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Salt-dependent binding of iron(II) mixed-ligand complexes containing 1,10-phenanthroline and dipyrido[3,2-*a*:2′,3′-*c*]phenazine to calf thymus DNA

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Abstract

The salt-dependent binding of racemic iron(II) mixed-ligand complex containing 1,10-phenanthroline (phen) and dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz), $[Fe(phen)_2(dppz)]^{2+}$ to calf thymus DNA (ct-DNA) has been characterized by UV–VIS spectrophotometric titration. The equilibrium binding constant (K_b) of the iron(II) complex to ct-DNA decreases with the salt concentration in the solution. The slope, $SK=(\delta\log K_b/\delta\log [Na^{2+}])$ has been found to be 0.49, suggesting that, in addition to intercalation, considerable electrostatic interaction is also involved in the ct-DNA binding of $[Fe(phen)_2(dppz)]^{2+}$. The calculation of non-electrostatic binding constant (K_t^o) based on polyelectrolyte theory has revealed that the non-electrostatic contribution to the total binding constant (K_b) increases significantly with the increase in $[Na^+]$ and reaches 36% at 0.1 M NaCl. On the other hand, the contribution of the non-electrostatic binding free energy (ΔG_t^o) to the total binding free energy change (ΔG^o) is considerably large, i.e. 87% at $[Na^+]=0.1$ M, suggesting that the stabilization of the DNA binding is mostly due to the contribution of non-electrostatic process. Moreover, the effect of specific ligand substitutions on ΔG^o has been rigorously evaluated using the quantity $\Delta\Delta G_t^o$, i.e. the difference in ΔG_t^o relative to that of the parent iron(II) complex, $[Fe(phen)_3]^{2+}$, indicating that each substitution of phen by dip and dppz contributes 7.5 and 17.5 kJ mol^{-1}, respectively to more favorable ct-DNA binding.

Keywords: DNA-binding; dppz; 1,10-phenanthroline; Iron(II); Salt-dependence

1. Introduction

The rational design of new DNA binders requires a detailed understanding of the DNA binding properties of the existing compounds. In addition to high resolution structural data of metal complex–DNA complexes, studies of the energetics of metal complex–DNA association are essential for a thorough understanding of the principle that governs the binding of metal complex to DNA [1]. Metal complex–DNA associations are induced by weak non-covalent forces, e.g. intercalation (π−π stacking) of ligand between DNA base pairs, van der Waals contacts, hydrogen bonds, hydrophobic interactions and electrostatic interactions [2,3]. These non-covalent forces depend on temperature, pH, pressure, salt concentration and many other environmental variables [4].

The effect of salt concentration on the binding free energy (ΔG) of protein–DNA complexes has been long known [4–7]. In general, salt effect arises from the reorganization of the ionic cloud around the DNA and binding ligand. Theoretically, the binding affinity of protein to DNA decreases when the salt concentration increases because ion-pair formation between protein and DNA is less favorable in the solution that contains a high concentration of salt [6]. Although the salt-dependence study of protein–DNA complex is quite a lot, in contrast the number of similar studies dealing with metal complex–DNA interaction is limited [8–11]. To date, studies on the DNA binding of metal complexes have been dominated by establishing the binding mode and the possible structure of their DNA complexes. In fact, by determining the salt effect of equilibrium binding constant (K_b) of the DNA binding of metal complexes

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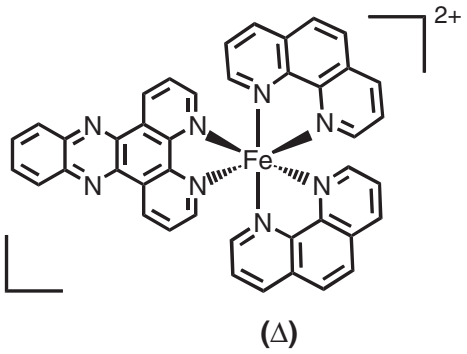

Fig. 1. Chemical structure of iron(II) mixed ligand complex containing phen and dppz. Only Δ-enantiomer is viewed.

and analyzing the results by polyelectrolyte theory [10–12], we may calculate the non-electrostatic contribution to the K_b and separate the binding free energy change (ΔG) into its electrostatic and non-electrostatic contributions. Furthermore, a comparison of non-electrostatic contributions of binding constant and free energy change allows us to evaluate the effect of specific constituents on net binding constant and free energy change without any interference from electrostatic contributions. Thus, this kind of comparison is purely based on the differences in substituents attached to the ligand of metal complexes [1,11].

In the present study, the salt-dependent binding of racemic iron(II) mixed-ligand complex containing 1,10-phenanthroline (phen) and dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz), $[Fe(phen)_2(dppz)]^{2+}$ (Fig. 1) to calf thymus DNA (ct-DNA) has been characterized by determining its DNA-binding constants at various concentrations of salt (NaCl) using spectrophotometric titration. The results obtained were then evaluated using polyelectrolyte theory to differentiate the electrostatic and non-electrostatic (intercalation) contributions of binding constant and free energy change. The data were further treated and compared with previous results to determine the effect of charge and specific substituents of the ligand on the stabilization of DNA binding events.

2. Experimental section

2.1. Chemicals

Tris(phen)iron(II) perchlorate $[Fe(phen)_3](ClO_4)_2$ was synthesized according to the literature procedure [13] and confirmed by elemental analysis and UV–Vis absorption spectroscopy. Iron(II) mixed-ligand complex, i.e. $[Fe(phen)_2(dppz)]I_2$ was prepared from tris-(phen)iron(II) perchlorate by ligand substitution reaction as reported previously [14] and the product was purified by semi-preparative HPLC followed by subsequent extraction and evaporation using a rotary evaporator. The concentration of $[Fe(phen)_2(dppz)]I_2$ for DNA-binding studies was determined spectrophotometrically using the molar absorptivity (ε) at its intraligand (IL) band, i.e. $\varepsilon_{376\ nm} = 13{,}356\ M^{-1}\ cm^{-1}$. Tris(2-amino-2-hydroxymethyl-1,3-propandiol) was purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan) and sodium chloride (NaCl) for adjusting ionic strength was from Wako Pure Chemical Industries (Japan). All chemicals and solvents were of analytical grade or higher and were used without further purification.

2.2. DNA sample

Calf thymus DNA (ct-DNA) was obtained from Sigma Chemicals Co. (USA) and used as received. The solid sodium salt of DNA samples was stored below 4 °C. A stock solution of ct-DNA was prepared and stored in 5 mM Tris–HCl buffer at pH 7.2. The concentration of ct-DNA solutions was determined spectrophotometrically using the reported molar absorptivity of $\varepsilon_{259\ nm} = 1.31 \times 10^4\ M^{-1}\ cm^{-1}$ [15] and the results were expressed in terms of base-pair equivalents per cubic decimeter. A solution of ct-DNA (ca. 10^{-5} M in base pair, bp) in Tris–HCl buffer gave a ratio of UV absorbance at 260 and 280 nm, $A_{260}/A_{280} \geq 1.9$, indicating that the ct-DNA was sufficiently free from protein.

2.3. Measurements of salt dependence in DNA binding

The equilibrium binding constant (K_b) of iron(II) complex to ct-DNA was determined by spectrophotometric titration over the concentration range 0.005 to 0.100 M NaCl. A fixed amount of iron(II) complex in 5 mM Tris–HCl buffer at pH 7.2 and various concentrations of NaCl was titrated with increasing amounts of ct-DNA stock solutions (10^{-6}–10^{-4} M) and the hypochromicity in the IL (360 and 376 nm) and MLCT (508 nm) bands due to metal complex–DNA interaction was monitored by a Jasco V-550 UV–VIS spectrophotometer equipped with a Jasco ETC-505T cell-temperature controller and a cell magnetic stirrer. Cell compartments were thermostated at 25±0.1 °C. The K_b values at various NaCl concentrations were calculated on the basis of the following equation [16–21]:

$$[DNA]_{total}/(|\varepsilon_A - \varepsilon_F|) = [DNA]_{total}/(|\varepsilon_B - \varepsilon_F|) + 1/\{K_b(|\varepsilon_B - \varepsilon_F|)\} \quad (1)$$

where ε_A, ε_F and ε_B correspond to $A_{obsd}/[complex]$, the molar absorptivity for the free iron(II) complex, and the molar absorptivity of the iron(II) complex in the fully bound form, respectively. In plots of $[DNA]/(|\varepsilon_A - \varepsilon_F|)$ versus [DNA], K_b is given by the ratio of the slope to the intercept. The salt concentration dependence of K_b for the DNA binding of the iron(II) complexes was then evaluated by plotting log K_b versus log $[Na^+]$ to obtain SK value, which is essential for polyelectrolyte analysis. Each measured point was the average value of at least three separate measurements with a relative standard deviation (RSD) normally less than 15%.

3. Results and discussion

3.1. Salt-dependence of binding constants

Fig. 2 shows the typical plots of [ct-DNA] versus $[ct\text{-}DNA]/(\varepsilon_A - \varepsilon_F)$ for the determination of binding constant (K_b) of $[Fe(phen)_2(dppz)]^{2+}$ to ct-DNA at various concentrations

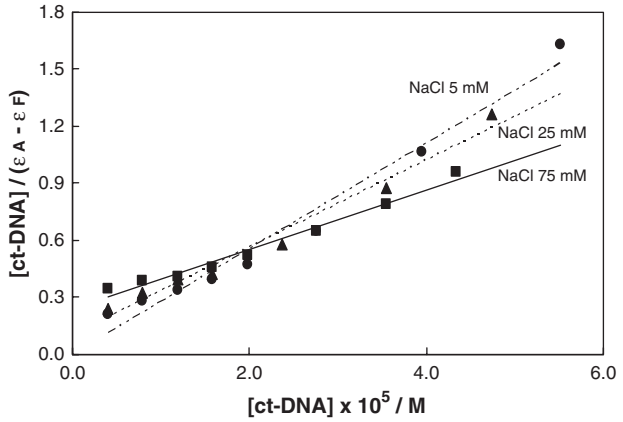

Fig. 2. Typical plots of $[\text{ct-DNA}]/(\varepsilon_A-\varepsilon_F)$ versus [ct-DNA] for the spectrophotometric titration of $[Fe(phen)_2(dppz)]^{2+}$ with increasing amount of [ct-DNA] in 5 mM Tris–HCl buffer pH 7.2 at 25 °C and various concentrations of NaCl.

of NaCl based on Eq. (1) and the detailed results are collected in Table 1. It should be noted that the calculated K_b value is the average value for Δ- and Λ-enantiomer of $[Fe(phen)_2(dppz)]^{2+}$, i.e. the K_b value for the racemic solution of $[Fe(phen)_2(dppz)]^{2+}$. The separation of the enantiomers or synthesis of the chiral complexes is not possible because the iron(II) complexes of phen and dppz are labile towards racemization in the solution [22,23]. In our separate study [24], a circular dichroism (CD) analysis of the dialysate solution comprised of the iron(II) complex and ct-DNA has revealed that the Δ-enantiomer is preferably bound to ct-DNA with the degree of selectivity of 2.13, which is defined as the molar ratio of Δ- to Λ-enantiomer in the solution, at a concentration of 50 mM NaCl. This finding is also consistent with the fact that the K_b value for the binding of Δ-$[Ru(phen)_2(dppz)]^{2+}$ to ct-DNA (3.2×10^5 M^{-1} per nucleotide) is slightly larger than that for Λ-$[Ru(phen)_2(dppz)]^{2+}$ (1.7×10^5 M^{-1} per nucleotide) [9]. The binding constants reported here were obtained over the concentration range 0.005 to 0.100 M NaCl in order to apply polyelectrolyte theory to the calculation of the non-electrostatic binding constants and separate the binding free energy change into its electrostatic and non-electrostatic contributions. The salt concentrations of 0.001–0.100 M were selected in this study because the polyelectrolyte theories which will be used for subsequent analysis are based on limiting laws that are strictly applicable to salt concentrations of lower than 0.100 M. It has been reported that the dependence of K_b on salt concentration becomes non-linear at higher concentrations of salt [9]. The plot of log $[Na^+]$ against log K_b for the binding of $[Fe(phen)_2(dppz)]^{2+}$ to ct-DNA is given in Fig. 3.

Table 1
Equilibrium binding constant (K_b) for the binding of $[Fe(phen)_2(dppz)]^{2+}$ to ct-DNA in 5 mM Tris–HCl buffer (pH 7.2) at 25 °C and various concentrations of NaCl

[NaCl] (M)	K_b (M^{-1} bp)	K_t^o (M^{-1}bp)	K_t^o/K_b (%)
0.005	3.54×10^5	2.83×10^4	8.01
0.025	1.88×10^5	3.37×10^4	18.0
0.050	1.32×10^5	3.37×10^4	25.5
0.075	0.95×10^5	2.99×10^4	31.4
0.100	0.79×10^5	2.88×10^4	36.5
Average = 3.09±0.26 (10^4)			

K_b is the equilibrium binding constant per DNA base pair as calculated by Eq. (1). All figures are average values of at least three separate measurements with RSD of less than 15%. K_t^o is the equilibrium binding constant contribution from non-electrostatic interaction to the observed (total) K_b and was calculated based on Eq. (2). The value of Z used in Eq. (2) was obtained from Fig. 3, i.e.: 0.560 (slope = $Z\Psi$ = 0.4932, where Ψ = 0.88 for B-form of double-stranded DNA). All values are determined at 25 °C.

It is clear from the plots that the binding constant decreases with increasing salt concentration. This is due to the stoichiometric amount of counterion release that follows the binding of charged ligand, i.e. iron(II) complex [12], suggesting that electrostatic interaction is involved in the DNA-binding event. Using the slope of linear fitting of Fig. 3, we may calculate non-electrostatic binding constant (K_t^o) at various concentrations of NaCl ($[M^+]$) according to the following polyelectrolyte theory [12]:

$$\ln K_b = \ln K_t^o + Z\xi^{-1}\{\ln(\gamma_\pm\delta)\} + Z\psi(\ln[M^+]) \quad (2)$$

where $Z\psi$ is estimated from the slope of the regression line in Fig. 3. Z is partial charge on the binding ligand involved in the DNA interaction as predicted by polyelectrolyte theory, ψ is the fraction of counterions associated with each DNA phosphate (ψ = 0.88 for double-stranded B-form DNA), $\gamma_\pm$ is the mean activity coefficient at cation concentration M^+, and the remaining terms are constants for double stranded DNA in B-form, i.e. ξ = 4.2 and δ = 0.56. Results of the calculations are summarized in Table 1 along with the percentage of K_t^o contribution to the total binding constants (K_b) at various concentrations of M^+, i.e. Na^+. These K_t^o can be taken as a measure how large the non-electrostatic forces stabilize the ligand–DNA interaction. In contrast to the K_b values which are

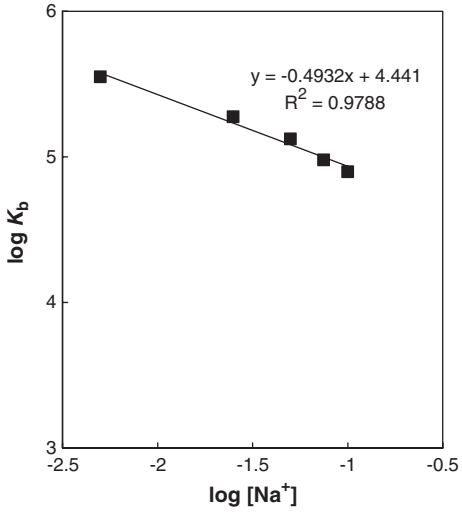

Fig. 3. Salt dependence of binding constant (K_b) for the binding of $[Fe(phen)_2(dppz)]^{2+}$ to ct-DNA. The slope of this plot corresponds to the SK quantity presented in Table 2.

salt-dependent, the magnitude of K_t^o is constant throughout the concentration of NaCl employed with the average value of 3.088×10^4 M^{-1} bp (RSD=8.4%). This is consistent with the expectation for the salt-independency of this parameter. Although the values of K_t^o are constant throughout the concentrations of salt, the percentage of K_t^o contributions to the K_b increases significantly and reach a maximum of 36.5% at $[Na^+]=0.1$ M. It can be expected that at higher concentrations of salt, e.g. at physiological condition ($Na^+\approx0.200$ M), the non-electrostatic forces would play a major role in the DNA binding of the iron(II) complex. The value of 25.5% for the non-electrostatic binding constant found in the DNA binding of $[Fe(phen)_2(dppz)]^{2+}$ at NaCl=0.05 M (see Table 2 for details) is considered to be high even when it is compared with those of other proven intercalators such as ethidium (12.3%) or daunomycin (8.6%) at the same ionic strength [8].

Further analysis is also possible to dissect the binding free energy change (ΔG^o) for the binding of $[Fe(phen)_2(dppz)]^{2+}$ to ct-DNA into its electrostatic (ΔG_{pe}^o) and non-electrostatic (ΔG_t^o) contributions at a given concentration of NaCl [1]. Table 2 summarizes the results of energetics calculation for the binding of $[Fe(phen)_2(dppz)]^{2+}$ to ct-DNA in 0.050 M NaCl along with those of other iron(II) complexes as well as proven organic intercalators [8,11] for the purpose of comparison. The total binding free energy changes listed in Table 2 were calculated based on the standard Gibbs relation:

$$\Delta G^o = -RT\ln K_b \tag{3}$$

where R is the gas constant and T is the temperature in Kelvin. The salt dependence of the binding constant (Fig. 3) is defined as the slope, SK:

$$SK = \delta\log K_b/\delta\log[Na^+] = -Z\psi. \tag{4}$$

The SK value can then be used to calculate the polyelectrolyte contribution of the free energy change (ΔG_{pe}^o) to the overall free energy change (ΔG^o) at a given NaCl concentration by the relation [1,11,25]:

$$\Delta G_{pe}^o = (SK)RT\ln[Na^+]. \tag{5}$$

The difference between the Gibbs free energy change (ΔG^o) and ΔG_{pe}^o is defined as the non-electrostatic free energy change (ΔG_t^o):

$$\Delta G_t^o = \Delta G^o - \Delta G_{pe}^o. \tag{6}$$

The quantity ΔG_t^o corresponds to the portion of the binding free energy change which is independent of salt concentrations and contains a minimal contribution from polyelectrolyte effects such as coupled ion release. Although the iron(II) complexes used formally have +2 charge, the local or partial charges involved in the interaction are not exactly the same due to the difference in structure and bulkiness of the iron(II) complexes. Therefore, before the effect of ligand substitution on the stabilization of DNA binding is compared, it is essential to first calculate K_t^o and dissect the ΔG^o values into ΔG_{pe}^o and ΔG_t^o contributions and then to use these two parameters to evaluate the effect of ligand charge and ligand substituents on the total binding free energy change, respectively.

3.2. Contribution of the ligand charge to the DNA binding

As shown in Eq. (4), SK is equal to $-Z\psi$, the slope of linear regression line of Fig. 3 where Z refers to the partial charge of the ligand involved in the DNA binding. Theoretically, since the iron(II) complexes used in this study have formal charge of +2, the value of SK should be $2\times0.88=1.76$ and that of ethidium and daunomycin would be $1\times0.88=0.88$ because these two organic intercalators are known to have +1 charge at neutral pH. The data shown in Table 2 consistently indicate that the charge (Z) involved in the DNA binding of either ethidium or daunomycin is in good agreement with theoretical expectation, i.e. approximately +1 (Z=SK/0.88). For iron(II) complexes, however, the Z values are much lower than those of theoretical prediction, especially for $[Fe(phen)_2(dppz)]^{2+}$. Although such lower values of SK and thus the lower value of Z are quite common for metal complexes containing phen and its derivatives [5,8], the reasons for these observations are always associated with the coupled anion release from the ligand, the counter cation release from double stranded DNA due to the increase in charge spacing resulted from the binding of cationic intercalator ligand and/or the changes in the hydration of ligand

Table 2
Thermodynamic parameters for the binding of iron(II) complexes and other ligands to ct-DNA[a]

Binding ligands	K_b (10^3 M^{-1} bp)	ΔG^o	SK	ΔG_{pe}^o	$K_t^o/10^3$ (%K_t^o/K_b) M^{-1} bp)	ΔG_t^o (%$\Delta G_t^o/\Delta G^o$)
$[Fe(phen)_3]^{2+}$ [b]	0.753	−16.3	0.97	−7.9	0.041 (5.4)	−8.40 (51.5)
$[Fe(phen)_2(dip)]^{2+}$ [b]	26.6	−25.1	1.1	−9.2	0.90 (3.4)	−15.9 (63.3)
$[Fe(phen)(dip)_2]^{2+}$ [b]	19.3	−24.7	0.89	−7.1	1.33 (6.9)	−17.6 (71.3)
$[Fe(phen)_2(dppz)]^{2+}$	132	−29.2	0.49	−3.6	33.7 (25.5)	−25.6 (87.5)
Ethidium[c]	494	−32.2	0.75	−5.0	61 (12.3)	−27.2 (84.5)
Daunomycin[c]	4900	−37.7	0.84	−5.9	422 (8.6)	−31.8 (84.4)

[a]In 50 mM NaCl, 5 mM Tris–HCl buffer (pH=7.2) at 25 °C. ΔG^o (kJ mol^{-1}) is the binding free energy change calculated by Eq. (3). The parameter SK ($Z\Psi$) is the absolute value of the slope obtained from the plots of Fig. 3. ΔG_{pe}^o and ΔG_t^o (kJ mol^{-1}) are the electrostatic and the non-electrostatic contributions, respectively. The electrostatic contribution was calculated using Eq. (5) and evaluated at $[Na^+]=50$ mM. The non-electrostatic portion of the free energy change was calculated by the difference. [b]Taken from our previous study [11]. [c]Taken from Ref. [8]; all values refer to the solution of 50 mM NaCl, 5 mM Tris buffer (pH=7.1) at 20 °C. The values in parentheses for K_t^o and ΔG_t^o correspond to the percentage of the non-electrostatic contributions to the overall binding constants (K_b) and free energy changes (ΔG^o), respectively.

Table 3
Energetics cost of ligand substitution in iron(II) complexes of phen and its derivatives

Iron(II) complexes	Ligand alteration	$\Delta\Delta G^{o}_{pe}$ (kJ/mol) [a]	$\Delta\Delta G^{o}_{t}$ (kJ/mol) [a]
$[Fe(phen)_3]^{2+}$	Reference compound	0.00	0.00
$[Fe(phen)_2(dip)]^{2+}$	One phen → one dip	−1.30	−7.50
$[Fe(phen)(dip)_2]^{2+}$	Two phens → two dips	+0.80	−9.20
$[Fe(phen)_2(dppz)]^{2+}$	One phen → one dppz	+4.30	−17.2

[a] The quantity of $\Delta\Delta G^{O}_{pe}$ and $\Delta\Delta G^{O}_{t}$ refer respectively to the difference in polyelectrolyte and non-electrostatic binding free energy changes relative to those of the reference compound, $[Fe(phen)_3]^{2+}$. The negative sign of $\Delta\Delta G^{o}$ indicates a *more favorable* DNA binding, while the positive one corresponds to a *less favorable* DNA binding. Abbreviations: phen=1,10-phenanthroline, dip=4,7-diphenyl-1,10-phenanthroline and dppz=dipyrido[3,2-*a*:2′,3′-*c*] phenazine.

or DNA upon binding each other [26,27]. No reports, to the best of our knowledge, have taken into account the differences in structural features between ethidium or daunomycin and metal complexes as well as in the location/position of positive charge on the binding ligand as factors responsible for such lower SK values. For the metal complexes like iron(II) complexes of phen and its derivatives, the positive charge of the complex is normally located at the central metal ion which is positionally far away from binding site, especially for the metal complexes with the bulkier ligand such as derivatives of phen, and is not involved directly in the DNA-binding event. Moreover, the positive charge at the central metal ion is distributed/transferred to the three ligands attached to it at different directions and in fact only one ligand, i.e. dppz is involved in a direct contact with DNA base pairs. Thus, the charge of octahedral metal complexes with bulkier ligand like $[Fe(phen)_2(dppz)]^{2+}$ only partially affects the DNA binding. Therefore, in addition to conventional reasons such as coupled anion release from the ligand and the hydration changes of the ligand or DNA upon binding, it is easy to understand that the SK values for the DNA binding of metal complexes are usually much lower than those predicted by theoretical values. In the case of either ethidium or daunomycin, the situation is different because their structures are more or less two dimensional planar and the positive charge in these molecules is involved directly in the ligand–DNA interaction, therefore their SK values are in good agreement with those of the theoretical predictions.

Since we have dissected the binding free energy change into electrostatic and non-electrostatic portions, the role of ligand charge in stabilizing the DNA binding may be also evaluated by comparing the quantity of ΔG^{o}_{pe} or more precisely $\Delta\Delta G^{o}_{pe}$ (Table 3), i.e. the difference in the electrostatic portion of the binding free energy change relative to the parent iron(II) complexes. All the iron(II) complexes listed in Table 2 are cationic complexes with the formal charge of +2. It has been revealed from Tables 2 and 3 that the electrostatic contribution of iron(II) complexes with the charge of +2 varies considerably as reflected in their ΔG^{o}_{pe} values, e.g.: from −3.6 to −9.2 kJ mol^{-1} in 0.050 M NaCl. The $\Delta\Delta G^{o}_{pe}$ for $[Fe(phen)_2(dip)]^{2+}$ is negative sign relative to parent complex, $[Fe(phen)_3]^{2+}$, indicating that $[Fe(phen)_2(dip)]^{2+}$ is electrostatically more favorable to the binding than its parent complex. In contrast, both $[Fe(phen)(dip)_2]^{2+}$ and $[Fe(phen)_2(dppz)]^{2+}$ have a positive sign of $\Delta\Delta G^{o}_{pe}$, suggesting that substitution of two phen for two dip or one phen for one dppz results in 0.8 and 4.3 kJ mol^{-1} loss of binding free energy, respectively to electrostatically yield less favorable of the DNA binding. However, it will be shown later that these electrostatic binding free energy losses are compensated by the large quantities of non-electrostatic binding free energy contributions. These tendencies are consistent with the binding mode of the first two complexes which is dominated by electrostatic interactions, while the other two complexes are known to be intercalated or partially intercalated into the base pair of DNA [9,14,24]. Therefore, it should be noted in the case of metal complexes that although the formal charge of metal complexes involved in the DNA binding is exactly the same, it affects differently the stabilization of the DNA binding depending on the structure and binding mode of the metal complexes.

3.3. Effect of ligand substitution on DNA binding

The free energy contribution of specific substituents of ligands to DNA binding can be evaluated using the quantity $\Delta\Delta G^{o}_{t}$, i.e. the difference in the non-electrostatic portion of the binding free energy change relative to the parent iron(II) complex, $[Fe(phen)_3]^{2+}$. The calculated $\Delta\Delta G^{o}_{t}$ values for $[Fe(phen)_2(dppz)]^{2+}$ and other iron(II) complexes are collected in Table 3 and shown graphically in Fig. 4. The rationalization for this approach has been discussed in detail by Chaires et al. [1]. Here the free energy contributions from reactant conformational changes as well as from the loss of rotational freedom due to DNA binding are likely the same for all the complexes studied because their structural features are quite similar, e.g. octahedral geometry. In such an approach, therefore, it can be assumed that the $\Delta\Delta G^{o}_{t}$ value of a particular complex corresponds primarily to the differences in its molecular interactions within the binding site, which are brought about by ligand substitution.

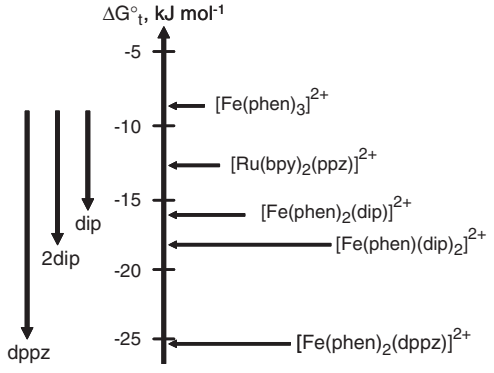

Fig. 4. Free energy diagram showing the effect of ligand substitution on the non-electrostatic portion of the binding free energy change. The magnitude of ΔG^{o}_{t} (kJ mol^{-1}) is shown on the axis. The arrows on the left side indicate the magnitude of the free energy difference ($\Delta\Delta G^{o}_{t}$) resulting from a specific ligand substitution.

Indeed, a negative value of $\Delta\Delta G_t^o$ is obtained in all cases, indicating that each ligand substitution gives rise to more favorable binding free energy relative to $[Fe(phen)_3]^{2+}$ in term of non-electrostatic interaction.

Probably the most striking result emerging from this study is a quantitative evaluation of the contribution of phenyl and phenazine moieties to the binding free energy. While the role of planar heterocyclic moieties in enhancing the DNA binding affinity has been well discussed [2,3], the quantitative evaluation involving the energetic contribution of such moieties is hardly reported. A comparison of the $\Delta\Delta G_t^o$ values for various iron(II) complexes in Table 3 gives an opportunity to quantitatively evaluate their contributions. Examining $\Delta\Delta G_t^o$ values of $[Fe(phen)_3]^{2+}$ and $[Fe(phen)_2(dip)]^{2+}$ reveals that the energetic stabilization of the binding is 7.50 kJ mol^{-1} for the substitution of one phen for dip, which is equal to the addition of 2 phenyl rings to the iron(II) complex and represents more than 40% of the total binding free energy change of $[Fe(phen)_3]^{2+}$. This additional free energy change, i.e. 7.50 kJ mol^{-1} corresponds to about 20-fold increase in the non-electrostatic binding constant. Further substitution of one phen for dip like in $[Fe(phen)(dip)_2]^{2+}$ consistently results in 9.20 kJ mol^{-1} of favorable free energy change which is 1.7 kJ mol^{-1} larger than that of $[Fe(phen)_2(dip)]^{2+}$ and corresponds to more than 55% of the total binding free energy change of its parent complex. This further substitution has enhanced about 30-fold the non-electrostatic DNA-binding constant of the iron(II) complex. The small increase in energetic increment for second substitution of phen for dip has been discussed in our previous report [11] in which it is attributed mainly to steric effect. Nevertheless, the substitution of the phenyl ring contributes substantially to the total binding free energy change and thus significantly stabilizes the binding of the iron(II) complexes to DNA.

A tremendous stabilization of the DNA binding is observed when one phen is replaced by dppz as found in $[Fe(phen)_2(dppz)]^{2+}$. An additional stabilization energy, 17.2 kJ mol^{-1}, which is equivalent to the total binding free energy of the parent complex, $[Fe(phen)_3]^{2+}$, is gained upon substitution of phen for dppz bearing phenazine moiety. This value corresponds to more than 800-fold increase in the non-electrostatic binding constant of its parent iron(II) complex. Such a tremendous change in the binding free energy change as observed for $[Fe(phen)_2(dppz)]^{2+}$ is readily understood because the addition of phenazine moiety (replacement of phen by dppz) dramatically changes the molecular interaction or binding mode of the iron(II) complex and ct-DNA, e.g. from electrostatic interaction in $[Fe(phen)_3]^{2+}$ [8,22] to full intercalation of dppz into the base pair of DNA [9,14]. In addition, it is noteworthy that the percent contribution of the non-electrostatic binding free energy change of $[Fe(phen)_2(dppz)]^{2+}$ to the total binding free energy change (87.5%) is comparable to those observed for proven organic intercalators such as ethidium or daunomycin, ca. 85% (Table 2) and thus the dppz ligand of the iron(II) complex interacts with double helical ct-DNA by classical intercalation similar to that established for ethidium or daunomycin.

4. Conclusion

We have demonstrated that measurements of the salt-dependence of the binding constant of iron(II) complexes allow us to calculate the non-electrostatic binding constant and to dissect the binding free energy change into its electrostatic and non-electrostatic components. Use of each component has provided a means to rigorously evaluate the contribution of charge and specific substituent to the binding free energy change. The result of quantitative analysis suggests that the main contribution to the stabilization of DNA binding comes from non-electrostatic interaction as indicated by their ΔG_t^o values ranging from 51.5% to 87.5% relative to the total binding free energy change. Specific moieties, e.g. two phenyl rings in dip and phenazine in dppz, contribute 7.5 and 17.2 kJ mol^{-1}, respectively to the total binding free energy change probably by providing more facilities for DNA binding via intercalation, hydrophobic or π–π stacking interaction. The results of this study clarify and quantify the contribution of specific substituents to the stabilization of the DNA binding and may provide a basic guidance for further design of new DNA binding agents of metal complexes.

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