Revisiting the Association of Cationic Groove-Binding Drugs to DNA Using a Poisson-Boltzmann Approach

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ABSTRACT Proper modeling of nonspecific salt-mediated electrostatic interactions is essential to understanding the binding of charged ligands to nucleic acids. Because the linear Poisson-Boltzmann equation (PBE) and the more approximate generalized Born approach are applied routinely to nucleic acids and their interactions with charged ligands, the reliability of these methods is examined vis-à-vis an efficient nonlinear PBE method. For moderate salt concentrations, the negative derivative, SK_{pred}, of the electrostatic binding free energy, ΔG_{el}, with respect to the logarithm of the 1:1 salt concentration, $[M^+]$, for 33 cationic minor groove drugs binding to AT-rich DNA sequences is shown to be consistently negative and virtually constant over the salt range considered (0.1–0.4 M NaCl). The magnitude of SK_{pred} is approximately equal to the charge on the drug, as predicted by counterion condensation theory (CCT) and observed in thermodynamic binding studies. The linear PBE is shown to overestimate the magnitude of SK_{pred}, whereas the nonlinear PBE closely matches the experimental results. The PBE predictions of SK_{pred} were not correlated with ΔG_{el} in the presence of a dielectric discontinuity, as would be expected from the CCT. Because this correlation does not hold, parameterizing the PBE predictions of ΔG_{el} against the reported experimental data is not possible. Moreover, the common practice of extracting the electrostatic and nonelectrostatic contributions to the binding of charged ligands to biopolyelectrolytes based on the simple relation between experimental SK values and the electrostatic binding free energy that is based on CCT is called into question by the results presented here. Although the rigid-docking nonlinear PB calculations provide reliable predictions of SK_{pred}, at least for the charged ligand-nucleic acid complexes studied here, accurate estimates of ΔG_{el} will require further development in theoretical and experimental approaches.

INTRODUCTION

Many important clinical drugs bind noncovalently to the minor groove of B-type DNA duplexes containing three or more consecutive AT basepairs (mG-binders) (1). These small organic drugs are used to treat many conditions, including cancer, genetic disorders, and viral and parasitic diseases. Various structural and biophysical studies have examined the noncovalent interactions that contribute to the binding affinity between the mG-binders and B-DNA (2–4). In particular, the complementarity of both shape and electrostatic potential, as discussed in the Supporting Material, between the drugs and the B-DNA as well as the short-range van der Waals and H-bonding contacts enhance binding affinity and contribute to the base sequence specificity (5–9). These studies, however, do not show the relative importance of these noncovalent interactions in stabilizing drug-DNA complexes (10–12). Understanding how these different interactions contribute to binding at the atomic level is critical to developing novel drugs with enhanced binding affinity, specificity, and biological activity.

Several experimental studies have observed that the binding affinities of mG-binders to B-DNA are very sensitive to small variations in salt concentration. In the literature, this observation has been interpreted to mean that nonspecific electrostatic interactions are important in the formation of these complexes (10,13,14). If K_{obs} is the experimental binding constant, and $[M^+]$ is the concentration of 1:1 salt in the bulk solution, then, in the absence of competing multivalent cations, $\log(K_{obs})$ is usually proportional to $\log[M^+]$ over a range of moderate salt concentrations (15,16). The slope of a linear $\log(K_{obs})$-$\log[M^+]$ plot is called SK_{obs} in the literature (17) and is negative for cationic drug-DNA complex formation. A constant negative SK_{obs} over a moderate salt range has historically been interpreted as a characteristic of the polyelectrolyte effect and originates from the high charge density of the negatively charged phosphate groups on the polyanionic DNA backbone (18–20). Because SK_{obs} is easy to obtain experimentally, predicting it is an ideal test of electrostatic models.

The first theoretical attempt to explain the binding of charged ligands to polyelectrolyte DNA was the counterion condensation theory (CCT) developed by Manning (18). The CCT was originally based on a coarse-grained model where the polyion (the DNA in our case) is treated as an infinite line charge representing the projection of the negatively charged phosphate groups onto the helical axis of the DNA. The ionic solvent is modeled as a uniform high dielectric medium, and the ions as point charges. The CCT was later extended by Fenley et al. (21) to account for the 3D arrangement of the phosphate groups obtained from structural data. More recently, others have considered more detailed nonuniform finite charge distributions within the framework of the CCT (22–24), but the CCT lacks

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features, like full atomic detail and the dielectric discontinuity between the interior and exterior of the molecule, that are important in some predictions of electrostatic properties, like sequence-dependent features of the electrostatic potential and counterion distributions surrounding nucleic acids (6,7,25). Therefore, the CCT may not reproduce experimental data for systems in which these effects are important (26).

According to the Manning CCT (18),

$$SK_{\text{obs}} = d(\ln K_{\text{obs}})/d\left(\ln\left[M^+\right]\right) = -z, \qquad (1)$$

where z is the charge on the cationic drug. Following similar assumptions, Record et al. (20) predict that $SK_{\text{obs}} = -z\psi$ where ψ is the thermodynamic binding fraction, which depends on the charge density of the nucleic acid. Both theories assume that the polyelectrolyte effect is purely entropic and arises when the ligand displaces counterions that were bound to the DNA before association.

Other interpretations of SK_{obs} have been discussed in the literature. For instance, Anderson and Record (17) express SK_{obs} in terms of preferential interaction coefficients, which take into account changes in the accumulation of cations and the exclusion of anions around the DNA and ligand during binding. If it is assumed that the only salt-dependent terms in the binding free energy are in the electrostatic component of the binding free energy, ΔG_{el}, then, following Sharp et al. (27), SK_{obs} relates to the change in the osmotic pressure on binding, $\Delta\Pi$, where Π is the osmotic pressure defined in the Methods section, by:

$$SK_{\text{obs}} = -\frac{d\Delta G_{\text{el}}}{d\,\ln\left[M^+\right]} = \Delta\Pi. \qquad (2)$$

The Poisson-Boltzmann equation (PBE) (6,28,29) can be solved numerically to find a potential that can be integrated by the methods of Sharp et al. (27) to calculate ΔG_{el}. Unlike the CCT, the PBE can make predictions of ΔG_{el} that include the 3D atomic structure of the biomolecules with low CPU cost due to the algorithmic advances made in the past decade. The PBE does not inherently include conformation change effects. The molecules in this study undergo only very small conformation changes on binding, as pointed out by Wilson et al. (4). Therefore, not including these conformational effects is a reasonable approximation, as we show in the Discussion. PBE methods include both the nonlinear PBE and its linear approximation, which is found by taking the first order approximation to the exponential term in the nonlinear PBE. The linear PBE is valid for small electrostatic potentials. The pairwise generalized Born (GB) method approximates the linear PBE by using an empirical Debye-Hückel term (30,31) to account for nonspecific salt effects.

Some theoretical studies have used the nonlinear PBE to investigate a limited number of drugs binding to nucleic acids (27,28,32–38), whereas several other groups have used either the linear PBE or GB model in lieu of the full nonlinear PBE to study different electrostatic effects in nucleic acids-charged ligand association processes (39–44). Wang and Laughton used molecular dynamics and the molecular mechanics/generalized Born approach to predict the relative affinity of the Hoechst 33258 ligand for different A/T-rich DNA sequences (41). In a newer follow-up study from the same laboratory it was found that predictions of the binding affinity of Hoechst 33258 to different DNA sequences are better when the molecular mechanics/Poisson-Boltzmann surface accessible approach is used as opposed to the molecular mechanics/generalized Born surface accessible approach (45). However, none of these studies have rigorously examined the validity of the linear PBE approximation for a large set of nucleic acid-charged ligand systems. Therefore, one of the main goals in this study is to determine whether the linear PBE provides an adequate approximation to the nonlinear PBE when investigating salt-dependent drug-nucleic acid interactions. Talley et al. (46) did address this question for protein-protein association, but their protein-protein complexes were generally of lower net charge than the complexes examined here. The complexes in this study are therefore expected to exhibit more pronounced nonlinear behavior. To confirm this expectation and to assess the ability of linear and nonlinear PBE analyses to reproduce experimental results, SK_{obs} was calculated with the linear and nonlinear PBE for the complexes in this study.

Unfortunately, extracting ΔG_{el} directly from the experimental data of the binding of charged ligands to nucleic acids is not possible. The CCT predicts that ΔG_{el} can be predicted by

$$\Delta G_{\text{el}} = -kT\,SK_{\text{obs}}\,\ln\left[\text{M}^+\right] + C, \qquad (3)$$

where C is a term that does not depend on the salt concentration. This equation is model-independent, as it is simply a thermodynamic identity. Manning then goes on to compute C by making several assumptions, including that the electrostatic potential can be given by the Debye-Hückel equation and that the atomic structure of neither the polyelectrolyte nor the binding ligand is important to ΔG_{el}. (18) These assumptions lead to the prediction that C is independent of the details of the binding partners and solely depend on the charge density of the polyelectrolyte. Because the PBE does consider this information, C is not necessarily independent of all parameters except the charge density of the polyelectrolyte, and as will be shown here, the PBE predictions of ΔG_{el} do indeed depend on these parameters.

Frequently, experimental groups (47–52) infer ΔG_{el} from the following equation:

$$\Delta G_{\text{el}} = -kT\,SK_{\text{obs}}\,\ln\left[\text{M}^+\right], \qquad (4)$$

which is a simplified version of Eq. 3. Once ΔG_{el} is calculated, the nonelectrostatic binding free energy, $\Delta G_{\text{non-el}}$ follows from:

$$\Delta G_{obs} = -kT \ln K_{obs} = \Delta G_{el} + \Delta G_{non-el}. \quad (5)$$

Whether the predictions of Eq. 4 agree with those of the PBE is not clear, however. For instance, from Eq. 4 a larger SK_{obs} indicates a larger ΔG_{el}, but this disagrees with the results of our recent PBE study (53). If Eq. 4 is not valid, then it is not possible to parameterize the PBE directly against the experimental data that has been reported without resolving the term C in Eq. 3. In this study, we report a detailed investigation of the behavior of ΔG_{el} with respect to $\ln[M^+]$ for a large number of DNA-drug complexes.

THEORETICAL METHODS

The DNA-drug complexes in this study are listed in Table S1, and the atomic coordinates of all the complexes are available in the RCSB Protein Data Bank (http://www.rcsb.org). The complexes were prepared as described in the Supporting Material.

The PBE calculations were carried out with an adaptive grid solver that is described elsewhere (A. Boschitsch and M. Fenley, unpublished) at 1:1 salt concentrations of 0.1-0.4 M at a temperature of 298 K. This PBE solver produced results that were comparable to the more commonly used APBS (54) PBE solver. The exterior dielectric constant, ε_{ext}, was set at 80, and the interior dielectric constant, ε_{int}, was fixed at 2. We discuss the effect of ε_{int} later. The dielectric boundary separating the solute and solvent regions was the solvent excluded, SE, surface. No ion-exclusion region was used because it has a consistent but small effect on our predictions of SK_{obs} (55). The dimensions of the grid were set to three times the largest dimension of the complex, and the fine grid spacing was 0.3 Å. The reader is referred to the Supporting Material for a more detailed description of the PBE calculations.

RESULTS AND DISCUSSION

Electrostatic binding free energy of drug-DNA complexes

In this study, SK_{pred} is considered rather than ΔG_{el} because ΔG_{el} is sensitive to the PBE parameters. This is illustrated in Fig. 1, where ΔG_{el} is plotted against ln[NaCl] for several values of ε_{int} for propamidine interacting with AT-rich B-DNA (PDB id: 102D). Unlike ΔG_{el}, which clearly exhibits significant change, the slope of the ΔG_{el} versus ln[NaCl] curves, which is proportional to SK_{pred}, is effectively the same for all ε_{int}. Comparable variations in ΔG_{el} are observed when varying the dielectric interface definitions used in the PBE calculations (data not shown), although, SK_{pred} is essentially invariant under such changes. Similar conclusions on different nucleic acid-charged ligand systems have been made in other PBE studies (56,57).

Intuitively, one would expect the desolvation cost to be unfavorable and the Coulombic interactions to be favorable for the association of unlike charges with the net ΔG_{el} remaining small. This expected anticorrelation between the Coulombic term and the reaction field term was observed for the complexes in this study (results not shown), where the Coulombic term is almost equal in magnitude but of opposite sign to the reaction field contribution. This compensation effect between the Coulombic and reaction field energies was first noted by Shaikh et al. (42) and Jayaram et al. (58) in a free energy component analysis of 25 minor groove drug-DNA complexes using a modified GB model (42,58). More recently, a molecular dynamics study of the essential subunit PA-PB1 interaction in the influenza virus RNA polymerase using the molecular mechanics/Poisson-Boltzmann surface accessible and molecular mechanics generalized Born surface accessible protocols also showed this compensation phenomena between the Coulombic and reaction field binding free energies (59). When the drug and the DNA are far apart, there is a net favorable electrostatic binding contribution, originating from the Coulombic term that is only weakly affected by the choice of PBE parameters. As they come in contact however, the unfavorable reaction field term grows and eventually dominates the Coulombic energy contribution. The sensitivity of ΔG_{el} to the parameters is largely attributable to the reaction field contribution. This desolvation energy is also what distinguishes the predictions of the CCT from those of the PBE in a simplistic sense. Because the CCT does not include a dielectric discontinuity, there is no desolvation cost, and therefore the C in Eq. 3 is not dependent on the details of the molecular surface.

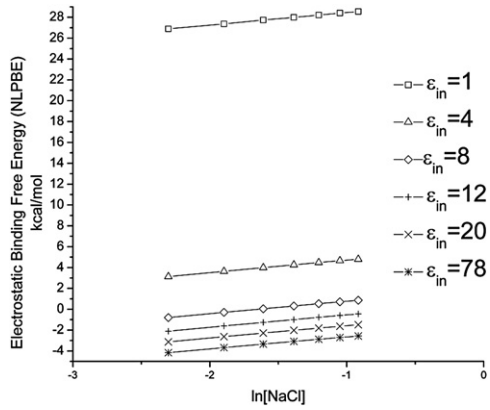

FIGURE 1 Electrostatic binding free energy, ΔG_{el} (kcal/mol), of the propamidine-B-DNA complex (PDB id: 102D) as a function of the logarithm of the concentration of a 1:1 salt, ln[NaCl] with different internal dielectric constants. ΔG_{el} is highly sensitive to the choice of interior dielectric constant, ε_{in}, changing from unfavorable to favorable. However, the slope of the lines is fairly constant.

Some attempts have been made to identify what surface definition should be used to construct the solute-solvent dielectric boundary in PBE calculations, but the results obtained by different groups are conflicting. In one study, it was found that the van der Waals surface reproduces the effects of charge mutations on the binding affinity of two different RNA-protein complexes better than the SE surface (56). On the other hand, a more recent PBE study of the association of mRNA cap analogs to the translation initiation factor eIF4E showed that both the van der Waals and

SE models provide similar predictions of the effects of mutations on the binding energetics (57). Based on these and our own PBE studies (53,60), we believe it is clear that one should be cautious when drawing any conclusions about whether electrostatics stabilizes or destabilizes binding because, by simply altering the dielectric boundary definition and the value of the interior dielectric constant, one can change ΔG_{el} from positive to negative.

To solve the parameterization problems noted above, one would like to use the reported experimental thermodynamic binding data, but, as mentioned before, the ability of Eq. 4 to predict ΔG_{el} is questionable. As can be seen in Fig. 2, ΔG_{el} and SK_{pred} were not correlated for the choice of PBE parameters listed in the Theoretical Methods section. As has been found in other PBE studies (36,38,61), ΔG_{el} is positive. The problem with Eq. 4 seems to be that it does not account properly for the dielectric discontinuity between the solute and solvent regions. In Fig. 3, ΔG_{el} was plotted against SK_{pred} where each quantity was calculated with an ε_{int} of 78, so that the dielectric discontinuity was nearly eliminated. This is the limit considered by the CCT, and we would therefore expect Eq. 4 to agree with the predictions of the PBE in this limit. We did not eliminate the dielectric discontinuity completely because this is not possible with our PBE solver, but this should illuminate the behavior in this limit. In this case, ΔG_{el} was strongly correlated with SK_{pred} with an $R^2 = 0.96$. This indicates that the primary difference between the predictions of ΔG_{el} by the CCT and those by the PBE arise from the dielectric discontinuity, whereas incorporating a realistic charge distribution is relatively unimportant. However, several studies have indicated that including a dielectric discontinuity is vital for reproducing other physical parameters (8,62,63), and we therefore do not feel that this choice of ε_{in} should be used.

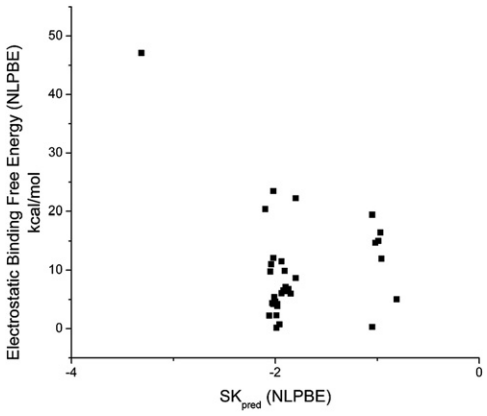

FIGURE 2 Electrostatic binding free energies, ΔG_{el} (kcal/mol), of all 33 drug-DNA complexes calculated using the nonlinear PBE with a dielectric constant of 2 versus $SK_{pred.}$ These two quantities are not correlated. Therefore, the experimental values of SK_{obs} should not be used to predict the value of ΔG_{el} using Eq. 4 in the main text.

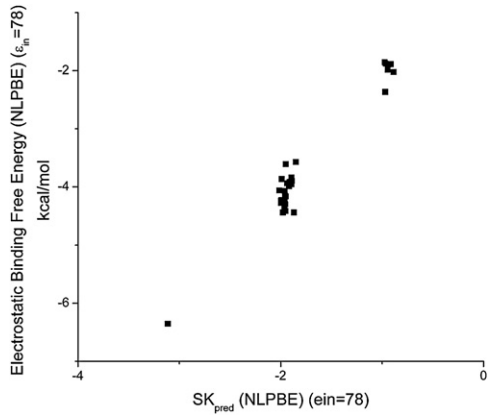

FIGURE 3 Electrostatic binding free energies, ΔG_{el} (kcal/mol), of all 33 drug-DNA complexes calculated using the nonlinear PBE with an internal dielectric constant of 78 versus $SK_{pred.}$ Unlike the previous figure, these two quantities are correlated, with an $R^2 = 0.96$. However, it is not clear whether the near lack of a dielectric discontinuity is reasonable.

Effects of the details of the charge distribution on SK_{pred}

SK_{pred} is fairly independent of changes in the 3D charge distribution that preserve the total charge on the complex, as can be seen from Fig. 4, where SK_{pred} calculated with a formal charge distribution is similar to that obtained using an all-atom charge assignment. These observations concur with an earlier study by Sharp et al. (27) that modeled the binding of DAPI to DNA as a cylinder-sphere interaction and compared the results to a classical all-atom DNA-drug model. They found that SK_{pred} predicted by all-atom models differs by $<3\%$ from that obtained with the coarse-grained models. Such errors are less than those in experimental estimates of SK_{obs}. Therefore consideration of full atomic detail does not appear to be necessary when computing SK_{pred} of charged ligand-nucleic acid complexes. Because the charge distribution does not seem to significantly affect SK_{pred} for the complexes in this study, it seems that the ionizable groups are the major contributors to SK_{pred}, with the dipolar groups playing a minor role. A thermodynamic study on the contribution of the closing basepair to the stability of RNA tetraloops supports this observation (64).

Comparing the predictions of the nonlinear PBE to experimental binding data

In Table 1, the available thermodynamic salt-dependent binding data for these complexes are compared to our nonlinear PBE predictions, and they are in excellent agreement. This strong correlation between the experimental thermodynamic data obtained from different laboratories and these nonlinear PBE predictions supports the use of the nonlinear PBE in accurately predicting SK_{obs} for these drug-DNA systems. Because the predictions of the linear PBE deviate strongly from the experimental data, the

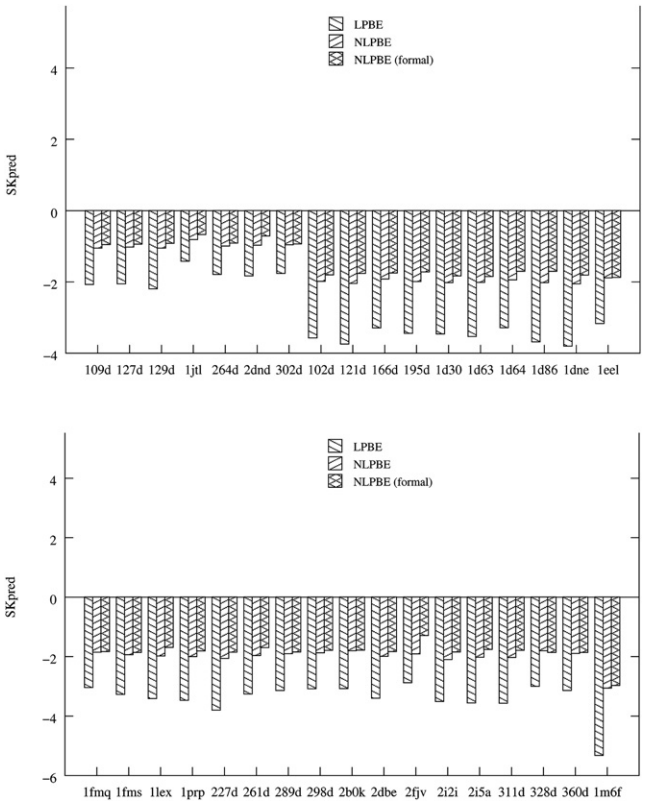

FIGURE 4 SK_{pred} of all 33 drug-DNA complexes considered here (identified by their PDB ids) and calculated using both the linear PBE and the nonlinear PBE are shown. The nonlinear PBE results obtained with the formal charge assignment for both drug and B-DNA are also shown. The complexes are grouped by net charge, with complexes 109D–302D having a charge of 1e, complexes 102D–360D having a charge of 2e, and complex 1M6F having a charge of 3e.

nonlinear PBE should be used for highly charged complexes like these.

Because predictions from static single-conformation PBE calculations accurately reproduce the experimental data, it appears that conformational flexibility can be neglected for *SK* predictions in these systems. The role of conformational dependence is more pronounced and its inclusion becomes necessary for more complicated nucleic acid systems where induced fit effects or intercalation are an integral part of the binding mechanism (65).

The reader is referred to the Supporting Material for a comparison of our PBE predictions of SK_{obs} to similar PBE results reported in the literature.

Linear versus nonlinear PBE predictions of SK_{pred}

SK_{pred} obtained with the nonlinear PBE is compared to that obtained with the linear PBE in Fig. 4. For all 33 DNA-drug complexes, the magnitude of SK_{pred} obtained from the linear PBE is larger than that obtained from the nonlinear PBE by at least 51%. This overestimation has also been observed in predictions of SK_{pred} for charged protein-protein complexes

TABLE 1 Theoretical predictions of the salt dependence of the binding affinity, SK_{pred}, using the NLPBE compared to the available experimental thermodynamic binding affinity data (SK_{obs}) for various minor groove drug-DNA complexes reported in the literature (12,49,74–80)

PDB name	Experimental SK_{obs}	SK_{pred} (NLPBE)
1D30	−2.3	−2.0
1D86	−1.51; −1.63	−2.0
1EEL	~−2	−1.9
227D	~−2	−2.1
264D	−0.90; −0.99 ± 0.02	−1.0
2DND	−0.79; −0.97	−0.9
2B0K	−1.50 ± 0.06	−1.8
2DBE	−1.45; −2.02	−2.0
2FJV	−1.8	−1.9
2I2I	−1.95 ± 0.02; −1.81 ± 0.01	−2.1
2I5A	~−2	−2.0

NLPBE, nonlinear PBE; PBE, Poisson-Boltzmann equation.

(46) and glutamine synthetase and glutaminyl synthetase bound to their cognate tRNA (66). For the protein-protein complexes considered by Talley et al. (46), the overestimation of the magnitude of SK_{obs} using the linear PBE compared with the nonlinear results is much smaller than for the drug-DNA complexes considered here. This is expected, given the larger charge densities of the drug-DNA complexes, and is consistent with the large differences between the linear and nonlinear PBE SK predictions obtained for the more highly charged tRNA synthetase-tRNA complexes examined by Bredenberg et al. (66). The reason why the linear PBE overestimates the magnitude of SK_{obs} is explained using a simple model in the Supporting Material.

To determine whether the difference between the linear and nonlinear PBE, SK_{pred} is predictable, the difference, $\Delta SK = SK_{pred}(\text{nonlinear PBE}) - SK_{pred}(\text{linear PBE})$, was calculated as a function of ligand charge. The result, shown in Fig. 5, suggests that ΔSK is proportional to the charge on the ligand. If this pattern holds for other complexes, then the predictions of the linear PBE could be corrected to agree more closely with those of the nonlinear PBE.

Comparing the nonlinear PBE predictions of SK_{pred} to those of the CCT

In Fig. 6, the values of SK_{pred} are plotted against the net charge of the cationic drug, and the magnitude of SK_{pred} is generally very close to the net charge on the drug, irrespective of the specific charge distribution and geometry of either the drug or the DNA. This result is in good agreement with the CCT and with the PBE analysis carried out by Rouzina and Bloomfield (67) that uses a coarse-grained DNA model. It is indeed striking to see the extent of agreement between the current and previous 3D PBE analyses and CCT predictions given that the 3D PBE and the CCT are based on very different physical models.

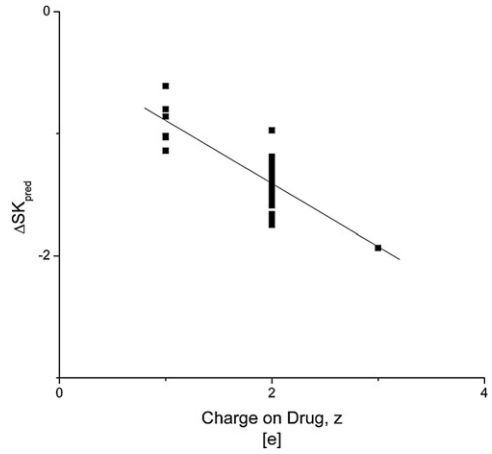

FIGURE 5 The difference, ΔSK_{pred}, between SK_{pred} calculated with the nonlinear PBE and SK_{pred} calculated with the linear PBE plotted against the charge on the ligand. These quantities seem to be proportional. Therefore, it might be possible to find a way to correct the linear solution to approximate that of the nonlinear PBE.

CONCLUSIONS

The binding of mG-binders was studied using the nonlinear PBE, and these results were used to assess the suitability of using the simpler linear PBE for modeling such systems. We believe the results show clearly that the linear PBE substantially overestimates the magnitude of SK_{pred} with large deviations from the experimental SK_{obs}. On the other hand, the nonlinear PBE provides SK_{pred} results that agree closely with experimental data as well as the predictions of the CCT. Hence, the linear PBE does not provide an adequate description of the electrostatic properties of these complexes.

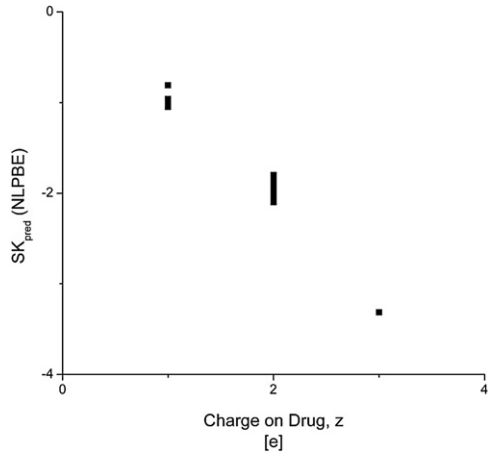

FIGURE 6 SK_{pred} calculated by the nonlinear PBE is plotted against the charge on the cationic drug, z, for all 33 drug-DNA complexes. SK_{pred} and the net drug charge are clearly correlated, as predicted by the CCT. The slope of the best fit line is -0.99 ± 0.02. This data is in good agreement with the CCT.

The nonlinear PBE predictions of SK_{pred} closely matched those of the CCT, whereas the PBE predictions of ΔG_{el} did not agree with those of the CCT. As indicated by our PBE results in the limit of no dielectric discontinuity, this is probably due to the lack of consideration of a low dielectric region in the CCT. The inclusion of a realistic charge distribution had a much smaller effect than the inclusion of a dielectric discontinuity.

This PBE analysis questions the popular method of extracting ΔG_{el} from experimental thermodynamic binding data of charged ligand-nucleic acid complexes, polysaccharides-proteins, and other charged biomolecular complexes (51,68,69). Because the PBE predictions do not agree with Eq. 4, extracting ΔG_{el} directly from thermodynamic binding data is not possible, even though Eq. 4 is used widely (51,69–71) to do this. The common practice of inferring that electrostatic interactions are more important when the magnitude of SK_{obs} is large (72), as implied by Eq. 4, is also questioned by these results, and these concerns should be reexamined by further experimental and theoretical investigations. Also, this work questions the value of obtaining SK_{obs}. Traditionally, it has been valued because of its presumed use to compute ΔG_{el}. If this is not possible, as indicated by these results, then the usefulness of determining SK_{obs} is debatable. The question as to whether electrostatic interactions favor or disfavor the binding of charged ligands to nucleic acids remains unanswered in light of the sensitivity of PBE predictions of ΔG_{el} to various input parameters. Proper parameterization of the PBE together with improved solvent descriptions must be developed and validated against reliable experimental data or all-atom molecular dynamics before these important questions can be answered.

In summary, the nonlinear PBE implementations to treat electrostatic interactions in DNA and DNA-ligand systems have evolved to a stage where reliable predictions of SK_{pred} can be made. However, further advances in a molecular level understanding of binding and, in particular, the effects of solvation (73) seem to be necessary before ΔG_{el} can be reliably computed for biomolecular complexes.

SUPPORTING MATERIAL

Two tables, two figures, and additional references are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00601-6.

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REFERENCES

1. Chaires, J. B. 1998. Drug—DNA interactions. *Curr. Opin. Struct. Biol.* 8:314–320.
2. Strekowski, L., and B. Wilson. 2007. Noncovalent interactions with DNA: an overview. *Mutat. Res.* 623:3–13.
3. Chaires, J. B. 1998. Energetics of drug-DNA interactions. *Biopolymers.* 44:201–215.
4. Wilson, W. D., F. A. Tanious, …, D. W. Boykin. 2008. Antiparasitic compounds that target DNA. *Biochimie.* 90:999–1014.
5. Xu, D., T. Landon, …, M. O. Fenley. 2007. The electrostatic characteristics of G.U wobble base pairs. *Nucleic Acids Res.* 35:3836–3847.
6. Boschitsch, A. H., and M. O. Fenley. 2004. Hybrid boundary element and finite difference method for solving the nonlinear Poisson-Boltzmann equation. *J. Comput. Chem.* 25:935–955.
7. Srinivasan, A. R., R. R. Sauers, …, W. K. Olson. 2009. Properties of the nucleic-acid bases in free and Watson-Crick hydrogen-bonded states: computational insights into the sequence-dependent features of double-helical DNA. *Biophys. Rev.* 1:13–20.
8. Rohs, R., S. M. West, …, B. Honig. 2009. The role of DNA shape in protein-DNA recognition. *Nature.* 461:1248–1253.
9. Xu, D., N. L. Greenbaum, and M. O. Fenley. 2005. Recognition of the spliceosomal branch site RNA helix on the basis of surface and electrostatic features. *Nucleic Acids Res.* 33:1154–1161.
10. Lane, A. N., and T. C. Jenkins. 2000. Thermodynamics of nucleic acids and their interactions with ligands. *Q. Rev. Biophys.* 33:255–306.
11. Neidle, S. 1997. Crystallographic insights into DNA minor groove recognition by drugs. *Biopolymers.* 44:105–121.
12. Mazur, S., F. A. Tanious, …, W. D. Wilson. 2000. A thermodynamic and structural analysis of DNA minor-groove complex formation. *J. Mol. Biol.* 300:321–337.
13. Hossain, M., and G. S. Kumar. 2009. DNA binding of benzophenanthridine compounds sanguinarine versus ethidium: comparative binding and thermodynamic profile of intercalation. *J. Chem. Thermodyn.* 41:764–774.
14. Sarkar, D., P. Das, …, N. Chattopadhyay. 2008. Binding interaction of cationic phenazinium dyes with calf thymus DNA: a comparative study. *J. Phys. Chem. B.* 112:9243–9249.
15. Nguyen, B., J. Stanek, and W. D. Wilson. 2006. Binding-linked protonation of a DNA minor-groove agent. *Biophys. J.* 90:1319–1328.
16. Jin, E., V. Katritch, …, D. S. Pilch. 2000. Aminoglycoside binding in the major groove of duplex RNA: the thermodynamic and electrostatic forces that govern recognition. *J. Mol. Biol.* 298:95–110.
17. Anderson, C. F., and M. T. Record, Jr. 1993. Salt dependence of oligoion-polyion binding: A thermodynamic description based on preferential interaction coefficients. *J. Phys. Chem.* 97:7116–7126.
18. Manning, G. S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.* 11:179–246.
19. Record, Jr., M. T., C. F. Anderson, and T. M. Lohman. 1978. Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. *Q. Rev. Biophys.* 11:103–178.
20. Record, Jr., M. T., W. Zhang, and C. F. Anderson. 1998. Analysis of effects of salts and uncharged solutes on protein and nucleic acid equilibria and processes: a practical guide to recognizing and interpreting polyelectrolyte effects, Hofmeister effects, and osmotic effects of salts. *Adv. Protein Chem.* 51:281–353.
21. Fenley, M. O., G. S. Manning, and W. K. Olson. 1991. Approach to the limit of counterion condensation. *Biopolymers.* 30:1191–1203.
22. Manning, G. S. 2008. Approximate solutions to some problems in polyelectrolyte theory involving nonuniform charge distributions. *Macromolecules.* 41:6217–6227.
23. Manning, G. S. 2002. Electrostatic free energy of the DNA double helix in counterion condensation. *Biophys. Chem.* 101–102:461–473.
24. Schurr, J. M., and B. S. Fujimoto. 2002. Extensions of counterion condensation theory I. Alternative geometries and finite salt concentrations. *Biophys. Chem.* 101–102:425–445.
25. Min, D., H. Li, …, W. Yang. 2008. Efficient sampling of ion motions in molecular dynamics simulations on DNA: variant Hamiltonian replica exchange method. *Chem. Phys. Lett.* 454:391–395.
26. Gold, B., L. M. Marky, …, L. D. Williams. 2006. A review of the role of the sequence-dependent electrostatic landscape in DNA alkylation patterns. *Chem. Res. Toxicol.* 19:1402–1414.
27. Sharp, K. A., R. A. Friedman, …, B. Honig. 1995. Salt effects on polyelectrolyte-ligand binding: comparison of Poisson-Boltzmann, and limiting law/counterion binding models. *Biopolymers.* 36:245–262.
28. Misra, V. K., K. A. Sharp, …, B. Honig. 1994. Salt effects on ligand-DNA binding. Minor groove binding antibiotics. *J. Mol. Biol.* 238:245–263.
29. Rocchia, W., S. Sridharan, …, B. Honig. 2002. Rapid grid-based construction of the molecular surface and the use of induced surface charge to calculate reaction field energies: applications to the molecular systems and geometric objects. *J. Comput. Chem.* 23:128–137.
30. Srinivasan, J., M. W. Trevathan, …, D. Case. 1999. Application of a pairwise generalized Born model to proteins and nucleic acids: Inclusion of salt effects. *Theor. Chem. Acc.* 101:426–434.
31. Onufriev, A., D. Bashford, and D. A. Case. 2000. Modification of the generalized Born model suitable for macromolecules. *J. Phys. Chem. B.* 104:3712–3720.
32. Misra, V. K., and B. Honig. 1995. On the magnitude of the electrostatic contribution to ligand-DNA interactions. *Proc. Natl. Acad. Sci. USA.* 92:4691–4695.
33. Baginski, M., P. Polucci, …, S. Martelli. 2002. Binding free energy of selected anticancer compounds to DNA—theoretical calculations. *J. Mol. Model.* 8:24–32.
34. Baginski, M., F. Fogolari, and J. M. Briggs. 1997. Electrostatic and non-electrostatic contributions to the binding free energies of anthracycline antibiotics to DNA. *J. Mol. Biol.* 274:253–267.
35. Chen, S. W., and B. Honig. 1997. Monovalent and divalent salt effects on electrostatic free energies defined by the nonlinear Poisson-Boltzmann equation: application to DNA binding reactions. *J. Phys. Chem. B.* 101:9113–9118.
36. De Castro, L. F., and M. Zacharias. 2002. DAPI binding to the DNA minor groove: a continuum solvent analysis. *J. Mol. Recognit.* 15:209–220.
37. Rohs, R., H. Sklenar, …, B. Roder. 2000. Methylene blue binding to DNA with alternating GC base sequence: a modeling study. *J. Am. Chem. Soc.* 122:2860–2866.
38. Kostjukov, V. V., N. M. Khomytova, …, M. P. Evstigneev. 2008. Electrostatic contribution to the energy of binding of aromatic ligands with DNA. *Biopolymers.* 89:680–690.
39. Spacková, N., T. E. Cheatham, 3rd, …, J. Sponer. 2003. Molecular dynamics simulations and thermodynamics analysis of DNA-drug complexes. Minor groove binding between 4′,6-diamidino-2-phenylindole and DNA duplexes in solution. *J. Am. Chem. Soc.* 125:1759–1769.
40. Chen, S. Y., and T. H. Lin. 2005. Molecular dynamics study on the interaction of a mithramycin dimer with a decanucleotide duplex. *J. Phys. Chem. B.* 109:9764–9772.
41. Wang, H., and C. A. Laughton. 2007. Molecular modelling methods for prediction of sequence-selectivity in DNA recognition. *Methods.* 42:196–203.
42. Shaikh, S. A., S. R. Ahmed, and B. Jayaram. 2004. A molecular thermodynamic view of DNA-drug interactions: a case study of 25 minor-groove binders. *Arch. Biochem. Biophys.* 429:81–99.
43. Shaikh, S. A., and B. Jayaram. 2007. A swift all-atom energy-based computational protocol to predict DNA-ligand binding affinity and ΔTm. *J. Med. Chem.* 50:2240–2244.

44. Treesuwan, W., K. Wittayanarakul, …, S. P. Mackay. 2009. A detailed binding free energy study of 2:1 ligand-DNA complex formation by experiment and simulation. *Phys. Chem. Chem. Phys.* 11: 10682–10693.

45. Wang, H., and C. A. Laughton. 2009. Evaluation of molecular modelling methods to predict the sequence-selectivity of DNA minor groove binding ligands. *Phys. Chem. Chem. Phys.* 11:10722–10728.

46. Talley, K., P. Kundrotas, and E. Alexov. 2008. Modeling salt dependence of protein-protein association: linear vs. non-linear Poisson-Boltzmann equation. *Commun. Comput. Phys.* 3:1071–1086.

47. Nguyen, B., D. Hamelberg, …, W. D. Wilson. 2004. Characterization of a novel DNA minor-groove complex. *Biophys. J.* 86:1028–1041.

48. Chaires, J. B. 2008. Calorimetry and thermodynamics in drug design. *Annu Rev Biophys.* 37:135–151.

49. Breslauer, K. J., D. P. Remeta, …, L. A. Marky. 1987. Enthalpy-entropy compensations in drug-DNA binding studies. *Proc. Natl. Acad. Sci. USA.* 84:8922–8926.

50. Haq, I., and J. Ladbury. 2000. Drug-DNA recognition: energetics and implications for design. *J. Mol. Recognit.* 13:188–197.

51. Prevette, L. E., M. L. Lynch, …, T. M. Reineke. 2008. Correlation of amine number and pDNA binding mechanism for trehalose-based polycations. *Langmuir.* 24:8090–8101.

52. Hossain, M., and G. Suresh Kumar. 2009. DNA intercalation of methylene blue and quinacrine: new insights into base and sequence specificity from structural and thermodynamic studies with polynucleotides. *Mol. Biosyst.* 5:1311–1322.

53. Bredenberg, J., and M. O. Fenley. 2008. Salt dependent association of novel mutants of TATA-binding proteins to DNA: predictions from theory and experiments. *Commun. Comput. Phys.* 3:1132–1153.

54. Baker, N. A., D. Sept, …, J. A. McCammon. 2001. Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc. Natl. Acad. Sci. USA.* 98:10037–10041.

55. Boschitsch, A. H., and M. O. Fenley. 2007. A new outer boundary formulation and energy corrections for the nonlinear Poisson-Boltzmann equation. *J. Comput. Chem.* 28:909–921.

56. Qin, S., and H.-X. Zhou. 2007. Do electrostatic interactions destabilize protein-nucleic acid binding? *Biopolymers.* 86:112–118.

57. Szklarczyk, O., J. Zuberek, and J. M. Antosiewicz. 2009. Poisson-Boltzmann model analysis of binding mRNA cap analogues to the translation initiation factor eIF4E. *Biophys. Chem.* 140:16–23.

58. Jayaram, B., K. McConnell, …, D. L. Beveridge. 2002. Free-energy component analysis of 40 protein-DNA complexes: a consensus view on the thermodynamics of binding at the molecular level. *J. Comput. Chem.* 23:1–14.

59. Liu, H., and X. Yao. 2010. Molecular basis of the interaction for an essential subunit PA-PB1 in influenza virus RNA polymerase: Insights from molecular dynamics simulation and free energy calculation. *Mol. Pharm.* 7:75–85.

60. Bredenberg, J. H., C. Russo, and M. O. Fenley. 2008. Salt-mediated electrostatics in the association of TATA binding proteins to DNA: a combined molecular mechanics/Poisson-Boltzmann study. *Biophys. J.* 94:4634–4645.

61. Rohs, R., and H. Sklenar. 2004. Methylene blue binding to DNA with alternating AT base sequence: minor groove binding is favored over intercalation. *J. Biomol. Struct. Dyn.* 21:699–711.

62. Elcock, A. H., and J. A. McCammon. 1996. The low dielectric interior of proteins is sufficient to cause major structural changes in DNA on association. *J. Am. Chem. Soc.* 118:3787–3788.

63. Honig, B., and A. Nicholls. 1995. Classical electrostatics in biology and chemistry. *Science.* 268:1144–1149.

64. Blose, J. M., D. J. Proctor, …, P. C. Bevilacqua. 2009. Contribution of the closing base pair to exceptional stability in RNA tetraloops: roles for molecular mimicry and electrostatic factors. *J. Am. Chem. Soc.* 131:8474–8484.

65. Frankel, A. D., and P. S. Kim. 1991. Modular structure of transcription factors: implications for gene regulation. *Cell.* 65:717–719.

66. Bredenberg, J., A. H. Boschtisch, and M. O. Fenley. 2008. The role of anionic protein residues on the salt dependence of the binding of aminoacyl-tRNA synthetases to tRNA: a Poisson-Boltzmann analysis. *Commun. Comput. Phys.* 3:1051–1070.

67. Rouzina, I., and V. A. Bloomfield. 1996. Influence of ligand spatial organization on competitive electrostatic binding to DNA. *J. Phys. Chem.* 100:4305–4313.

68. Ahl, I. M., B. H. Jonsson, and L. A. E. Tibell. 2009. Thermodynamic characterization of the interaction between the C-terminal domain of extracellular superoxide dismutase and heparin by isothermal titration calorimetry. *Biochemistry.* 48:9932–9940.

69. Richard, B., R. Swanson, and S. T. Olson. 2009. The signature 3-O-sulfo group of the anticoagulant heparin sequence is critical for heparin binding to antithrombin but is not required for allosteric activation. *J. Biol. Chem.* 284:27054–27064.

70. Lin, P. H., S. J. Tong, …, W. Y. Chen. 2009. Thermodynamic basis of chiral recognition in a DNA aptamer. *Phys. Chem. Chem. Phys.* 11:9744–9750.

71. Chernatynskaya, A. V., L. Deleeuw, …, A. N. Lane. 2009. Structural analysis of the DNA target site and its interaction with Mbp1. *Org. Biomol. Chem.* 7:4981–4991.

72. Bhadra, K., M. Maiti, and G. S. Kumar. 2008. Berberine-DNA complexation: new insights into the cooperative binding and energetic aspects. *Biochim. Biophys. Acta.* 1780:1054–1061.

73. Reddy, C. K., A. Das, and B. Jayaram. 2001. Do water molecules mediate protein-DNA recognition? *J. Mol. Biol.* 314:619–632.

74. Wang, L., A. Kumar, …, W. D. Wilson. 2002. Comparative thermodynamics for monomer and dimer sequence-dependent binding of a heterocyclic dication in the DNA minor groove. *J. Mol. Biol.* 317:361–374.

75. Wilson, W. D., F. A. Tanious, …, L. Strekowski. 1990. DNA sequence dependent binding modes of 4′,6-diamidino-2-phenylindole (DAPI). *Biochemistry.* 29:8452–8461.

76. Haq, I., J. E. Ladbury, …, J. B. Chaires. 1997. Specific binding of Hoechst 33258 to the d(CGCAAATTTGCG)2 duplex: calorimetric and spectroscopic studies. *J. Mol. Biol.* 271:244–257.

77. Miao, Y., M. P. H. Lee, …, W. D. Wilson. 2005. Out-of-shape DNA minor groove binders: induced fit interactions of heterocyclic dications with the DNA minor groove. *Biochemistry.* 44:14701–14708.

78. Pilch, D. S., M. A. Kirolos, …, K. J. Breslauer. 1995. Berenil [1,3-bis (4′-amidinophenyl)triazene] binding to DNA duplexes and to a RNA duplex: evidence for both intercalative and minor groove binding properties. *Biochemistry.* 34:9962–9976.

79. Tanious, F. A., W. Laine, …, W. D. Wilson. 2007. Unusually strong binding to the DNA minor groove by a highly twisted benzimidazole diphenylether: induced fit and bound water. *Biochemistry.* 46: 6944–6956.

80. Miao, Y. 2006. Shape-dependent molecular recognition of specific sequences of DNA by heterocyclic cations. *In* Chemistry. Georgia State University, Atlanta, GA.