Calculation of the binding affinity of the anticancer drug daunomycin to DNA by a statistical mechanics approach

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Equilibrium binding constants of the anticancer drug daunomycin, bound to several GC containing polymeric DNAs (G represent guanine and C cytosine), are calculated by means of a microscopic statistical mechanics approach and based on observed x-ray crystal structures. Our calculation shows base sequence specificity of daunomycin in agreement with the observations. We find the drug binding constant to be sensitive to the base composition of the host sequence. The binding stability decreases in the order of CGTACG, CGATCG, and CGGCCG, which is consistent with observations (T represents thymine and A adenine). This binding specificity arises from sequence specific hydrogen bond and nonbonded interactions between the drug and a host DNA. These interactions are affected by sequence specific structural features exhibited from x-ray crystallography. The agreement between our calculations and experiments shows that our method is of practical application in analyzing sequence specific binding stability of anticancer drugs. [S1063-651X(97)01506-7]

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INTRODUCTION

Daunomycin is an anticancer drug used for the treatment of such diseases as acute leukemia, hematologic malignancies, and a variety of solid tumors [1,2]. The target of this drug is DNA and it is known to inhibit both DNA replication and transcription through binding and intercalation into DNA base pairs [3]. Because of its biological mode of action and pharmacological application, the DNA binding properties of daunomycin have been a subject of intensive investigations.

A focus of recent investigation is on the base sequence specificity of daunomycin binding [4–10]. Such a study is important in probing the mechanism of preferential binding of an anticancer drug to a particular DNA site. An understanding of this binding selectivity is of importance in facilitating the design of new anticancer drugs. Several experimental studies have revealed that daunomycin binding is sensitive to base composition and sequence of the host DNA. While each experiment gives somewhat different sequence specificity duo to differences in experimental conditions, the following descending order in binding stability is commonly found in these experiments: AC $>$ AG $>$ GC or GG [5,6,10] (A represents adenine, G guanine, and C cytosine). A detailed analysis on these experiments suggested that the optimal daunomycin binding site may be a sequence containing ATGC or ATCG base pairs [7] (T represents thymine). The structural feature of the minor groove amino group of guanine and the difference in stacking patterns are attributed as the origin of the observed sequence specificity of daunomycin binding [10].

An earlier theoretical study by Chen, Gresh, and Pullman on the interaction energies in several model daunomycin-DNA systems showed that the observed base specificity can be explained by the intricate interplay between different components of interactions between DNA and binding daunomycin [8]. Both hydrogen bonds (H bonds) and nonbonded interactions were found to be the primary source of sequence specificity. However the structural models used in this study were constructed based on the assumption that they have the same internal geometry as in the observed daunomycin-d$(CGTACG)_2$ structure. X-ray diffraction studies have shown an apparent sequence specific structural difference in different daunomycin bound DNA crystals. In addition thermal effects are neglected in this study. It is not clear how such neglect will affect the calculated binding specificity.

In another theoretical study carried out by Cieplak *et al.*, free energy perturbation–molecular dynamics calculations were carried out to compare daunomycin binding stability in CGTACG, CGCACG, and CATACG double helical hexamers [9]. The calculated free energy changes in these hexamers show daunomycin binding preference in qualitative agreement with relevant experimental data. However, it was reported that the calculations for the second base-pair perturbation is less decisive than other perturbation calculations. As a result, this study was restricted to single base-pair perturbations.

An analysis that includes sequence specific structure, thermal effects, and an unrestricted sequence can be made possible by the use of the microscopic statistical mechanics approach we have developed [11,12] combined with the use of observed x-ray crystal or NMR structures. In the present work we carry out such a study to determine daunomycin binding specificity of several GC containing DNA sequences using their respective x-ray crystal structures. The equilibrium binding constant, which measures thermodynamic stability of binding, of daunomycin bound to these DNA polymers are calculated and compared with observations. The

binding specificity revealed by these binding constants will be analyzed. We will also examine the role of both H bonds and nonbonded interactions in daunomycin binding specificity. Such a study also serves as a test of the applicatability of our method in analysis of anticancer drug binding to random sequence DNA.

THEORETICAL METHOD AND COMPUTATION PROCEDURES

The DNA polymers studied in the present work are Poly d(CGTACG)·Poly d(CGTACG), Poly d(CGATCG)·Poly d(CGATCG) and Poly d(CGGCCG)·Poly d(CGGCCG). These DNA polymers are selected because of the availability of relevant x-ray crystal structural data for daunomycin-bound complex. Each of these polymers is an infinitely long helix with a six base-pair repeating sequence. The coordinates for daunomycin-bound duplexes are generated from the x-ray crystal structures of respective oligomers deposited in the Brookhaven protein data bank (PDB files pdb1d11.ent, pdb1d10.ent, pdb110d.ent) [13–15]. In these drug-bound polymers one daunomycin is intercalated into the space between two base pairs in every CG step. Therefore, there are two drugs bound to every repeating sequence and the drug–base-pair ratio is 3 base pairs (bp)/drug.

The equilibrium binding constant of daunomycin bound to these DNA polymers are calculated by a microscopic statistical mechanics method. In this approach the binding stability is derived from an statistical analysis of drug dissociation motions. The anharmonic motions of the drug leading to dissociation are modeled by a modified self-consistent harmonic theory [11,12]. The details of our method is described in our earlier papers. In the present paper only a brief summary is given.

The dissociation of daunomycin from DNA can be divided into two stages. The first is the disruption of H bonds, which involves motions on a scale of $\sim 1/10$ Å. The second is the translational separation of the bulk drug from DNA, which involves motions in the range of $\sim$ Å. Because the bulk of daunomycin is stacked between neighboring base pairs, the second stage can also be called a drug-base unstacking process. These events can be assumed to be statistically independent events. Hence, the dissociation probability P_D of daunomycin can be given by

$$P_D = P_{st} \prod_i P_i , \tag{1}$$

where P_{st} is the drug-base unstacking probability and P_i is the probability for the disruption of a drug-DNA H bond. There are usually several drug-DNA H bonds, the probability for the disruption of all these bonds is the product of the P_i of each bond.

Equation (1) implies no explicit dependence of unstacking probability on the H bond probability and vice versa. The coupling between stacking interaction and H bond motions, however, is implicitly included in these probabilities. This is because these probabilities are calculated from the normal modes of the self-consistent harmonic equation which involve the collective motions of both stacking and H bond displacement. In our earlier work on the melting calculation of DNA [20,21] the coupling between H bond and stacking is also explicitly introduced in thermal expansion terms. This, however, is negligible at premelting temperatures because of the small value of probabilities, and thus is not included in the present work.

Using the drug dissociation probability P_D, the equilibrium binding constant K_{eq} of the drug can be determined from the fact that the sum of dissociation and association probability equals to one. We therefore obtain

$$K_{eq} = \frac{1 - P_D}{P_D} . \tag{2}$$

Given initial microscopic structure and force fields, the disruption probability P_i of individual H bonds and daunomycin unstacking probability P_{st} can be computed by the procedures described below.

Hydrogen bond disruption probability

H bond disruption probability can be derived from a statistical mechanics calculation of microscopic bond fluctuational motions. Given the initial coordinates and force constants one can solve the equation of bond motion from the following effective Hamiltonian:

$$\begin{aligned} H_0 = & \sum_{atoms} \frac{P^2}{2m} + \sum_{bonds} \frac{1}{2} K_r (r - r_{eq})^2 + \sum_{angles} \frac{1}{2} K_\theta (\theta - \theta_{eq})^2 \\ & + \sum_{dihedrals} \frac{1}{2} K_\Phi (\Phi - \Phi_{eq})^2 + \sum_{\mathrm{H}\ bonds} \frac{1}{2} K_i (r_i - \langle r_i \rangle)^2 \\ & + \sum_{nonbond} \frac{1}{2} K_{NB} (r_{ij} - \langle r_{ij} \rangle)^2 + V_{eq} . \end{aligned} \tag{3}$$

This effective Hamiltonian is self-consistently adjusted to the real system by the minimization of the Bogoliubov free energy expansion $F = F_0 + \langle H - H_0 \rangle$, where H is the Hamiltonian of the original system, and F and F_0 are the free energy of the original and effective system, respectively. V_{eq} is the static part of the Hamiltonian, i.e., the potentials at equilibrium positions. K_r and K_θ are covalent bond stretch and angle bending force constants. These force constants are refined from observed vibrational spectra [16,17]. Since the changes in dihedral angles are small for motions leading to H bond disruption, K_Φ can be given by the second derivative of the relevant potential from AMBER (a software package for the simulation of DNA, proteins, and organic molecules) [9,18]. The nonbonded force constant K_{NB} is from a simple empirical algorithm [19]. The H bond force constant K_i is given by [20,21]

$$K_i = (1 - P_i) \frac{\int_{r_c}^{\infty} dr \, \frac{d^2 V_i(r)}{dr^2} e^{-(r - \langle r_i \rangle)^2 / 2 \langle u_i^2 \rangle}}{\int_{r_c}^{\infty} dr \; e^{-(r - \langle r_i \rangle)^2 / 2 \langle u_i^2 \rangle}} , \tag{4}$$

where r_c is the innerbound cutoff determined from $V_i(r_c) = 2 | V_i(r_i^0) |$ (r_0^i is the potential minimum position). Our analysis indicates that the calculations are not sensitive to the exact choice of the cutoff. The scaling factor $(1 - P_i)$ is introduced to take into effect of disrupted bonds in a statistical description of the force constant and P_i is the

disruption probability of the bond given below. $\langle r_i \rangle$ is determined by an empirical thermal expansion and by appropriately choosing the potential minimum such that $\langle r_i \rangle$ agrees with the x-ray observed length at room temperature [20,21]. $\langle u_i^2 \rangle$ is the mean square vibrational amplitude of the bond derived based on a self-consistent harmonic approach [20,21]

$$\langle u_i^2 \rangle = \sum_l s_{il}^2 \frac{\hbar}{2M\omega_l} \coth\left[\frac{\hbar\omega_l}{2k_BT}\right], \tag{5}$$

where ω_l and l is the frequency and the index of the normal modes, respectively. T is the temperature, k_B Boltzmann's constant, and $\hbar$ Planck's constant divided by 2π.

The self-consistent harmonic approach also gives rise to statistical probability distribution functions of finding a particular H bond with a particular length. From these distribution functions one can determine the probability of finding an H bond fluctuating beyond a certain breakdown point, i.e., the disruption probability of this H bond. This probability is given by

$$P_i = \int_{L_{max}}^{\infty} dr\ e^{-(r-\langle r_i \rangle)^2/2\langle u_i^2 \rangle}, \tag{6}$$

where L_{max} is the maximum stretch length (breakdown point) which can be found in our previous publication [20].

The newly calculated force constants are then substituted into Eq. (3) to start another round of calculation. Such a process continues until every output force constant matches the input force constant, judged by the condition $\Delta K_i / K_i < 0.01$. The self-consistent solution corresponds to a minimized Bogoliubov free energy expansion.

Drug-DNA unstacking probability

As pointed out in our earlier work [11], daunomycin-base unstacking probability can be calculated by assuming that, after the disruption of drug-DNA H bonds, the drug can oscillate along the orientation of the ring system of the aglycon chromophore group in a composite drug-DNA interaction potential well. Such an oscillating motion can then be described by an effective one dimensional effective harmonic Hamiltonian

$$H_0 = \frac{P^2}{2M} + \frac{1}{2}K_{st}(R - \langle R \rangle)^2, \tag{7}$$

where M is the total mass and $\langle R \rangle$ is the equilibrium position of the drug given below. K_{st} is the effective force constant determined by minimizing the Bogoliubov free energy expansion $F = F_0 + \langle H - H_0 \rangle$. F_0 is the free energy of the effective harmonic system in Eq. (7): $F_0 = -k_BT \ln[\exp(-H_0/k_BT)]$. 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

$$K_{st} = (1 - P_{st}) \frac{\int_{u_m}^{\infty} du\ e^{-u^2/2D} \frac{d^2}{du^2} V_{st}(\langle R \rangle + u)}{\int_{u_m}^{\infty} du\ e^{-u^2/2D}}, \tag{8}$$

where V_{st} is the drug-DNA stacking potential and u_m is the inner bound of the hard core of the potential chosen as -3 Å. $1 - P_{st}$ is used to scale the force constant to take into consideration the effect of dissociated drugs in a statistical description of the force constant of drug motions. The mean square vibrational displacement D can be given from another Stationary condition $\partial F / \partial K_{st} = 0$, which gives

$$D = \frac{\hbar}{2M\omega} \coth\left[\frac{\hbar\omega}{2k_BT}\right], \tag{9}$$

where $\hbar$ is the Plank's constant divided by 2π and ω is the frequency obtained from

$$M\omega^2 = K_{st}. \tag{10}$$

The mean position $\langle R \rangle$ 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

$$V_{st}(\langle R \rangle + \mu) = V_{st}(\langle R \rangle - \mu). \tag{11}$$

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_{st} can be determined by the distribution function $\exp(-u^{-2}/2D)$ as

$$P_{st} = \int_{L_{max}}^{\infty} du\ \exp(-(u - \langle R \rangle)^2/2D), \tag{12}$$

where L_{max} is the maximum displacement before dissociation. It is chosen as the first inflection point of the potential.

TABLE I. Comparison between calculated and observed equilibrium daunomycin binding constant K_{eq} in several host DNA polymers. Only those with similar base sequence and base-pair–drug ratio are used for comparison. Calculated and observed K_{eq} is at 293 K and 298 K, respectively. Observed values are from Remeta *et al.* [10]

Host DNA polymer	Base pair–drug ratio	K_{eq}	Method
Poly d(CGTACG)·Poly d(CGTACG)	3.0	6.94×10^7	theor.
Poly d(AC)· Poly d(GT)	3.0	7.99×10^7	expt.
Poly d(CGATCG)·Poly d(CGATCG)	3.0	2.34×10^7	theor.
Poly d(AG)·Poly d(CT)	3.5	1.39×10^7	expt.
Poly d(CGGCCG)·Poly d(CGGCCG)	3.0	8.60×10^6	theor.
Poly(dG)·Poly(dC)	3.5	1.01×10^7	expt.

TABLE II. Helical parameters for daunomycin-DNA complexes studied in this work.

Sequence	Base pair	Helical twist angle (deg)	Rise (Å)
CGTACG	C1-G12	34.95	5.277
	G2-C11	30.85	3.362
	T3-A10	34.48	3.695
	A4-T9	30.85	3.362
	C5-G8	34.95	5.277
	G6-C7		
CGATCG	C1-G12	35.36	5.142
	G2-C11	31.56	3.545
	A3-T10	31.99	3.365
	T4-A9	31.56	3.545
	C5-G8	35.36	5.142
	G6-C7		
CGGCCG	C1-G12	37.50	5.492
	G2-C11	28.69	3.501
	G3-C10	34.49	3.020
	C4-G9	28.69	3.501
	C5-G8	37.50	5.492
	G6-C7		

The newly calculated stacking force constant K_{st} is substituted into Eq. (7) to start another round of calculation. Such a process continues until the input K_{st} matches the output K_{st}. The self-consistent solution corresponds to the minimized Bogoliubov free energy expansion.

RESULTS AND DISCUSSIONS

Daunomycin binding specificity

The calculated equilibrium binding constant K_{eq} of daunomycin bound to Poly d(CGTACG)·Poly d(CGTACG), Poly d(CGATCG)·Poly d(CGATCG), and Poly d(CGGCCG)·Poly d(CGGCCG) is given in Table I. Hereafter these polymers will be named CGTACG, CGATCG, and CGGCCG polymer, respectively. These host DNA polymers are considered because of the availability of their daunomycin-bound structure. All these daunomycin-bound DNAs have a base-pair–drug ratio of 3 bp/drug. For comparison the observed K_{eq} for host DNA duplexes with sequence and base-pair–drug ratio close to these three duplexes are included. Our calculated K_{eq}s are in fair agreement with observations. For instance, the calculated K_{eq} for the CGTACG polymer is 6.94×10^{7}. The observed value for the DNA polymer with closest sequence is 7.99×10^{7} for Poly d(AC)·Poly d(GT) at 3 bp/drug [10]. Our calculated K_{eq} for the CGATCG polymer is 2.35×10^{7} which is compared to observed value of 1.39×10^{7} for Poly d(AG)·Poly d(TC) at 3.5 bp/drug [10]. The calculated K_{eq} for the CGGCCG polymer is 8.6×10^{6} which is also close to the observed value of 1.01×10^{7} for Poly(dG)·Poly(dC) at 3.5 bp/drug [10].

In addition to the fair agreement between calculated and observed binding constants, our calculation also predicts sequence dependent binding specificity in agreement with observations and other theoretical analysis. From Table I we find that the strength of daunomycin binding descends in the order of CGTACG > CGATCG > CGGCCG. This order is equivalent to GT > GA > GG or AC > AG > GG. The observed order is AC > AG > GG in a recent study by Remeta *et al.* [10]. Although earlier expermental studies give a somewhat different binding specificity duo to differences in sample conditions, a commonly found order is AC > AG > GC [5,6]. Therefore, our calculation is consistent with the binding specificity probed by these experiments. In the three GC containing DNA sequences studied, we predict that CGTACG is the preferential binding sequence for daunomycin. Such a prediction is consistent with an earlier study which suggested that the optimal daunomycin binding site is a ATGC or ATCG sequence [7].

Our predicted sequence preference is slightly different from molecular mechanics study of Chen, Gresh, and Pullman [8]. In that study an order of CGATCG > CGTACG > TATATA > CGCGCG > TACGTA is given. Our calculation predicts that CGTACG > CGATCG. This discrepancy arises most likely from the use of different structural models. In the study of Chen, Gresh, and Pullman all daunomycin-bound DNA sequences are assumed to have the same conformation as that of the daunomycin-DNA d(CGTACG)$_2$ complex determined by x-ray crystallography. In contrast we use x-ray crystal structure for every sequence. From Table II we find that the helical parameters of daunomycin-bound DNA are different from sequence to sequence. Although the difference is relatively small between the CGTACG and CGATCG sequence, our analysis indicates that it is sufficient to affect specific H bonds and nonbonded interactions between daunomycin and host DNA sequence, which in turn affects the binding preference.

Apart from this difference, the same trend of binding preference is predicted by both Refs. [8] and [9] and our group, although different approaches ranging from molecular mechanics and statistical mechanics are used. Experiments have shown that, except for pure AT or AU sequences (U represents uracil), daunomycin binding is predominantly enthalpy driven [10,22]. Therefore it is not surprising that a similar binding preference can be obtained from molecular mechanics and statistical mechanics calculations. It is pointed out that the entropic effect, as well as sequence specific structural features, does affect the binding stability to a certain extent. These effects need to be considered to quantitatively determine the binding preference.

Origin of sequence dependent binding preference

Chen, Gresh, and Pullman have shown that both drug-DNA H bonds and nonbonded interactions play an important

TABLE III. All drug-DNA H-bond disruption probability $P_H=\Pi_i P_i$, drug unstacking probability P_{st}, drug dissociation probability P_D, and equilibrium drug binding constant K_{eq} in several daunomycin-bound DNA polymers at 293 K.

System	P_H	P_{st}	P_D	K_{eq}
CGTACG	3.27×10^{-4}	4.40×10^{-5}	1.44×10^{-8}	6.94×10^{7}
CGATCG	7.36×10^{-4}	5.81×10^{-5}	4.27×10^{-8}	2.34×10^{7}
CGGCCG	3.93×10^{-4}	2.95×10^{-4}	1.16×10^{-7}	8.60×10^{6}

TABLE IV. Disruption probability P_i and bond length $\langle r_i \rangle$ of drug-DNA H bonds in several daunomycin-bound DNA polymers at 293 K. Only the bonds related to daunomycin bound between C5—G8 and G6—C7 are included in this table.

System	Bond	P_i	$\langle r_i \rangle$ (Å)
CGTACG	N2 G 8 — O7 DM 13	0.1232	3.1760
	O6 DM 13 — O2 C 5	0.3221	3.3350
	O9 DM 13 — N3 G 8	0.0294	2.7490
	O11 DM 13 — N3 C 7	0.2808	3.3560
	N3* DM 13 — O2 C 5	0.2568	3.2930
CGATCG	N2 G 8 — O7 DM 13	0.5531	3.2660
	O6 DM 13 — O2 C 5	0.4659	3.4570
	O9 DM 13 — N3 G 8	0.0282	2.7440
	O11 DM 13 — N3 C 7	0.3505	3.3340
	N3* DM 13 — O4* C 5	0.1273	3.1780
CGGCCG	N2 G 8 — O7 DM 13	0.2575	3.3290
	O6 DM 13 — N9 G 6	0.1621	3.2670
	O9 DM 13 — N3 G 8	0.0238	2.8320
	O11 DM 13 — O2 C 7	0.3964	3.4040

role in the sequence dependent binding preference [8]. The importance of nonbonded interactions was also revealed by the free energy perturbation–molecular dynamics study of Cierpiak *et al.* [9]. We have also studied the contribution of these interactions on binding stability. Table III gives our calculated drug dissociation probability P_D and its components, unstacking probability P_{st}, and all drug-DNA H bond disruption probability $P_H = \Pi_i P_i$, as well as the equilibrium binding constant K_{eq}. These probabilities give good indication of the contribution from H bond and nonbonded stacking interactions to the binding stability.

The P_{st} of the CGTACG polymer is similar in value to that of CGATCG sequence. This results from a similar drug-DNA stacking interactions in these two sequences. From Table II one can see that both helical twist angles and rises are very similar in these two sequences. On the other hand the P_H of the CGTACG polymer is substantially smaller than that of the CGATCG polymer. From Table IV we find that several drug-DNA H bonds in CGTACG are slightly more stable than those in CGATCG. These H bonds collectively contributes to a more stable binding of daunomycin to the CGTACG polymer than to the CGATCG polymer.

The lower binding affinity in the CGGCCG polymer arises primarily from nonbonded stacking interactions between daunomycin and DNA. From Table III one can see that the P_H for the CGGCCG polymer is comparable to that of CGTACG and larger than that of CGATCG. In contrast, the P_{st} of this sequence is an order of magnitude larger than that in the other two sequences. This is due to a much weaker drug-DNA stacking interaction in the CGGCCG polymer. From Table II one can see that the helical twist angle of the CG step in CGGCCG polymer is more than 2° larger than that in a CGTACG or CGATCG polymer. Moreover, the helical rise of this polymer is more than 0.2 Å larger than that of the other two polymers. The substantially larger twist angle and rise in the binding site naturally result in a weaker stacking interaction between daunomycin and DNA. The difference in these stacking interactions is significant to the extent that the K_{eq} of the CGGCCG polymer is an order of magnitude larger than those of the two other polymers studied.

CONCLUSION

Sequence-dependent binding specificity of daunomycin arises from sequence specific drug-DNA hydrogen bond and nonbonded interactions. The thermodynamics of these interactions can be studied by a microscopic statistical mechanics method we have developed. The equilibrium drug binding constant in several GC containing host DNAs is calculated by means of this approach and based on microscopic structure from x-ray crystallography. The fair agreement between our calculation and observed sequence specificity of daunomycin binding shows the potential application of our method in analysis of binding preference of anticancer drugs to DNA sites with random sequences.

Our calculation is based on observed x-ray crystal structures. Effect associated with crystal packing may affect our calculated binding constant to a certain extent. But this effect is likely to be small based on the agreement between our calculation and observations.

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