Binding stability of a cross-linked drug: Calculation of an anticancer drug cisplatin-DNA complex

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(Received 18 October 1996)

One of the binding modes of anticancer and antibiotic drugs bound to DNA is the formation of a cross link, i.e., binding is made through the formation of covalent bonds between a binding drug and DNA. In this work we present a computational method to calculate the binding stability of a drug cross linked to DNA. Our method is based on the modified self-consistent harmonic approach in which the disruption probability of the cross-linked bonds as well as hydrogen bonds is calculated from a statistical analysis of microscopic thermal fluctuational motions. A Morse potential with appropriate parameters is used to model the cross-linked covalent bonds. Our method is applied to an anticancer drug cisplatin-DNA oligomer d(CTCTAGTGCTCAC)·d(GTGAGCACTAGAG) complex. We calculated the equilibrium binding constant of a cisplatin bound to this DNA oligomer. Our method can also be used to analyze the effect of drug binding on DNA base-pair thermal stability. We find that, despite the disruption of certain interbase H bonds, the thermal fluctuational opening probability P^{op} of base pairs in the cisplatin binding region is enhanced by the formation of non-Watson-Crick H bonds as well as cross-linked covalent bonds. Although the entire DNA helix is bent by cisplatin binding, the stability of the base pairs outside the binding region is only slightly affected by this deformation. [S1063-651X(97)05805-4]

PACS number(s): 87.15.By, 87.15.Kg, 63.70.+h

I. INTRODUCTION

DNA is a major target of anticancer and antibiotic drugs [1,2]. Experimental structural studies have revealed that drugs bind to DNA through a few well-defined modes. These include (i) intercalation, in which a drug is inserted into the inner space between base pairs [1]; (ii) groove binding, in which a drug resides in one of the two grooves on the side of DNA molecule [2]; and (iii) cross linking, in which a drug forms strong covalent bonds with DNA [3]. A key task in computer-aided drug design is to estimate the dynamical binding stability of a drug. In principle, the dynamical stability of binding can be studied both from a dissociation process and from an association process. However, because of complicated effects related to association, it is difficult to study binding stability from this process. Therefore, a practical way to study binding is from the analysis of the dissociation process. As dissociation is a rare event, molecular-dynamics simulation would have to run for prohibitively long times to probe it. In our earlier studies we have developed computational methods, based on a self-consistent statistical-mechanics approach, to calculate the rare probability of dissociation and hence the binding stability of drugs. We have applied our methods to study the first and second types of drugs [3,4]. It is the aim of the present work to extend our method to determine the binding stability of cross-linked drugs.

A typical cross-linked drug is cisplatin. Despite its simple structure, this drug is one of the most widely used anticancer drugs [5,6]. The effectiveness of this drug against a number of cancers, particularly testicular cancer, has made it the focus of many investigations. An important element in the effectiveness of a drug is the dynamical stability of binding. An analysis of this stability is therefore important in probing the underlying mechanisms and drug-design principles. Although cisplatin has been under intensive investigation, to our knowledge, little work has been done on the binding stability of cisplatin-DNA or other cross-linked drug-DNA systems. In this work the cisplatin binding stability and its effect on host DNA base pairs will be analyzed.

The dynamical binding stability of a cisplatin-DNA system is determined by cross-linked covalent bonds, nonbonded interactions, and solvation effects. Because of the small size of cisplatin, which is composed of only three atoms in the DNA-bound state, the second and third interactions are expected to be much smaller than the first. Therefore the overall binding stability of cisplatin is predominantly determined by cross-linked bonds. As a good approximation one can neglect the second and third interactions in the calculation of the binding stability of cisplatin. The disruption probability of cross-linked bonds can be determined from an analysis of thermal fluctuational motions of these bonds. As the harmonic potential normally used to model these covalent bonds does not permit disruption, a more appropriate potential needs to be introduced. In this work we use the Morse potential to model cross-linked bonds. The parameters of this potential are derived based on data from experiments and quantum-mechanical calculations. The Morse potential has been proposed to empirically model covalent bonds [7,8]. Our recent work on protein disulfide bonds indicated that this potential gives the covalent bond free energy in fair agreement with experiments [9].

In addition to the blocking of the active site of a biomolecule, drugs can perform their task by enhancing the dynamic stability of the target biomolecule. This enhanced stability hinders those biological processes that involve a conformation change or an induced fit. Cisplatin binding results in two changes in a host DNA. One is the formation of the non-Watson-Crick chemical bonds (H bonds and cross-linked bonds) coupled by the disruption of several Watson-Crick interbase H bonds in the binding region. The other is the bending of the entire host DNA oligomer. The effect of these changes on the base-pair stability of host DNA will be analyzed from our calculated base-pair opening probability $P^{\rm op}$ in the drug-bound oligomer.

II. THEORETICAL MODEL

The system studied in the present work is a cisplatin-bound DNA oligomer d(CTCTAG*TG*CTCAC)·d(GTGAGCACTAGAG). G* represents the base cross linked to the drug. The structure of the corresponding drug-free DNA oligomer is generated from fiber coordinates [10]. The nomenclature of the bases in this oligomer is C1T2C3T4A5G6T7G8C9T10C11A12C13 on one strand and G14T15G16A17G18C19A20C21T22A23G24A25G26 on the other. The cross-linked bases are therefore G6 and G8. The coordinates of this complex is from the NMR structure deposited in the Brookhaven protein data bank (PDB file pdb1da4.ent). The internal motions of this complex can be modeled by the Hamitonian

$$
\begin{aligned}
H=&\sum_{\text{atoms}}\frac{P^2}{2m}+\sum_{\text{bonds}}\frac{1}{2}K_r(r-r_{\rm eq})^2+\sum_{\text{angles}}\frac{1}{2}K_\theta(\theta-\theta_{\rm eq})^2\\
&+\sum_{\text{dihedral}}\frac{V_n}{2}[1+\cos(n\phi-\gamma)]\\
&+\sum_{\text{cross link}}[V_0(1-e^{-a(r-r_0')})^2-V_0]\\
&+\sum_{\text{H bonds}}[V_0(1-e^{-a(r-r_0')})^2-V_0]\\
&+\sum_{\text{nonbond}}\left[\frac{A_{ij}}{r_{ij}^{12}}-\frac{B_{ij}}{r_{ij}^{6}}+\frac{q_iq_j}{\epsilon_{ij}r_{ij}}\right]. \qquad (1)
\end{aligned}
$$

Except for the cross-linked and H-bond terms, the above Hamiltonian is the same as that used in molecular-dynamics simulations of biomolecules [11]. The covalent force constants are from refinements with respect to observed vibrational spectra [12,13]. The nonbonded parameters are from AMBER [11], a computer software package for simulation of DNAs, proteins, and organic molecules. We use a Morse potential to empirically describe cross-linked and H-bond interactions. The H-bond potential is between the donor and acceptor heavy atoms [14]. This potential is applied to every donor-acceptor pair whose separation is less than 3.5 Å. To further simplify the calculation we use an extended united atom representation in which no hydrogen atoms are explicitly expressed and the mass and charge are added to their parent atoms.

The cross-linked covalent bond parameters are determined as follows. The potential depth $V_0=14$ kcal/mol is from self-consistent molecular orbital calculations [15]. The parameter $a=3.44$ Å^{-1} is determined by matching the calculated second derivative of the potential at potential minimum to the ir force constant [16]. r_0' is the potential minimum for a strained cross-linked bond. These bonds are strained due to strong cross-bond forces and this is evidenced by the observed variation of bond lengths (from 1.99 Å to 2.09 Å). r_0' can be divided into two terms $r_0'=r_0+\delta r$, where r_0 is the potential minimum for an unstrained bond and δr is the strain induced by cross-bond stress. $r_0=2.038$ Å is from a neutron diffraction study on L-cystine [17,18]. The value of δr is determined by a variety of interactions across the bond. It can be determined empirically by equating the calculated thermal average bond length $\langle r\rangle$, $\langle r\rangle=r_0+\delta r+dr$, with the observed length in x-ray crystal structure. In this way $\delta r=r_x-r_0-dr$, where r_x is the x-ray length and dr is the thermal expansion determined from the zero average force condition $\langle V'\rangle=0$. Notice that effects associated with forces other than the cross-linked bond are shifted into δr. These forces are relatively unchanged over the small displacement leading to the disruption of the cross link. Thus only the cross-linked bond potential needs to be included in the zero force calculation. The calculated δr for the cross-linked bonds is in the range between -0.062 Å and 0.038 Å.

H-bond parameters are given as follows. The potential depth $V_0=3.5$ kcal/mol is the average from those used in our study of H-bond breaking in DNA [19] and also those used in simulation studies [16]. The parameter $a=1.22$ Å^{-1} is from *ab initio* calculations [14]. r_0' is the potential minimum for a strained H bond. H bonds are strained because of strong cross-bond static forces. These forces are responsible for the observed wide variety of bond lengths (from 2.5 Å to 3.5 Å) in biomolecules. r_0' can be divided into two terms $r_0'=r_0+\delta r$, where r_0 is the potential minimum for an unstrained H bond and δr is the strain induced by the cross-bond stress. Based on values used in our DNA calculations [19] and in simulations [20] we tentatively assign $r_0=2.89$ Å. The exact value of r_0 will not affect the calculation as any error will be compensated for by δr, which is adjusted to the observed x-ray crystal bond length. The value of δr is determined by many factors including hydrophobic, Coulomb, and van der Waals interactions. It can, however, be determined empirically by equating the calculated thermal average bond length $\langle r\rangle$ with the observed length in x-ray crystal structure. In this way $\delta r=r_x-r_0-dr$, where r_x is the x-ray length and dr is the thermal expansion determined from zero average force condition $\langle V'\rangle=0$. Notice that effects associated with forces other than H bond are shifted into δr. These forces are relatively unchanged over the small displacement of H-bond disruption. As a result, only the H-bond Morse potential needs to be included in the zero force calculation. The calculated δr for the H bonds is in the range of -0.021 to 0.031 Å.

III. SELF-CONSISTENT CALCULATION OF CROSS-LINKED BOND AND H-BOND DISRUPTION

Our algorithm is similar to the self-consistent phonon theory of anharmonic lattice dynamics developed for the

study of quantum crystals [21] and later applied to H-bond disruption in DNA polymers [19,22]. This approach is based on the Bogoliubov variational theorem, which states that the free energy F of a system can be approximated by the solutions of an effective Hamiltonian [23]. From the Bogoliubov inequality

$$F \leqslant F_0 + \langle H - H_0 \rangle \tag{2}$$

one can self-consistently adjust the parameters of the trial Hamiltonian H_0 with respect to the true Hamiltonian H to find a trial system that minimizes the left-hand-side terms and thus best approaches the true free energy. Here F_0 is the free energy of the trial Hamiltonian system. Both the free energies and Hamiltonians have two components, one static and one dynamic. The dynamic component is the internal thermal fluctuational vibrational energies. For small displacement thermal fluctuational motions up to the point of chemical bond disruption, the hydrophobic forces are relatively unchanged and the changes in dihedral and nonbonded van der Waals and Coulomb interactions are small. Therefore, we can use a normal mode Hamiltonian as the dynamic component for H_0. The effective Hamiltonian H_0 can then be given by

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

where V_{st} is the static part of the Hamiltonian, i.e., the potentials at equilibrium positions. Since the changes in dihedral angles are small, K_Φ can be given by the second derivative of the relevant potential. The nonbonded force constant K_{NB} is from a simple empirical algorithm [24]. The cross-linked bond and H-bond force constant K_{CL} and K_{H} are determined by minimization of the free-energy expansion in Eq. (2), which gives

$$K = (1 - P) \frac{\int_{r_c}^{\infty} dr \frac{d^2 V(r)}{dr^2} e^{-(r - \langle r \rangle)^2 / 2 \langle u^2 \rangle}}{\int_{r_c}^{\infty} dr e^{-(r - \langle r \rangle)^2 / 2 \langle u^2 \rangle}}, \tag{4}$$

where r_c is the inner-bound cutoff determined from $V(r_c) = 2V_0$. Our analysis indicates that the calculations are not sensitive to the exact choice of the cutoff [$V(r_c) = 0$ and $V(r_c) = 4V_0$ give similar results]. The scaling factor $(1 - P)$ is introduced to take into account the disrupted bonds in the statistical ensemble and P is the disruption probability of the bond. $\langle u^2 \rangle$ is the mean-square vibrational amplitude of the bond given by

$$\langle u^2 \rangle = \sum_l s_l^2 \frac{\hbar}{2M\omega_l} \coth \left[\frac{\hbar \omega_l}{2k_B T} \right], \tag{5}$$

where ω_l and l are 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 gives rise to statistical probability distribution functions for finding a particular cross-linked bond with a particular length. From these distribution functions one can determine the probability of finding a cross-linked bond fluctuating beyond a certain breakdown point, i.e., the disruption probability of an individual cross-linked bond. This probability is given by

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

where $L_{\max}$ is the maximum stretch length (breakdown point) of the cross-linked bonds. It is determined as the potential inflection point [where $V'' = 0$, which gives $L_{\max} = r_0' + (1/a)\ln 2$]. Given the bond disruption probability, the free-energy change ΔG associated with the disruption can be deduced from the Boltzmann relation

$$\Delta G = -RT \ln P. \tag{7}$$

The computation procedure is as follows. Starting from an initial set of force constants and given the structure of a drug-DNA complex, the equations of motions derived from the Hamiltonian in Eq. (3) are solved to determine the normal modes. The calculated normal modes are then used to calculate $\langle u^2 \rangle$ and P from Eqs. (5) and (6). These are then used to calculate the parameter r' using the method given in the structure and force fields section and a new set of force constants from Eq. (4). The newly calculated force constants are then used to restart another round of calculation. Such a process continues until every output force constant matches the input force constant, judged by the condition $\Delta K/K < 0.01$. The self-consistently determined P's are then used to calculate ΔG's from Eq. (7). Our computations were carried out on IBM RS6000 servers at Purdue computing center. The diagonalization of the dynamic matrix from Eq. (3) was performed by the LAPACK routine DSYEV.

IV. RESULTS AND DISCUSSIONS

A. Cisplatin-binding constant

There are two cross-linked bonds between cisplatin and DNA. These are the PT-N7 bonds between the drug and base G6 and between the drug and base G8, respectively. The calculated disruption probability P of these bonds is given in Table I along with bond length $\langle r \rangle$ and bond disruption free energy ΔG. We have not found any relevant experimental data to compare with our results; nontheless, we can compare our calculated ΔG with observed values for cross-linked disulfide bonds in proteins. These disulfide bonds are covalent bonds of similar length and energy. Included in Table I are the observed ΔG for disulfide bonds in various

TABLE I. Calculated mean bond length $\langle r \rangle$, disruption probability P, and disruption free energy ΔG of the cross-linked bonds in cisplatin-DNA oligomer d(CTCTAGTGCTCAC)·d(GTGAGCACTAGAG). For comparison the observed ΔG for the disulfide bond in BPTI [24], ribonuclease T1 [25], α-lactalbumin [26], and ribonuclease A [27] is given. The ΔG for the CYS 6–CYS 103 bond in ribonuclease T1 is deduced from the observed value for the two disulfides in the protein. The ΔG for ribonuclease A is the per bond average from the observed value of 19 kcal/mol for all four disulfide bonds.

Method	System	Bond	$\langle r \rangle$ (Å)	P	ΔG (kcal/mol)
MSHA	cisplatin-DNA	PT CPT 1–N7 G 6	2.05	3.450×10^{-5}	5.99
		PT CPT 1–N7 G 8	2.05	3.453×10^{-5}	5.99
EXPT	BPTI	SG CYS 14–SG CYS 38			5
	α-lactalbumin	SG CYS 6–SG CYS 120			3.12
	ribonuclease T1	SG CYS 2–SG CYS 10			3.4
		SG CYS 6–SG CYS 103			3.8–5.9
	ribonuclease A	each disulfide bond			4.75

proteins. Our calculated P is $\sim$5.99 kcal/mol, which is close to the observed values ranging from 3.12 to 5.9 kcal/mol [25–28].

As discussed in the Introduction, nonbonded interactions can be neglected in the calculation of binding stability. Therefore, the dissociation probability P_D of cisplatin can be derived from the P for individual cross-linked bonds:

$$P_D = P_{\text{PT-N7(G6)}} \times P_{\text{PT-N7(G8)}} . \tag{8}$$

The calculated P_D for the cisplatin-DNA complex studied is 1.19×10^{-9}. The equilibrium binding constant in terms of P_D is

$$K_{\text{eq}} = \frac{1-P_D}{P_D} \approx \frac{1}{P_D} . \tag{9}$$

Substitution of the calculated P_D into this equation gives a binding constant $K_{\text{eq}} = 8.4\times10^{8}$. We have not found a reported binding constant for cisplatin. Nontheless, our calculated K_{eq} is of similar order to the observed K_{eq} of the groove binding drug netropsin ($\sim10^{8}$) [29] and that of intercalating drug daunomycin ($\sim10^{7}$) [30].

In our study we used a Morse potential to describe a cross-linked bond. The Morse potential has been suggested as a potential for the covalent bond stretch [7,8] as well as for the H-bond stretch [14]. All the potential parameters are determined by a simple scheme based on AMBER molecular-dynamics simulation force fields and molecular-orbital calculations. The statistical-mechanical algorithm is developed based on Bogoliubov variational theorem. Therefore, our calculation should give a reasonable estimate of the disruption probability of a covalent bond. Our earlier analysis on protein disulfide bonds indicates that the calculated dissociation free energy ΔG is not sensitive to a small variation of the parameters.

B. Effect of cisplatin binding on base-pair thermal stability

The thermal stability of a base pair can be described by the base-pair opening probability P^{op}. This probability can be determined from the individual hydrogen-bond disruption probability. For base pairs without a direct cross link to the drug, their P^{op} is given by [19]

$$P^{\text{op}} = \prod_i P_i , \tag{10}$$

where i is the index of the H bonds in a base pair and P_i is the individual H-bond disruption probability. In cisplatin-bound oligomer the G6 and G8 bases in G6-C21 and G8-C19 base pairs are cross linked to cisplatin. The separation of these base pairs occurs only after the disruption of the respective cross-linked bond. Therefore, the P^{op} of these base pairs is given, respectively, by

$$P^{\text{op}}_{\text{G6-C21}} = P_{\text{PT-N7(G6)}} \prod_i P_i ,$$

$$P^{\text{op}}_{\text{G8-C19}} = P_{\text{PT-N7(G8)}} \prod_i P_i . \tag{11}$$

TABLE II. Opening probability P^{op} of the base pairs in drug-free and cisplatin-bound DNA oligomer d(CTCTAGTGCTCAC)·d(GTGAGCACTAGAG).

Base pair	P^{op} drug-free	P^{op} cisplatin-bound
C1-G26	4.57×10^{-6}	7.21×10^{-6}
T2-A25	3.12×10^{-3}	1.62×10^{-3}
C3-G24	4.63×10^{-6}	5.23×10^{-6}
T4-A23	3.28×10^{-3}	1.67×10^{-3}
A5-T22	3.27×10^{-3}	1.68×10^{-3}
G6-C21	4.64×10^{-6}	2.49×10^{-9}
T7-A20	3.15×10^{-3}	1.73×10^{-3}
G8-C19	4.59×10^{-6}	1.36×10^{-10}
C9-G18	4.56×10^{-6}	6.01×10^{-6}
T10-A17	3.35×10^{-3}	1.55×10^{-3}
C11-G16	4.56×10^{-6}	3.05×10^{-6}
A12-T15	3.10×10^{-3}	1.70×10^{-3}
C13-G14	4.56×10^{-6}	3.85×10^{-6}

TABLE III. Calculated mean bond length $\langle r \rangle$, disruption probability P, and disruption free energy ΔG of the H bonds (H) and cross-linked bonds (C) in the cisplatin binding region of DNA oligomer d(CTCTAGTGCTCAC)·d(GTGAGCACTAGAG). Only those H bonds with length smaller than 3.5 Å are included in this table.

System	Bond	Type	$\langle r \rangle$ (Å)	P	ΔG (kcal/mol)
G6-C21	N1 G 6–N3 C 21	H	2.85	4.16×10^{-2}	1.85
	N2 G 6–O4 T 7	H	2.93	4.19×10^{-2}	1.85
	O6 G 6–N6 A 20	H	2.75	4.14×10^{-2}	1.86
	N7 G 6–PT CPT 1	*C*	2.05	3.45×10^{-5}	5.99
T7-A20	O4 T 7–N2 G 6	H	2.93	4.19×10^{-2}	1.85
	N6 A 20–O6 G 6	H	2.75	4.14×10^{-2}	1.86
G8-C19	N1 G 8–N3 C 19	H	2.89	2.89×10^{-3}	2.66
	N2 G 8–O2 C 19	H	2.94	3.51×10^{-2}	1.95
	O6 G 8–N4 C 19	H	2.88	3.87×10^{-2}	1.89
	N7 G 8–PT CPT 1	*C*	2.05	3.45×10^{-5}	5.99

The calculated $P^{\rm op}$'s for all the base pairs in the cisplatin-bound as well as in drug-free DNA oligomer are given in Table II. We found that, except for the base pairs in the binding region (T7-A20, G6-C21, and G8-C19 base pairs), the $P^{\rm op}$ of other base pairs changes only slightly by cisplatin binding even though the oligomer bends considerably. Our analysis indicates that, although cisplatin binding induces bending of the entire host DNA oligomer, such a deformation does not result in a significant change in the hydrogen-bond configuration in the base pairs outside cisplatin binding region. In addition, the change in the base stacking pattern is also small. Therefore, the thermal stability of these base pairs is not expected to change significantly. There are, however, exceptions. As shown in Table II, the $P^{\rm op}$ of C1-G26, C3-G24, and C9-G18 bass pairs is increased slightly as a result of cisplatin binding. It is not clear whether this is an artifact arising from a particular x-ray crystal structure used in our study.

The stability of base pairs in the cisplatin binding region is significantly affected. As shown in Table III, several interbase H bonds in this region are disrupted. However, there are additional H bonds, which do not exist in drug-free DNA, formed with other bases. These non-Watson-Crick H bonds not only compensate for the broken interbase H bonds but also enhance thermal stability of the base pairs in this region. In the G6-C21 base pair there are two broken interbase H bonds. However, G6 base forms two additional H bonds with neighboring bases apart from the cross-linked bond. These include N2(G6)-H-O4(T7), O6(G6)-H-N6(A20), and N7(G6)-PT(CPT1) bonds. These bonds have to be disrupted to allow for large-amplitude motions needed for the separation of G6-C21 base pair. Hence the $P^{\rm op}$ of the G6-C21 base pair is the product of P of all these bonds. Using the P's in Table III, we found a $P^{\rm op}$ of 2.49×10^{-9} for the G6-C21 base pair, which is compared to 4.64×10^{-6} for the drug-free case. Therefore, the thermal stability G6-C21 base pair is significantly increased by these non-Watson-Crick H bonds as well as the cross-linked bond.

A similar situation occurs in the T7-A20 base pair. As this base pair is sandwiched between two bases cross linked to cisplatin, all the interbase H bonds in this base pair are disrupted due to severe bending of the double helix in the region. However, there are two H bonds formed to the G6 base: one is O4(T7)-H-N2(G6) and the other is N6(A20)-H-O6(G6). To allow for the large-amplitude motions of the T7 and A20 base needed to separate these bases, these two H bonds have to be disrupted. Therefore, one can define the $P^{\rm op}$ of the T7-A20 base pair as the product of the P of these two H bonds. Our calculated $P^{\rm op}$ for this base pair is 1.73×10^{-3}, which is compared to 3.15×10^{-3} in the drug-free oligomer. One can see that despite of the disruption of interbase H bonds, the bases are stablized to a similar extent as in the drug-free configuration.

All the interbase H bonds in the G8-C19 base pair are intact. Moreover, there is no additional H bond formed with other bases apart from the N7(G8)-PT(CPT1) cross-linked bond. Therefore, the $P^{\rm op}$ of this base pair is the product of the P of the cross-linked bond and those of the interbase H bonds. The calculated $P^{\rm op}$ is 1.36×10^{-10}, which is compared to 4.59×10^{-6} for drug-free base pair. Therefore, the thermal stability of this base pair is significantly enhanced by cisplatin binding.

V. CONCLUSION

A microscopic statistical-mechanics method is developed to calculate the equilibrium binding constant of a drug cross linked to DNA. This method is based on a self-consistent harmonic approach. The cross-linked bonds are modeled by a Morse potential with parameters determined from AMBER force fields and molecular obital calculations. Our method can give reasonable estimate of drug binding stability as well as individual chemical bond disruption free energy without parameter fitting. Therefore, it has potential application in computer-aided drug design. Our method can also be used to analyze the effect of drug binding on base-pair stability.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of K.C. Wong Education Foundation Hong Kong, the National Science Foundation of the People's Republic of China, and the Ministry of Personnel of the People's Republic of China. We also thank the Computing Center of Academia Sinica for providing us with their computer facilities.

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