Relation between the change in DNA elasticity on ligand binding and the binding energetics

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The widespread use of tweezers for measurement of ligand-DNA binding parameters is based on the McGhee–von Hippel treatment of the DNA contour and persistence length as a function of concentration. The McGhee–von Hippel approach contains the basic assumption that the binding constant K is independent of the number of already bound ligands. However, the change in elasticity of DNA on binding affects the entropic part of the Gibbs free energy and, hence, the K value in a concentration-dependent manner, making the whole approach inconsistent. In the present work we show that the energetic effect of DNA stiffening on noncovalent binding of small ligands is negligible with respect to the net energy of reaction, whereas the DNA stiffening on binding of large ligands must always be considered in each particular case.

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I. INTRODUCTION

The past decade has been marked by the use of optical and magnetic tweezers in probing the stretching behavior of biological macromolecules such as DNA [1,2]. The method is based on measuring the force versus extension curve, $F(x)$, which allows insight into changes of the mechanical properties of DNA under the influence of various factors in terms of the persistence length a_P and the contour length L_C. Tweezers have been effectively applied for measurement of the DNA binding parameters for various biologically important molecules, such as the equilibrium binding constant K, the mode of binding, and the number of excluded sites [2–5]. The approach used in the majority of published works is based on employing the standard McGhee–von Hippel equation for numerical analysis of the dependence of the DNA contour length on the concentration of the ligand, derived from the $F(x)$ data. A fundamental assumption behind the McGhee–von Hippel approach is that the binding constant K is independent of the number of already bound ligands (e.g., see Ref. [5]), i.e., it is equal for all binding densities r. However, stretching experiments show that some ligands strongly affect the elasticity of DNA via changing a_P as a function of the ligand concentration C_0, in highly nonlinear fashion. In particular, for some intercalating ligands the $a_P(C_0)$ curve initially increases, and then, at a certain critical ligand concentration, decays abruptly (e.g., see Ref. [6]). Such behavior affects the entropy of the DNA molecule bound to ligands in a concentration-dependent manner. It follows that the ligand-DNA binding constant must depend on the binding density r, suggesting that the use of the McGhee–von Hippel equation with the $F(x)$ data appears to be incorrect in the general case.

With the aim of understanding the degree of "incorrectness" of the McGhee–von Hippel equation, in the present work an estimation of the Gibbs free energy change due to changes in the persistence and contour lengths is performed for typical DNA-binding ligands.

II. ESTIMATION OF THE ENTROPIC COST OF DNA STIFFENING ON LIGAND BINDING

A. Binding of small ligands

In a first approximation let us model the distribution of the end-to-end distances h of the polymer chain with a standard Gauss function:

$$W(h) = \left(\frac{3}{2\pi N\ell^2}\right)^{3/2} \exp\left(-\frac{3h^2}{2N\ell^2}\right), \tag{1}$$

where ℓ is the length of a chain segment and N is the total number of segments. Assuming further that the behavior of the DNA molecule follows the wormlike chain model, in the extreme limit $N\to\infty$ the mean square chain length $\langle h^2\rangle$ can traditionally be expressed as

$$\langle h^2\rangle = N\ell^2 = N_A A^2 = 2a_P L_C, \tag{2}$$

where A and N_A are the length of the Kuhn statistical segment and their total number, respectively.

The substitution of Eq. (2) into Eq. (1) yields

$$W(h) = \left(\frac{3}{4\pi a_P L_C}\right)^{3/2} \exp\left(-\frac{3h^2}{4a_P L_C}\right). \tag{3}$$

The molar entropy of the chain with average length $h = \sqrt{\langle h^2\rangle}$ can be written as

$$S = R\ln W(h) = \frac{3}{2}R\left(\ln\frac{3}{4\pi a_P L_C} - 1\right). \tag{4}$$

Within a group of small molecules, which have negligible dimensions compared with the DNA receptor, and noncovalently bind with DNA by intercalation or major (minor) groove modes, the intercalation results in most significant changes in either a_P or L_C [1]. Although the DNA stretching data under conditions of ligand intercalation are so far scarce, daunomycin is considered to be a molecule affecting the elasticity of DNA to a large extent as compared to other intercalators [1,6]. Taking

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the values from Ref. [6] measured for daunomycin binding with λ-DNA, under the critical ligand concentration a_P reaches a maximum at $a_P = 280$ nm and $L_C = 18$ μm. The same parameters for bare λ-DNA were measured as $a_{P0} = 50$ nm and $L_{C0} = 16.5$ μm. Hence the entropy change, associated with the change in λ-DNA elasticity can be calculated from Eq. (4) as

$$\Delta S_n = \frac{3}{2} R \ln \frac{a_{P0} L_{C0}}{a_P L_C} = -22.6 \text{ J/mol K}. \quad (5)$$

The value (5) is associated with the molar entropy of binding of a certain number of ligands, n. Hence, the value (5) must be divided by n. Knowing that each intercalated daunomycin molecule increases the contour length of the complex by 0.31 nm [7], it is possible to estimate the number n of bound molecules as $n = (L_C - L_{C0})/(0.31 \text{ nm}) \approx 4839$. Now, the molar Gibbs free energy change associated with the change in DNA elasticity on ligand binding related to a single binding event ($n = 1$) at $T = 300$ K takes the value $\Delta G_1 = -T\Delta S_1 = 1.4$ J/mol. The energetics of noncovalent binding of small molecules to DNA typically ranges between 20 000 and 80 000 J/mol [8], which is four orders of magnitude higher than the ΔG_1 calculated above. Although the ΔG_1 value was estimated employing a rough approximation of the statistical behavior of the DNA molecule by means of a Gauss distribution function, the negligible magnitude of ΔG_1 suggests that a more accurate analysis is not required.

Taken as a whole, the results obtained indicate that the overall DNA elasticity change during binding of small molecules does not affect the equilibrium binding constant to any notable degree, which gives an answer to the principal question of this work. It also means that the application of the McGhee–von Hippel equation to DNA stretching experiments appears to be valid.

B. Binding of large ligands

Another question concerns the ΔG_1 value if a large ligand, e.g., a protein, which has dimensions comparable with the receptor itself, binds with DNA. Assuming that the ligand binding is the strongest possible, converting the wormlike DNA chain with parameters a_{P0} and L_{C0} into a stiff rod with the largest persistence length, one can estimate the maximum possible change in entropy and Gibbs free energy from Eq. (4) as $\Delta G_1 = -TS = 34.2$ kJ/mol, which is comparable with the total energy of daunomycin binding with DNA [7,8] and some proteins [9] in aqueous solutions. Although seeming relatively large, it is necessary to note that such stiffening of DNA is possible only if the ligand dimensions are comparable with those of DNA itself. The binding reactions in aqueous solution are known to be characterized by compensation of solute-solute and solute-solvent interactions [9,10], resulting in relatively weak dependence of the net free energy of binding on the molecular weight of the ligand. This means that the energetics of binding of large ligands with DNA may either be much higher than or commensurate with ΔG_1, suggesting that the energetic effect of DNA stiffening on noncovalent binding of large ligands must always be considered in each particular case. This information is important for understanding the relative contribution of various physical factors to the net Gibbs free energy change during the complexation process.

C. Implications regarding the mechanism of molecular recognition of DNA on ligand binding

During the past decade a new concept of the molecular recognition of nucleic acids by various ligands has emerged in molecular biology as a result of an accumulation of theoretical and experimental data on the changes of statistical-mechanical properties of nucleic acids on ligand binding (see Ref. [11] and references therein). Briefly, according to this concept, the recognition of DNA or RNA may occur as a result of conformational and/or flexibility changes of nucleic acids right at the site of binding and in its vicinity. The conformational changes are often associated with local bending or other distortions of DNA [12], whereas the flexibility changes are sometimes related to changes in the degrees of freedom and rigidity of various components of nucleotides at the binding site [13,14], often inferred from molecular dynamics simulations. The changes in the rigidity of nucleotides, when accumulated over the whole DNA molecule, will inevitably contribute to alteration of the overall elasticity of DNA expressed in terms of the persistence length. The results of the present work, however, show that, at least for small ligands binding with DNA, the contribution of DNA stiffening to the energetics of binding on the level of one bound ligand appears to be negligible. This means that interpretations of molecular recognition phenomenon in terms of local flexibility changes should be treated with caution.

III. CONCLUSION

In the present work we discuss the hidden assumption behind the application of the methods of manipulation of DNA at the single-molecule level (such as DNA stretching experiments using magnetic tweezers) in order to extract ligand-DNA binding parameters. The McGhee–von Hippel approach used conventionally for their computation does not account for the change in elastic properties of DNA on ligand binding, thereby potentially containing a hidden error in the determination of the binding parameters. Using the classical model of a wormlike chain compared with published experimental data on the binding of typical small molecules with DNA, it was shown that the effect of DNA stiffening on ligand binding per one bound ligand is negligible as compared to the typical Gibbs free energy change in the complexation reaction. Hence, no significant error exists in application of the McGhee–von Hippel approach to stretching experiments. This result has also led to the view that the concept of the effect of local flexibility changes on ligand binding with DNA as a potential mechanism for DNA recognition must probably be reconsidered in view of the fact, described in the present work, that the overall elastic response of DNA per one bound ligand appears to be small.

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