. Both hydrogen bond disruption probabilities and drug unstacking probability are determined self-consistently. These probabilities are then used to determine temperature dependent base pair opening probabilities and the drug binding constant. The calculated base pair opening probabilities and drug binding constant are found to be in fair agreement with experiments carried out at room temperature. Our calculation shows cooperative base pair disruption and drug dissociation at certain critical temperatures close to the observed melting temperatures for similar helices. We find that the temperature dependence of the drug binding constant fits well to the van't Hoff relation, in agreement with observations. Our calculation indicates the occurrence of a premelting transition in the drug-bound DNA helix. Some comments are made about this premelting transition.

Key words: Base pair opening – Chemical bond disruption – DNA melting – Drug-DNA interaction – Drug dissociation

Introduction

Daunomycin is an anthracycline antibiotic drug widely used in the treatment of various cancer diseases. This drug and the closely related adriamycin drug are effective drugs currently available for cancer chemotherapy. Daunomycin is known to inhibit both DNA replication and RNA transcription through binding and intercalation between DNA base pairs (DiMarco et al. 1975). In recent years there have been extensive investigations on daunomycin-DNA systems using a variety of techniques, including crystallographic structural analyses (Quigley et al. 1980; Frederick et al. 1990; Nunn et al. 1991), footprinting studies (Chaires and Herrera 1990), and thermodynamic measurements (Phillips et al. 1978; Chaires et al. 1982; Remeta et al. 1993). Many theoretical studies have also been carried out (Crothers 1971; McGhee 1976; Chen et al. 1985; Cieplak et al. 1990; Chen and Prohofsky 1994a; Misra et al. 1994). A major focus of these studies is the investigation of the effect of the intercalating drug on the dynamic stability of the drug-bound base pairs.

In the process of replication and transcription the base pairs of the DNA double helix have to be separated. It is thus expected that binding of daunomycin inhibits the unbinding process through both direct blocking and enhancement of 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 observations of a significant increase in the melting temperature of a number of daunomycin-bound DNAs (Phillips et al. 1978; Chairs et al. 1982; Remeta et al. 1993). In an earlier study (Chen and Prohofsky 1994a) we found that, at a premelting temperature, the opening probability of a base pair in a daunomycin-bound DNA is indeed substantially decreased with respect to that of the drug-free DNA. Using the calculated disruption probability of the drug-base H-bonds and the unstacking probability of the drug we predicted a daunomycin binding constant in good agreement with observed values for similar daunomycin-DNA complexes. In the present work we extend our earlier study to calculate the temperature behavior of the drug-binding constant and base pair opening probabilities of both daunomycin-bound and drug-free Poly d(CGTA) · Poly d(TACG) complex. We also determine the melting temperatures of these complexes and compare with observations for similar complexes. This particular DNA sequence is chosen because of the availability of conformational information on the daunomycin-DNA complexes with this sequence.

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To calculate temperature dependent base pair opening probabilities and drug unstacking probability of both drug-bound and drug-free DNA polymer, we employ a microscopic statistical approach – the cooperative modified self-consistent phonon approximation (MSPA) theory we developed earlier (Chen and Prohofsky 1994b). The cooperative MSPA theory is a theory that models individual bonds or atom-atom interactions by effective harmonic potentials self-consistently determined from realistic potentials and based on X-ray determined microscopic structures (Prohofsky 1985). The thermal fluctuational disruption probability of H-bonds can be determined by statistical means within the framework of the theory (Chen et al. 1991). The unstacking probability between stacked groups can be estimated by a similar self-consistent approach in which the motion of the groups, after the disruption of the connecting H-bonds, is considered in the composite stacking potential between these groups (Chen and Prohofsky 1994a). Experimental measurements and Ising model helix-coil transition theory have shown the existence of cooperative effects in long DNA helices. This effect is incorporated into our theory by including disruption probabilities as order parameters for melting (Chen and Prohofsky 1994c). Our calculations indicated the existence of a critical temperature at which opening probabilities show dramatic increases and it is identified as the helix melting temperature. Our studies on a variety of DNA sequences showed that our calculated melting temperatures (Chen and Prohofsky 1994b) as well as premelting base pair opening probabilities (Chen et al. 1991; Chen and Prohofsky 1992) are in agreement with observations. Moreover good agreement is found between the calculated and observed binding constant of daunomycin bound to DNA at room temperature (Chen and Prohofsky 1994a). We will show in the present work that our calculation predicts the melting temperature and the temperature dependence of drug binding constant of a daunomycin-bound DNA polymer in fair agreement with observations. We also examine the premelting behavior of the drug-bound base pairs and predict the occurrence of a premelting transition.

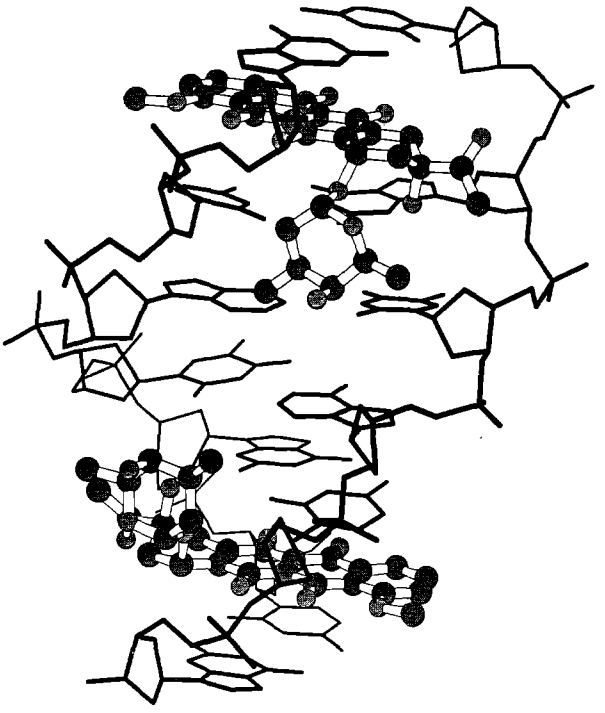

Fig. 1. X-ray crystal structure of a daunomycin-d(CGTACG) complex. Two daunomycins (*ball and stick*) intercalate into the two CG sections of the DNA oligomer (*line drawing*). This graphic was generated by MOLSCRIPT (Kraulis, J Appl Crystallogr **24**, 946 (1991))

Theory

Structure and force fields

The coordinates of the B-DNA Poly d(CGTA)·Poly d(TACG) are generated from standard fiber B-structures (Chandrasekaran and Arnott 1989). The coordinates of the daunomycin-bound Poly d(CGTA)·Poly d(TACG) are generated directly from the x-ray crystal structure of a daunomycin-d(CGTACG) complex (Wang et al. 1987). The structure of this crystal complex is shown in Fig. 1. In this crystal structure there are two drugs intercalating into the two 5′-CG-3′ steps. These two sites for the drug were found to be equivalent because of the symmetry of the oligomer. Our daunomycin-DNA complex is extracted from the section of CGTA of the crystal structure. Since there is one daunomycin bound to every GC segment the drug-base pair ratio of our system is 4 bp/drug. The two polymers generated are linear helices containing an infinite number of base pairs with a repeating sequence of CGTA plus the drug. In dealing with these infinite repeating sequences we exploit helical symmetry to reduce the calculation to a number of calculations each of which has the dimensionality of a unit cell that contains the residues of a single repeating sequence. The number of heavy atoms in a unit cell of the drug-free and drug-bound systems is 164 and 205 and the dimensionality of the equation of motion is 492×492 and 615×615 respectively. The bases in a unit cell are labeled such that the bases on one strand are C1, G2, T3 and A4 and the complementary bases on the opposite strand are G8, C7, A6 and T5 respectively. The daunomycin molecule in the unit cell is denoted as D.

The force fields of the two polymers 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 spectral study (Tsuboi et al. 1973) and those of the DNA back-bones are from a separate 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 can be found in another paper (Chen and Prohofsky 1994a).

The crystal x-ray measurements revealed the existence of a number of water bridged H-bonds between daunomycin and the bases (Wang et al. 1987). Our earlier study (Chen and Prohofsky 1994a) showed that these water bridged connections are essentially disrupted as their disruption probabilities are in the order of more than $\frac{1}{2}$. The contribution of these water-bridged bonds to the dynamic stability of the base pairs and the drug is therefore negligibly small as compared to the contribution from that of the direct H-bonding and drug stacking. Therefore as a good approximation we can neglect these water-bridged H-bonds in the determination of base pair opening probabilities and drug dissociation probability.

In addition to the valence 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 earlier studies (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 1994b). Here, P_l^{op} and $P_{l'}^{op}$ are base pair opening probabilities of the two neighboring pairs. For the two GC pairs that are sandwiched between the drug aglycon chromophore (AC) residue and a base pair one of the P_l^{op}s is replaced by the drug dissociation probability (Chen and Prohofsky 1994a). In calculating the Coulomb force constants the effective charges of the atoms in DNA and in daunomycin are from Miller (1979) and from Newlin et al. (1984) respectively. The x-ray crystal measurement showed that for every drug there is a sodium ion coordinating with the drug and DNA 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 that a bulk water dielectric constant is used in this case.

The base pairs and the daunomycin drug in a daunomycin-DNA complex are stabilized by a number of H-bonds and stacking interactions. The disruption of a base pair and the dissociation of the drug can therefore be determined by analyzing the disruption probabilities of these H-bonds and the unstracking probabilities. The calculated drug dissociation probability can then be used to determine the drug binding constant which can be compared with experiments. We showed in our earlier work (Chen and Prohofsky 1994a) that the stacking interaction between the bases behaves differently from the stacking interactions between the daunomycin drug and the bases. Therefore different approaches can be used in the determination the probabilities. We have demonstrated that the stacking interaction between the bases in a base pair in a double helix is disrupted along 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 stacking interactions between the intercalating daunomycin and the bases contribute substantially to the dynamic stability of the drug. Therefore the stacking interaction has to be included in the determination of the drug dissociation probability.

We have demonstrated in our earlier work (Chen and Prohofsky 1994a) that the unstacking probability of daunomycin can be estimated from a single self-consistent calculation of the oscillating motion of the drug in the composite van der Waals potential well after the disruption of drug-base H-bonds. In the present study the same method is employed to determine the temperature dependent drug dissociation probability and the binding constant. As with the earlier study the AMBER van der Waals parameters (Weiner et al. 1986; Cieplak et al. 1990) are used to determine the composite potential between the drug and DNA.

Base pair opening probability

Hydrogen bond disruption probabilities and base pair opening probabilities at a given temperature are computed statistically from the self-consistently determined normal mode eigenfrequencies and eigenvectors of a cooperative MSPA microscopic effective harmonic equation (Chen and Prohofsky 1993a, 1994b). These probabilities can be further used to determine drug dissociation probability discussed in the next subsection. The basic procedure for this self-consistent computation is summarized below:

Step 1: Given the coordinates and initial force constants one can solve the equation of motion of a repeating sequence helix in the phase (mementum) space:

$$\left(\sum_k B_k^{+}(\theta)\Phi_k(\theta)B_k(\theta)-\omega_\lambda(\theta)^2 I\right)q^\lambda(\theta)=0 \tag{1}$$

where k is the index for the type of harmonic motions (valence bond stretch, angle bending, torsion, out of plane bending, H-ond breathing and motions induced by non-bonded van der Waals and Coulomb interactions). λ is the index for the vibrational normal modes. θ is the phase shift between the motions in neighboring unit cells. $B_k(\theta)=\sum_l B_{0lk}R^l e^{-il\theta}$ is the transformation matrix (B matrix) from all the unit cells in mass weighted Cartesian coordinates to the 0th unit cell in internal coordinates. R is the matrix of the rotational part of the screw operation and the expression for the matrix B_{0lk} can be found in the literature (Califano 1976). $\Phi_k(\theta)=\sum_l \Phi_{lk}e^{-il\theta}$ is the matrix of force fields. $\omega_\lambda(\theta)$ and $q^\lambda(\theta)$ are the eigenfrequency and eigenvector in mass weighted Cartesian coordinates respectively.

Step 2: Using the eigenfrequencies and eigenvectors one can determine the mean square vibrational amplitude D_{li} and mean length R_{li} of a bond by minimization of the free energy of the system:

$$D_{li}=\sum_\lambda \int_0^\pi d\theta\,\frac{|s_{li\lambda}(\theta)|^2}{2\pi\omega_\lambda(\theta)}\coth\left(\frac{\hbar\omega_\lambda(\theta)}{2K_BT}\right) \tag{1a}$$

$$R_{li} = (1 - P_l^{uc})\left[r_{li}^0 + \frac{1}{a_{li}} \ln(\cosh(\mu_{li} a_{li})) \right] + P_l^{uc}\left(L_{li}^{\max} + 2P_{li}\sqrt{2D_{li}} \right) \quad (2)$$

where k_B is the Boltzmann's constant, $\hbar$ is planck's constant divided by 2π, l is index for base pair or drug-base connection in a unit cell and i is the index of the bonds in the system. $L_{li}^{\max}$ is the maximum bond stretch length before disruption, $s_{li\lambda}(\theta)$ is the internal stretch coordinates for the bond, $\mu_{li} = 2\sqrt{2D_{li}\ln 2}$ and r_{li}^0 and a_{li} are the parameters of the Morse potential that models H-bond interaction between the two bond-end atoms (Chen and Prohofsky 1993a). P_l^{uc} is the base pair unconstrained probability (Chen and Prohofsky 1994b) used to introduce cooperativity and it will be given below.

Step 3: The calculated D_{li} and R_{li} can then be used to determine the statistical bond disruption probability P_{li}:

$$P_{li} = C_{li} \int_{L_{li}^{\max}}^{\infty} dr \exp\{-(r - R_{li})^2 / 2D_{li}\} \quad (3)$$

where $C_{li}^{-1} = \int_{L_{li}^{\max}}^{\infty} dr \exp\{r - R_{li}^2 / 2D_{li}\}$ and $r_{li}^{\min}$ is the bond hard-core inner boundary. Base pair opening probabilities, P_l^{op}s can then be given by the P_{li}s. For a drug-free base pair (Chen and Prohofsky 1994b)

$$P_l^{op} = \prod_i P_{li} \quad (4)$$

In daunomycin bound Poly d(CGTA)·Poly d(TACG) the P_l^{op} of the G2-C7 pair should be replaced by (Chen and Prohofsky 1994a):

$$P_{G2\text{-}C7}^{op} = P_{N2(G2)-H-O9(D)} \times P_{N3(G2)-H-O9(D)} \times \prod_i P_{G2\text{-}C7,i} \quad (5)$$

The calculated P_l^{op}s and the drug dissociation probability, to be given later, can then be used to calculate P_l^{uc}.

$$P_l^{uc} = (P_{l-1}^{op} P_l^{op} P_{l+1}^{op})^{1/3} \quad (6)$$

For a base pair adjacent the intercalating drug P_{l-1}^{op} or P_{l+1}^{op} is replaced by the drug dissociation probability.

Step 4: To complete the iteration loop new force constant for the bonds are calculated from D_{li}, R_{li} and probabilities:

$$\phi_{li}^{\text{int}} = C_{li} \int_{r_{li}^{\min}}^{\infty} dr \exp\{-(r - R_{li})^2 / 2D_{li}\} \frac{d^2 V(r)}{dr^2} \quad (7)$$

where $r_{li}^{\min}$ is given in the footnote of Eq. (3) and $V(r)$ is the Morse potential for the bond end-atoms. These force constants and the nonbonded force constants are then scaled by the calculated probabilities to introduce cooperativity as described in the last section. The scaled force constants are then used to repeat the calculation from step 1 through step 4. This iteration process continues until a self-consistent solution is found for the force constants. In our calculations a self-consistency is assumed if the difference between the force constant of the current iteration and previous iteration divided by the force constant is less than 0.003 at premelting temperatures and less than 0.006 near the melting temperature.

Daunomycin dissociation probability

We have shown in our earlier study that the dissociation of daunomycin from DNA involves two elements: disruption of the drug-base H-bonds and water bridged drug-base H-bonds followed by the translational dissociation or unstacking of the bulk drug from the bases. In the present work the water bridged drug-base H-bonds are neglected because they contribute little to the dynamic stability (Chen and Prohofsky 1994a). The dissociation probability P_D can then be given by:

$$P_D = P_{N2(G2)-H-O9(D)} \times P_{N3(G)-H-O9(D)} \times P_{st} \quad (8)$$

where P_{st} is the drug-base unstacking probability which can be further divided as $P_{st} = P_{st}^{ac} \times P_{st}^{as}$ and P_{st}^{ac} and P_{st}^{as} is the probability for the drug aglycon chromophore (AC) group and amino sugar (AS) group respectively. Since the stacking between the drug AS group and the bases is weak $P_{st}^{as} \approx 1$ at levels of excitations where the other opening probabilities are appreciable.

The P_{st}^{ac} can be calculated self-consistently 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 composite potential between the drug and DNA (Chen and Prohofsky 1994a). The computation procedure is summarized below:

Step 1: The oscillating motion of the drug, after disruption of drug-base H-bonds, are described by an effective one dimensional harmonic Hamiltonian:

$$H_0 = \frac{1}{2} M \dot{u}^2 + \frac{1}{2} \phi u^2 \quad (9)$$

where M is the total mass and u is the displacement around an equilibrium position R. $R = R_0 + \Delta R$ where R_0 is the x-ray observed position set to be zero, ΔR is the mean displacement and ϕ is the effective force constant of the drug. By solving the equation of motion one obtains the eigenfrequency $\omega = \sqrt{\phi / M}$ and eigenvectors s for the effective harmonic motion.

Step 2: The mean square vibrational displacement D can be given from ω and s by minimization of the free energy of the oscillating drug:

$$D = \frac{s^* s}{2M\omega} \coth\left[\frac{\hbar\omega}{2k_B T} \right] \quad (10)$$

The mean displacement ΔR of the drug is given by:

$$\Delta R = (1 - P_{uc})\Delta R_b + P_{uc}\Delta R_d \quad (11)$$

where ΔR_b is the mean displacement for the bounded drug determined by $V(\Delta R_b + \mu) = V(\Delta R_b - \mu)$ with $V(r)$ the composite potential and $\mu = 2\sqrt{2D\ln 2}$. $\Delta R_d = L_{\max} + 2P_{st}\sqrt{2D}$ is the mean displacement for the dissociated drug and $L_{\max}$

is the maximum displacement before drug dissociation (Chen and Prohofsky 1994a). P_{uc} is the drug unconstrained probability used to introduce cooperativity and it is given below.

Step 3: P_{st}^{ac} can be determined from the calculated D and ΔR as:

$$P_{st}^{ac} = \int_{L_{\max}}^{\infty} du \exp[-(u-\Delta R)^2/2D] \tag{12}$$

and P_{uc} is calculated from P_{st}^{ac} and the P^{op}s of adjacent base pairs as:

$$P_{uc} = (P_{l-1}^{op} P_{st}^{ac} P_{l+1}^{op})^{1/3} \tag{13}$$

Step 4: To complete the self-consistent iteration loop a new force constant, with cooperative effect included, is determined from:

$$\phi = (1-P_{st}^{ac}) \frac{\int_{u_m}^{\infty} du e^{-u^2/2D} \frac{d^2}{du^2} V(\Delta R+u)}{\int_{u_m}^{\infty} du e^{-u^2/2D}} \tag{14}$$

where u_m is the inner bound of the hard core of the potential chosen as –3 Å. This new force constant is then used to repeat the calculation from step 1 through step 4 until self-consistency is reached.

Given the drug dissociation probability P_D, the equilibrium binding constant K_{eq} of the drug can be determined by the condition that the sum of dissociation and association probability equals one. We therefore obtain:

$$K_{eq} = \frac{1-P_D}{P_D} \tag{15}$$

Results and discussion

Effect of daunomycin binding on the melting of DNA

In Fig. 2 we plot our calculated temperature dependent base pair opening probabilities of the base pairs of both daunomycin-bound and drug-free DNA Poly (CGTA) · Poly d(TACG). The two dashed lines in the figure correspond to the probabilities for the drug-free DNA polymer. The upper dashed line is for the AT pairs and the lower one is for the GC pairs. The four solid lines are the probabilities for the daunomycin-bound DNA polymer. The uppermost solid line is for the T3-A6 pair, the second one is for the A4-T5 pair, the third is for the C1-G8 pair and the bottom line is for the G2-C7 pair. From the figure one finds that the probabilities for both drug-free and daunomycin-bound show cooperative disruption such that at a critical temperature all the probabilities in a helix rise sharply from a small value to 1. This critical temperature indicates the onset of massive disruption of base pairs and it is taken as the melting temperature T_m of that helix.

The T_m for the drug-free helix is found from Fig. 2 to be 355 K. We have not found an observed T_m for B-DNA

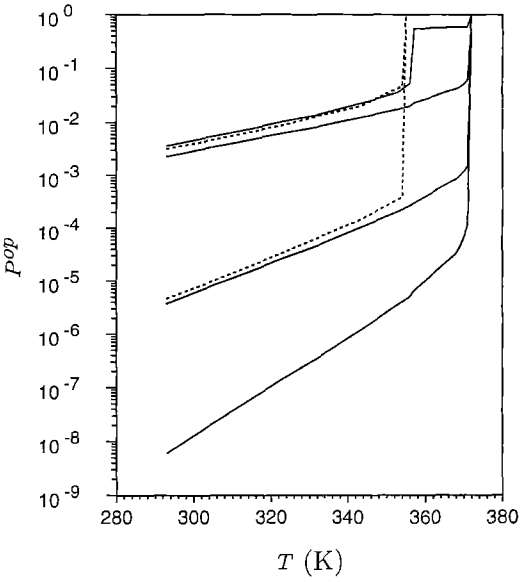

Fig. 2. Calculated opening probability P^{op} of the base pairs in drug-free and daunomycin-bound Poly d(CGTA) · Poly d(TACG) as a function of temperature. The upper (*lower*) dashed line corresponds to the P^{op} of the AT (GC) pairs of the drug-free helix. The solid lines correspond to the P^{op}s of the base pairs in the drug-bound helix. The upper most solid line is for the T3-A6 pair, the second line is for the A4-T5 pair, the third line is for the C1-G8 pair and the bottom line is for the G2-C7 pair

Poly d(CGTA) · Poly d(TACG). We selected this polymer because it is one of a few sequences whose daunomycin-bound structure is known from x-ray crystallography. Nonetheless we can compare our calculation with the observed T_m for a similar B-DNA alternating GC-AT polymer at the same salt concentration as in our calculation. The salt concentration in our calculation is 0.05 M NaCl (Chen and Prohofsky 1993b). The observed T_m for Poly d(TG) · Poly d(CA) at the same concentration is 356 K (Wells et al. 1970). This shows that our calculated T_m for the drug-free helix is in good agreement with available experimental measurements.

The T_m of a free DNA helix for varying salt concentration has been calculated using the cooperative MSPA method and the results are in agreement with observations (Chen and Prohofsky 1993b). The calculations of this work are carried out at 0.05 M Na^+ as the calculation is simplest for this value.

From Fig. 2 one finds that the T_m of the daunomycin-bound helix is increased significantly as compared to the drug-free helix, in agreement with observed behavior. Apparently the increase in the T_m results from significantly enhanced stability of the G2-C7 base pair. Table 1 shows the comparison between our calculated T_ms for daunomycin-bound as well as drug-free helix and the observed T_ms for several sequences that are similar to our helix and have a similar base pair to drug ratio (Chaires 1983; Remeta et al. 1993). From the Table one finds that the conditions under which the experiments were carried out are substantially different from those of our calculation. The effective Na^+ concentration in our calculation is 0.05 M, whereas the Na^+ concentrations used in the experiments

Table 1. Comparison between calculated and observed melting temperature for drug-free (T_m^{free}) and daunomycin-bound (T_m^{bound}) DNA polymers. C is the Na^+ concentration and ΔT_m is the difference between the melting temperatures of daunomycin-bound and drug free DNA polymer

Method	Sample	C (M)	T_m^{free} (K)	bp-drug ratio (bp/drug)	T_m^{bound} (K)	ΔT_m (K)	Reference
Theory	Poly d(CGTA)·Poly d(TACG)	0.05	355[a]	4.0	372	17	
UV	salmon tests DNA	0.015	340	3.7	363	23	Remeta et al.
UV	Poly d(AC)·Poly d(GT)	0.015	346	3.0	371	25	Remeta et al.
UV	Poly d(GC)·Poly d(GC)	0.015	371	3.7	384	13	Remeta et al.
UV	Poly d(GC)·Poly d(GC)	0.0045	348	4.0	363	15	Chaires (1983)

[a] When scaled to 0.015 M Na^+ is 346 K

range from 0.0045 M to 0.015 M. The lower salt concentrations used in these experiments resulted in substantially lower T_ms. One can, however, extrapolate our calculated melting temperature for the drug-free helix to lower salt concentration by using the following equation with parameters determined based on the analysis of a number of DNA sequences (Blake and Delcourt 1990):

$$T_m = 17 \log_{10}[Na^+] + 377 \quad (16)$$

By using this equation we find an extrapolated T_m of 346 K for our drug-free helix at 0.015 Na^+ concentration which is compared to 340 K for a salmon testes DNA and 346 K for Poly d(TG)·Poly d(CA) at the same salt concentration.

Although the melting temperature of a drug-free and a daunomycin-bound DNA polymer depends sensitively on salt concentration, the shift of T_m upon binding of daunomycin seems to be much less sensitive to salt concentration. From Table 1 one finds that the observed melting temperature shift ΔT_m of Poly d(GC)·Poly d(GC) at 0.015 M Na^+ concentration is roughly the same as that at 0.0045 M Na^+. Since the shift in melting temperature is insensitive to salt concentration we can compare our calculation directly with observations which were carried out at different salt concentrations. Our calculated ΔT_m for Poly d(CGTA)·Poly d(TACG) is 17 K as compared to the observed value of 23 K for a salmon testes DNA (Remeta et al. 1993) and the values of 13 K and 15 K for Poly d(GC)·Poly d(GC) (Chaires 1983; Remeta et al. 1993). Our calculated ΔT_m is 2 to 4 K larger than that of Poly d(GC)·Poly d(GC) and it is 6 K smaller than that of salmon testes DNA. This is the expected behavior. Although in our system the AC group of daunomycin is intercalated between the space of two alternating GC pairs, each two GC pairs are separated by two AT pairs instead of two GC pairs as in the case of Poly d(GC)·Poly d(GC). Experiments on a host of DNA sequences (Remeta et al. 1993) showed that the ΔT_m of a DNA decreases with increasing GC content. The largest ΔT_ms appear in sequences containing AT or AA sequences and sequences containing AC or AG sequences. One then expects the ΔT_m for Poly d(CGTA)·Poly d(TACG) to be larger than that of Poly d(GC)·Poly d(GC). On the other hand the salmon testes DNA is composed of random sequences. This DNA should contain many consecutive AC and AG sequences as well as consecutive AT sequences which could result in a higher ΔT_m for salmon testes DNA than that of Poly d(CGTA)·Poly d(TACG).

Temperature dependence of daunomycin binding constant

We have illustrated in our earlier work (Chen and Prohofsky 1994a) that the daunomycin equilibrium binding constant K_{eq} can be estimated from the drug unstacking probability, determined by a simple analysis of the oscillating motion of the drug in a composite stacking potential well, and the drug-base H-bond disruption probabilities, determined by the full MSPA self-consistent calculation. The calculated K_{eq} at room temperature from that work is in good agreement with the observations. In the present work we use the same formulation to determine the temperature dependent K_{eq} for daunomycin-bound Poly d(CGTA)·Poly d(TACG) at temperatures from room temperature to melting temperature. In this study we neglect water bridgd drug-base H-bonds because of high disruption probabilities in these bonds determined in our earlier study.

The calculated ln K_{eq} as a function of the inverse temperature 1/T is shown in Fig. 3. The numerical value of K_{eq} as well as the drug dissociation probability, unstack-

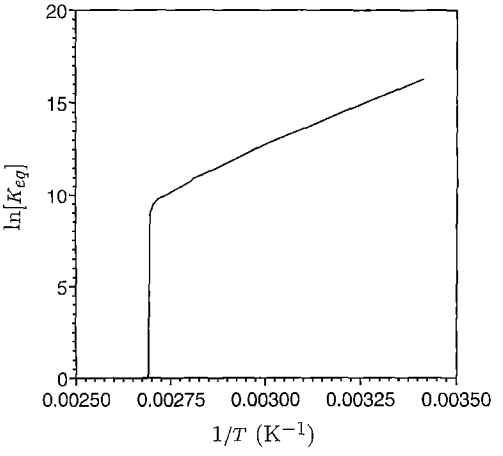

Fig. 3. The logarithm of the daunomycin equilibrium binding constant ln K_{eq} as a function of inverse temperature 1/T

Table 2. Daunomycin equilibrium binding constant K_{eq}, dissociation probability P_D, unstacking probability P_{st} and drug-base H-bond disruption probabilities $P_{N2(G2)-H-O9(D)}$ and $P_{N3(G2)-H-O9(D)}$ of daunomycin-bound Poly d(CGTA) · Poly d(TACG) at several premelting temperatures. K_{eq} is derived from $K_{eq} = (1 - P_D)/P_D$ and P_D is given by $P_D = P_{st} \times P_{N2(G2)-H-O9(D)} \times P_{N3(G2)-H-O9(D)}$

T (K)	K_{eq}	P_D	P_{st}	$P_{N2(G2)-H-O9(D)}$	$P_{N3(G2)-H-O9(D)}$
293	1.18×10^7	8.51×10^{-8}	5.86×10^{-5}	0.458	0.0032
313	1.84×10^6	5.43×10^{-7}	1.66×10^{-4}	0.536	0.0061
333	3.61×10^5	2.77×10^{-6}	4.07×10^{-4}	0.591	0.0115
353	6.83×10^5	1.46×10^{-5}	1.07×10^{-3}	0.633	0.0217
368	1.76×10^4	5.70×10^{-5}	2.03×10^{-3}	0.667	0.0422
371	7.56×10^3	1.32×10^{-4}	3.88×10^{-3}	0.672	0.0506
372	1.59×10^{-2}	0.984	1.0	0.994	0.9904

ing probability and drug-base H-bond disruption probability at different temperatures are also listed in Table 2. As in our earlier work the calculated K_{eq} at room temperature is in fair agreement with observations for similar helices at room temperature. There is a slight difference between the calculated K_{eq} from this work and that of our earlier work. This difference arises because of the introduction of the cooperative effect and the neglect of water bridged drug-base H-bonds in this work. It is interesting to note that the temperature dependence of ln K_{eq} follows approximately a linear curve with respect to 1/T, particularly at temperatures below 357 K. This temperature behavior is consistent with an experimental observation that the daunomycin equilibrium binding constant fits well to the standard van't Hoff relation at premelting temperatures (Chaires 1985). The standard van't Hoff relationship is (Remeta et al. 1993):

$$\ln K_{eq} = \ln K_h + \Delta H_b/R\,(1/T_m - 1/T) \qquad (17)$$

where K_h is the apparent drug binding constant at the melting temperature and R is the molar gas constant. ΔH_b is the daunomycin-DNA binding enthalpy and this is assumed to be independent of temperature. Our ln K_{eq} curve at temperatures below T_m indeed fits quite well with the van't Hoff relationship. This correlation is indicative of the proper treatment of the elements of thermal expansion and the cooperative effect in our MSPA formulation. On the other hand our numerical calculation offers a theoretical proof that the daunomycin binding constant should obey the van't Hoff relationship.

The values of K_h and ΔH_b for our daunomycin-Poly d(CGTA) · Poly d(TACG) can be estimated from the ln K_{eq} − 1/T curve in Fig. 3. We find a value of 7.56×10^3 for K_h and a value of −17.4 kcal/mol for ΔH_b. We have not found a published value for K_h. The experimentally estimated value for ΔH_b varies significantly from one experiment to another. In one experiment the estimated value is −27.0 kcal/mol at 0.05 M Na^+ and −16.0 kcal/mol at 0.1 M Na^+ for a calf-thymus DNA (Chaires 1985). In another experiment the estimated value is −9.24 kcal/mol for a salmon testes DNA and −8.65 kcal/mol for Poly d(GC) · Poly d(GC) at 0.015 M Na^+ (Remeta et al. 1993). Our calculated ΔH_b is somewhere between the estimated values from these experiments. Taking into consideration of the large variation in experimentally estimated ΔH_bs our result is in reasonable agreement with these experiments.

There is a small feature in the K_{eq} curve at 357 K and it coincides with the small features found in the P^{op} curves in Fig. 2 for the drug-bound base pairs at the same temperature. These features are caused by a sudden decrease in the stability of one of the AT pairs. As shown in Fig. 2 the P^{op} of the T3-A6 base pair increases abruptly from 0.051 at 356 K to 0.51 at 357 K. The reason for the abrupt disruption of about half of the A3-T6 base pairs before melting of the entire drug-helix complex will be discussed in the next section. From Eqs. (8), (11), (13) and (15) one finds that K_{eq} is dependent on the drug unconstrained probability P^{uc} which is in turn dependent on the P^{op}s of the neighboring GC pairs. As a result K_{eq} is expected to show a small feature in accordance with the P^{op} of the GC pairs.

At temperatures above 357 K K_{eq} drops more and more rapidly when approaching the melting temperature. In this region K_{eq} departs from the linear van't Hoff curve. At the melting temperature the K_{eq} changes rapidly from 7.56×10^3 at 371 K to 0 at 372 K, indicating a complete dissociation of daunomycin from DNA at melting.

Temperature dependence of base pair opening probabilities

The calculated values of the P^{op} for the base pairs in both drug-free and daunomycin-bound Poly d(CGTA) · Poly d(TACG) at several premelting temperatures are given in Table 3. The calculated disruption probabilities of the individual interbase H-bonds for the two helices are given in Table 4. As in our earlier work our calculated P^{op}s for the free helix at room temperature are in fair agreement with measurements from imino proton exchange carried out at room temperature. From both Table 3 and Fig. 2 we find that, except for one base pair, the P^{op}s of the drug-bound DNA are close to those in the drug-free DNA at temperatures below the T_m of the free helix. All the P^{op}s monotonically increase with temperature. The P^{op}s for the AT pairs are in the range 10^{-3} to 10^{-2} at premelting temperatures, in agreement with the observed value for some AT(AU) pairs (Gueron et al. 1987) but substantially different from the observed value of 10^{-5} for other AT pairs (Gueron et al. 1987). While a big discrepancy is found in the observed P^{op} for AT pairs, all the observed P^{op} for GC pairs are of the order of 10^{-6}. We have pointed out that the discrepancy in the observed P^{op} for AT pairs arises be-

Table 3. Opening probability P^{op} of the base pairs in daunomycin-bound and drug-free Poly d(CGTA) · Poly d(TACG) at several premelting temperatures

Base pair	T (K)	P^{op}	
		Drug-bound	Drug-free
C1-G8	293	3.71×10^{-6}	4.64×10^{-6}
	313	1.36×10^{-5}	1.71×10^{-5}
	333	4.89×10^{-5}	6.94×10^{-5}
	353	2.09×10^{-4}	3.46×10^{-4}
	368	8.33×10^{-4}	
G2-C7	293	4.09×10^{-9}	4.63×10^{-6}
	313	3.60×10^{-8}	1.71×10^{-5}
	333	2.84×10^{-7}	6.85×10^{-5}
	353	3.60×10^{-6}	3.45×10^{-4}
	368	3.32×10^{-5}	
T3-A6	293	3.61×10^{-3}	3.25×10^{-3}
	313	7.20×10^{-3}	6.29×10^{-3}
	333	1.46×10^{-2}	1.34×10^{-2}
	353	3.64×10^{-2}	3.98×10^{-2}
	368	5.81×10^{-1}	
A4-T5	293	2.29×10^{-3}	3.18×10^{-3}
	313	4.47×10^{-3}	6.17×10^{-3}
	333	8.62×10^{-3}	1.34×10^{-2}
	353	1.78×10^{-2}	3.88×10^{-2}
	368	4.17×10^{-2}	

Table 4. Disruption probability P_i of the interbase H-bonds of the GC pairs in daunomycin-bound and drug-free Poly d(CGTA) · Poly d(TACG) at several premelting temperatures

Base pair	Bond	T (K)	P_i	
			Drug-bound	Drug-free
C1-C8	N4-H-O6	293	0.0046	0.0049
		323	0.0117	0.0125
		353	0.0308	0.0364
	N3-H-N1	293	0.0354	0.0371
		323	0.0517	0.0547
		353	0.0781	0.0874
	O2-H-N2	293	0.0227	0.0254
		323	0.0435	0.0491
		353	0.0870	0.1087
G2-C7	O6-H-N4	293	0.0050	0.0049
		323	0.0127	0.0125
		353	0.0344	0.0364
	N1-H-N3	293	0.0387	0.0371
		323	0.0564	0.0547
		353	0.0849	0.0874
	N2-H-O2	293	0.0216	0.0253
		323	0.0418	0.0489
		353	0.0838	0.1083

cause of a minor groove hydration spine found in certain AT sequences (Chen and Prohofsky 1992). This hydration spine substantially enhances the dynamic stability of the base pairs to which it is attached. The AT pairs with lower P^{op} are the ones that have a hydration spine attached to them, and those with higher P^{op} have no hydration spine.

Table 5. Disruption probability P_i of the interbase H-bonds of the AT pairs in daunomycin-bound and drug-free Poly d(CGTA) · Poly d(TACG) at several premelting temperatures

Base pair	Bond	T (K)	P_i	
			Drug-bound	Drug-free
T3-A6	O4-H-N6	293	0.0427	0.0396
		323	0.0839	0.0755
		353	0.1949	0.2031
	N3-H-N1	293	0.0847	0.0820
		323	0.1231	0.1190
		353	0.1870	0.1960
A4-T5	N6-H-O4	293	0.0346	0.0400
		323	0.0648	0.0755
		353	0.1275	0.2021
	N1-H-N3	293	0.0665	0.0804
		323	0.0961	0.1168
		353	0.1392	0.1920

In our drug-free and drug-bound DNA complex there is no hydration spine attached to the minor groove of the AT pairs. Therefore the P^{op} of the these AT pairs is expected to be in the range 10^{-3} to 10^{-2}. Our calculated P^{op} for the GC pairs in the free DNA helix is also in good agreement with observations (Gueron et al. 1987). The P^{op} of the G2-C7 base pair in the drug-bound DNA is found to be three orders of magnitude smaller than that in the free helix in this temperature range. We have pointed out (Chen and Prohofsky 1994a) that this significant change arises because of strong hydrogen bonding between the O9 atom of the drug and the N3 atom of the G2 base.

We find from Fig. 2 that the P^{op} of the T3-A6 base pair in the drug-bound DNA is slightly larger than its counterpart in the free helix at temperatures below 348 K. On the other hand the P^{op}s of the other pairs in the drug-bound DNA are all smaller than their counterparts in the free helix. It is surprising to see that the P^{op} of one of the AT pairs in the drug-bound DNA is larger than its counterpart in the free helix. Because of enhanced stability from daunomycin binding, one excepts the P^{op} of this base pair to become smaller as does the other base pairs in the drug-bound DNA. This unexpected increase of P^{op} arises because of the unique stacking feature of the T3-A6 pair in the structure of our drug-bound DNA complex.

Our drug-DNA complex is generated directly from an x-ray crystal structure of the daunomycin-DNA d(CGTACG) complex. In this crystal structure the rise between the two AT pairs is 3.695 Å. This is compared to the rise of 3.362 Å between an AT and a GC pair in the complex and the rise of 3.4 Å for standard B-conformation DNA base pairs. On the other hand the daunomycin-bound GC pairs in the crystal structure tilt in such a way that the G bases are displaced away from the drug AC group and the complementary C bases are drawn closer to the drug AC group. This structural feature results in an opposite stacking pattern in the two AT-GC interfaces in our daunomycin-Poly d(CGTA) · Poly d(TACG) complex. A unit cell of our helix is extracted from the CGTA section of the crystal daunomycin-DNA d(CGTACG) complex.

Therefore between the T3-A6 and G2-C7 pairs the G2-C7 pair is tilted such that G2 is moved away and C7 is drawn closer to the T3-A6 pair. Between the A4-T5 pair and the C1-G8 pair in an adjacent unit cell the C1-G8 pair is tilted such that the C1 base is moved away and G8 base is drawn closer to the A4-T5 base pair.

The larger separation between the adjacent AT pairs results in a weaker stacking between these pairs as compared to free helix. Since the size of a G base is substantially larger than that of a C base, the stacking between two base pairs with a G base tilted towards the other base pair should be enhanced and the stacking between base pairs with a C base tilted towards the other base pair should be reduced. Therefore the stacking between T3-A6 and G2-C7 pairs is weaker than that in the free helix. On the other hand, the stacking between an A4-T5 pair and a C1-G8 pair in an adjacent unit cell is stronger than that in the free helix. The combined weaker stacking between the T3-A6 and its two neighboring base pairs (A4-T5 and G2-C7) is responsible for the reduced stability or the larger P^{op} of this base pair in the drug-bound system. As for the A4-T5 base pair, the enhanced stacking between it and its neighboring C1-G8 pair seems to at least balance or even outweigh the reduced stacking between it and the T3-A6 pair. This coupled with enhanced stability from daunomycin binding results in a smaller P^{op} for the A4-T5 pair at premelting temperatures.

The premelting transition

Our calculation seems to indicate the existence of a premelting transition in the drug-bound helix. We find from Fig. 2 that the P^{op} of the T3-A6 base pair increases from 0.051 at 356 K to 0.54 at 357 K, while the P^{op}s of the other base pairs increases only moderately. This premelting transition occurs at a temperature 15 K below the final T_m and 2 K above the T_m of the free helix. The transition involves the disruption of half of the T3-A6 base pairs or 1/8 of the total number of base pairs. Although the number of disrupted pairs is substantial, these disrupted pairs are separated by an average of three intact base pairs plus the drug. In addition, each of the disrupted base pairs is still bound by the daunomycin AS residue located in its minor groove. Apparently the occurrence of this premelting transition is a direct result of the weaker stacking in the T3-A6 base pairs. The effect of this weaker stacking seems to outweigh the stabilizing effect associated with daunomycin binding and ultimately leads to the disruption of the T3-A6 base pairs at temperatures a few degrees above the T_m of the free helix.

It would be interesting to see if this premelting transition really exists, or if it is an artifact of calculation, or is introducted as a result of the use of the particular crystal structure which might be different from the structure in solution. We have not found a relevant experimental observation that can be used to clarify the existence of this premelting transition. It is worthwhile to point out that several differential UV measurements (Chaires 1983; Remeta et al. 1993) have shown that in the presence of non-saturating amounts of daunomycin DNA helices melts biphasically, i.e. a fraction of the base pairs disrupt at a temperature below the final melting temperature. The observed temperature of the first transition is dependent on the drug-base pair ratio. The higher the ratio of drug to base pair the closer the first transition temperature is to the final melting temperature. This biphasic transition is most prominent at low drug ratios. It becomes less and less observable as the drug ratio increases and at drug saturation this biphasic melting disappears. The reported first transition temperature for Poly d(GC) · Poly d(GC) at a drug-base pair ratio of 4 bp/drug is only 3 K below the final T_m and 12 K above the T_m of the free helix (Chaires 1983). For Poly d(AT) · Poly d(AT) at 5 bp/drug the observed first transition occurs at a temperature 20 K below the final T_m and 14 K above the T_m of the free helix (Remeta et al. 1993).

The comparison between our premelting transition and the observed UV transition is however not straightforward. Our premelting transition is characterized by a disruption of certain type of base pairs scattered at individual sites separated by several intact base pairs and attached by an intact drug. On the other hand, UV spectroscopy measures the UV absorbance that arises from the $\pi-\pi^*$ electronic transition in both purine and pyrimidine bases. An increase in the absorbance reflects a change in the electronic configuration of the bases due to the decrease in base-base stacking. Therefore the UV spectroscopy more likely measures base-base unstacking involving several unpaired bases instead of scattered single base pair disruption. This conclusion is supported by the observation that the UV biphasic transition is observable only at lower drug to base pair ratios.

Biphasic melting phenomena in drug-DNA systems were also predicted by the helix-coil transition theory (McGhee 1976). The biphasicity was found to be noncooperative, which is in agreement with the behavior of our premelting transition. The helix-coil transition theory also predicted that after the disruption of more than 50% of the base pairs the bound drugs will be expelled from their complexes and the free drugs are subsequently redistributed. In our premelting transition, in which 12.5% of the base pairs are disrupted, no significant drug dissociation occurs. Massive drug dissociation does occur at the melting temperature where more than 50% of the base pairs are disrupted. The final melting as shown in our calculation is abrupt which is typical for a long repeating helix. The redistribution of the drugs is not expected to alter the dynamics significantly in this abrupt melting process.

Conclusion

We have used a microscopic statistical mechanical approach, combined with an effective harmonic theory, to determine bond disruption probabilities and to analyze the motion of daunomycin dissociation from the bases. Our calculated temperature behavior of base pair opening probabilities and the drug binding constant for a daunomycin-bound DNA is in good agreement with observations. The probabilities show the onset of massive base pair separation and drug dissociation at temperatures close to observed melting temperatures. Our calculation also indicates a premelting transition in the drug-bound helix which might be related to the observed biphasic melting in many nonsaturated drug-DNA systems.

The agreement between observations and our calculations demonstrates that our approach is an effective and computationally efficient approach in determination of bond disruption and unstacking melting in biological macromolecules. Although the formulation rests on a harmonic approximation, nonlinear effects and thermal expansion in bonding and stacking interactions can be incorporated into the theory through a well established self-consistent approach at atomic level of detail. This approach allows the determination of the thermodynamic variables such as disruption probabilities as function of temperature that is accurate up to melting temperature. One important advantage of this method is that disruption probabilities are determined by statistical mechanical means. Collective bond disruption and unstacking dissociation are on a time scale of milliseconds or larger which is beyond the capabilities of stimulation.

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