CORRELATION BETWEEN THE THERMODYNAMIC STABILITY OF DNA DUPLEXES AND THE INTERACTION AND SOLVATION ENERGIES OF DNA BUILDING BLOCKS

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The unwinding Gibbs energy (or duplex dissociation energy) is an important measure of the thermodynamic stability of DNA oligomers. This value can be measured experimentally or predicted by empirical models parametrised on experimental data. Our previously developed model based on accurate DFT-D calculations of interaction energies between nucleic acid bases corrected for solvation contribution. This work was successfully extended to cover variable lengths of oligomers. This model was further applied to oligomers containing inosine, an unnatural base. The results, however, are not satisfactory and it is clear that the model does not take into account all variables contributing to DNA stability. Inclusion of the backbone deformation energy did not improve the model. We also compared models based on DFT-D and forcefield calculations. Forcefield performs well in this application, because the systematic error in interaction energies is cancelled in the fitting procedure.
Keywords: DNA stability; Density functional theory; Stacking interactions; Solvation; DNA hybridization; DFT calculations; Thermodynamics.

The melting temperatures and unwinding Gibbs energies evidence the thermodynamic stability of DNA. This stability depends, among other factors, also on the sequence of nucleic acid bases. Prediction of the stability of DNA duplexes from the sequence is very topical, e.g. in assessing the relative stability of ribonucleotide probes used in DNA sequencing and genome characterisation techniques. Doktycz et al.[1] have recently determined the melting temperatures and unwinding Gibbs energies for 140 octamer duplexes. Furthermore, they have predicted their stabilities on the basis of purely empirical correlation not reflecting the physical nature of noncovalent interactions between DNA building blocks.

In our previous paper[2], we predicted the unwinding Gibbs energies of DNA octamer duplexes using a model based on calculated accurate interaction energies between DNA bases (H-bonded, intra- and interstrand stacked

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pairs) and solvation Gibbs energies. Our model predicted the unwinding Gibbs energies with a smaller root mean square error (RMSE) than the fully empirical nearest-neighbour model, which uses a considerably higher number of fitted parameters. This fact, which sugests the importance of non-covalent interactions inside DNA, stimulated us to extend the original model, which was parametrised only on DNA octamers.

First, we successfully extended the present model of DNA oligomers of various lengths, using data measured and compiled by Sugimoto et al.[3] The second step was more complicated as we attempted to apply our model to oligomers containing unnatural bases. Our goal was to develop a model able to predict the stability of oligomers containing bases which was not included in the training set used for its parametrisation.

We selected inosine, a purine base capable of pairing with all natural nucleobases. The experimental data, along with the nearest-neighbour model parametrisation, were published by Watkins and SantaLucia[4].

Since our initial results were not satisfactory, we improved our model to include additional contributions. Introducing an unnatural base pair into a DNA double helix could lead to a change of its conformation. To cover this effect, we prepared our structures not only by geometry optimisation but also by using a molecular dynamics (MD) simulation, allowing the structure to achieve its natural conformation. Substantial changes of the structure will also affect the energy of the DNA backbone, a variable which was assumed to be constant in DNA containing only natural bases. This effect is now included through an additional molecular mechanics (MM) calculation.

METHODS

Sequences and Experimental Data

In this work, we have used experimental data for 65 DNA oligomers measured or compiled by Sugimoto et al.[3] The length of the oligomers ranges from 5 to 16 nucleotides. Fifty randomly selected sequences were used as a training set for fit, whereas the remaining fifteen structures were used for validation. For DNA containing inosine, we have used the data measured by Watkins et al.[4] All experimental Gibbs energies were measured in 1 M NaCl and are reported as $\Delta G°_{310}$.

Forcefield Parameters for Inosine

Molecular mechanics is used to refine generated structures of DNA oligomers. We have used the ff99 forcefield[5] to describe the DNA. Parameters for inosine have been derived by standard methodology described in ref.[6] to make them consistent with the forcefield. The tool *antechamber* from the AMBER [7] software package was used to automatize the procedure.

Structure Preparation

Since the only input is the sequence of the DNA oligomer, its geometry was built using the *nucgen* program, a part of the AMBER [7] package. Oligomers containing inosine were generated by replacing the matching base with inosine in proper orientation or replacing the whole base pair with an average structure from a short MD equilibration. All structures were then optimised in continuous solvent using a generalised Born model (GBM) as implemented in the AMBER code.

Interaction Energies

Our model is based mainly on the calculation of all pairwise interaction energies between DNA bases in a given oligomer, i.e. sugars and phosphates are not considered. Interaction energies are calculated as the difference between energy of a complex and energies of isolated subsystems. For a reliable and well-balanced description of these interactions, the selected method must be able to cover dispersion interaction. Efficiency is also crucial, because all the neighbouring base pairs are calculated in many oligomers. To satisfy these requirements, we have used the RI-DFT-D method[8,9], recently developed in our laboratory, which is a combination of advanced DFT calculation with an empirical dispersion term. The parameters in the damping function (associated with the dispersion term) were adjusted to reproduce accurate CCSD(T)/complete basis set results. The RI-DFT-DI procedure thus yields highly accurate interaction energies, geometries and other properties of molecular clusters. RI stands for resolution of identity approximation, which significantly increases the efficiency. Meta-GGA functional TPSS [10] with a triple-zeta basis set TZVP is used in all RI-DFT-D calculations in this study. Correction of the basis set superposition error (BSSE) is included in the parametrisation of the dispersion term in the DFT-D method, which is the reason why DFT-D interaction energies have not been cor-

rected for the BSSE. The calculations are performed using the Turbomole[11] package.

As mentioned above, the method provides very accurate results and can be trusted as a benchmark in our application but could be too demanding for practical application. Consequently, we also tested molecular mechanics for the calculation of interaction energies using the AMBER package[7]. The parameters for isolated bases were derived using the *antechamber* according to the recommended procedure.

Contribution of Solvation

The changes in solvation of bases upon duplex dissociation play an important role in the energetics of DNA denaturation. They are a factor which significantly reduces the difference in the strength of AT and GC hydrogen bonding in terms of Gibbs energy. Moreover, and this is probably even more important, solvation/desolvation phenomena significantly affect the strength of H-bonded pairs, whereas stacked pairs remain practically unchanged. Consequently, stacking contributes more to the overall DNA stability than H-bonding. As we have shown in our previous work[2], it is necessary to include the solvation contribution in order to be able to predict DNA duplex stability.

However, it is not possible to directly calculate this value for large molecules with the required precision directly. In order to overcome this limitation, we devised a scheme making it possible to estimate the total $\Delta\Delta G$ for any given sequence based on the calculation of smaller models.

The scheme is built on the following approximation:

1) The structure of a single strand of DNA in solution is generally unknown. For shorter oligomers, it is expected (and confirmed by our pilot MD simulations) to be a single strand with retained stacking of bases. In our model, we use the same geometry as for a strand in a double helix.

2) Since the stacking is conserved, the only change is associated with solvation in the hydrogen-bonding region. We believe that this value is sequence-independent and can thus be separated into the contributions of single base pairs.

3) For the purpose of our model, the relative values of Gibbs energy of solvation are sufficient. Only the differences between base pairs are calculated.

4) The access of water to the bases is limited by the adjacent bases and the DNA backbone and is different in the middle and at the end of a strand. Trimer is the smallest model that can describe these effects.

The C-PCM method[12] at the HF/6-31G(d) level is used to evaluate the solvation Gibbs energies of model systems. The default set of solvent parameters and recommended UAHF radii (United atom topological model radii optimized for HF/6-31G(d) level) are used. The Gaussian software package Version 03 [13] was used to perform the calculations. This method yields not only the electrostatic contribution to the Gibbs energy, but includes an estimate of non electrostatic terms (cavitation energy and solute–solvent dispersion).

Model trimers CXC/GYG, where XY is every studied base pair (AT, GC, IA, IC, IG, IT), were built. The phosphate groups were protonized in order to improve the reliability of the C-PCM calculations. This will not affect the resulting relative value, because the environment of the phosphates does not change upon dissociation, while it allows us to avoid problems associated with continuous solvent calculation of a charged system. The solvation Gibbs energy difference on duplex dissociation $\Delta\Delta G^{solv}$ was calculated as

$$\Delta\Delta G^{solv} = \Delta G^{solv}(\text{duplex}) - \Delta G^{solv}(\text{strand A}) - \Delta G^{solv}(\text{strand B}). \quad (1)$$

The solvation contribution of each pair XY (denoted as $\Delta G^{solv}(XY)$), relative to an AT pair, was calculated as

$$\Delta G^{solv}(XY) = \Delta\Delta G^{solv}(XY) - \Delta\Delta G^{solv}(AT). \quad (2)$$

The environment at the end of the duplex is different and was modelled separately. The scheme described above was applied also to end base pairs. As a model, dimers AC/GT and GC/GC are used (there is no inosine at the end of a duplex in our set of sequences), obtaining the value $\Delta G^{solv}(AT,end)$

$$\Delta G^{solv}(AT,end) = \Delta\Delta G^{solv}(AT,end) - \Delta\Delta G^{solv}(AT). \quad (3)$$

The total contribution of solvation to the ΔG consists of the CG-contents-dependent value $\Delta G^{solv}(sequence)$ and the correction for the end pairs $\Delta G^{solv}(ends)$

$$\Delta G^{solv}(sequence) = \sum N_{XY} * \Delta G^{solv}(XY) \quad (4)$$

$$\Delta G^{solv}(ends) = N_{AT,end} * \Delta G^{solv}(AT,end) \quad (5)$$

where N_{XY} is the number of XY pairs in the duplex and $N_{AT,end}$ is the number of the end AT pairs.

Backbone Deformation Energy Calculation

For a better description of sequence-dependent irregularities in the double helix structure, we introduced a new parameter into our model, the energy of the sugar–phosphate backbone. In our study of stretched DNA [14], we have shown that the backbone is flexible and the energetic effect small, but we wanted to examine how it affects the overall DNA stability. The energy of the backbone was calculated by applying MM on the structure of the oligomer with the nucleobases removed. The glycosidic bond was cut without any special treatment. This can be done only because we are interested in relative energy values which are not affected by this simplification. The backbone energy of the same sequence before optimisation E^{model} is subtracted from the calculated value E^{opt}, resulting in deformation energy which is dependent on the sequence. For the relative backbone energy E_{BB} to be obtained, the average deformation energy for a given length of oligomer $E(N)^{\mathrm{av}}$ is subtracted, which yields the final equation

$$E_{\mathrm{BB}} = (E^{\mathrm{opt}} - E^{\mathrm{model}}) - E(N)^{\mathrm{av}} . \qquad (6)$$

This approach was justified by pilot calculation at RI-DFT-D level for two model trimer with purine–purine mismatch in the middle introducing distortion in the backbone. For RI-DFT-D calculation, the glycosidic bonds were terminated with hydrogen atoms. Energy relative to an undistorted structure was calculated. The AMBER energy, which amounts to 1.7 kcal/mol, is in good agreement with RI-DFT-D value of 2.0 kcal/mol.

Estimating the ΔG of DNA Duplex Dissociation

For oligomers of variable length, we have expanded the constant K in our model for octamers into a fixed part K and a part K_{N} scaling with the length of the oligomer N

$$\Delta G = K + K_{\mathrm{N}} * N + c_{\mathrm{h}} * (E_{\mathrm{h}} + \Delta G^{\mathrm{solv}}(\mathrm{sequence}) + \Delta G^{\mathrm{solv}}(\mathrm{ends})) +$$

$$+ c_{\mathrm{i}} * E_{\mathrm{i}} + c_{\mathrm{s}} * E_{\mathrm{s}} \qquad (7)$$

where c_{h}, c_{i} and c_{s} are weighting coefficients for the sums of interaction energies E_{h} (hydrogen bonding), E_{i} (interstrand stacking) and E_{s} (intrastrand stacking). The interaction energy of hydrogen bonding is corrected for solvation by ΔG^{solv} terms prior to weighting by c_{h}. The same equation was used for oligomers containing unnatural bases, the different properties of

these bases being included in the calculated energies and solvation term ΔG^{solv}(sequence).

The variables K, K_N, c_h, c_i and c_s were optimised using the least squares method to fit the experimental Gibbs energies for all sequences in the training set.

RESULTS AND DISCUSSION

Estimating the ΔG of DNA Duplex Dissociation

As we have shown in our previous work, it is necessary to include all calculated contributions so as to be able to obtain good correlation with the experimental value of ΔG. For discussion on partial models not including some of the terms, see the original paper[2].

Our first model (labelled as Model 1 in Table I) is thus fitted on a training set of 50 structures, with all coefficients optimised. The results of the fit are summarised in Table I and plotted against the experimental values of ΔG in Fig. 1. The RMSE over the training set is 0.65 kcal/mol, which is about twice the value which we have achieved in our previous work on DNA octamers. This is mainly caused by the broader range of ΔG values covered by the training set. For the validation set of structures, we got an RMSE of 0.49 kcal/mol, which indicates a good prediction ability of the model.

Analysis of the coefficients provides further insight into DNA stability. The length-independent constant K is quite small when compared with the next term $K_N * N$, which describes the sequence-independent effect of oligomer length on the stability. Both these terms are positive, which

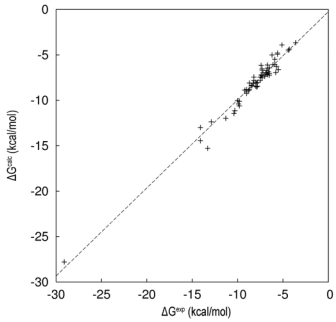

FIG. 1
Correlation between experimental and calculated Gibbs energies for RI-DFT-D based Model 1

TABLE I
Fit results based on RI-DFT-D calculations. Model 1 is parametrised on oligomers containing only natural bases

Parameter	Model 1	Model 2	Model 3	Model 4
K	2.37 ± 0.74	–2.11 ± 1.88	2.38 ± 0.77	3.60 ± 5.26
K_N	2.76 ± 0.38	0.95 ± 0.45	1.31 ± 0.30	–0.25 ± 1.31
c_n	0.06 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.10 ± 0.05
c_i	0.23 ± 0.04	–0.05 ± 0.04	0.03 ± 0.03	–0.11 ± 0.14
c_s	0.22 ± 0.03	–0.07 ± 0.03	0.05 ± 0.02	–0.08 ± 0.14
RMSE (all)	1.77	1.90	1.45[a]	1.71
RMSE (training)	0.65[a]	2.47	1.18	1.48
RMSE (validation)	0.49	1.38	0.68	0.93
RMSE (IA)	2.01	1.92	1.73	1.52[a]
RMSE (IC)	2.08	1.26	1.31	1.16
RMSE (IG)	3.30	1.43	2.19	2.29
RMSE (IT)	2.03	1.32	1.62	2.57
RMSE (IX)	2.40	1.33[a]	1.66	1.97
$\langle R^2 \rangle$	0.39	0.53	0.55	0.51
	Model 5	Model 6	Model 7	
K	6.74 ± 3.58	–6.00 ± 3.22	–0.13 ± 2.37	
K_N	–0.22 ± 0.64	0.92 ± 0.91	0.74 ± 0.58	
c_n	0.02 ± 0.04	0.07 ± 0.03	0.10 ± 0.02	
c_i	0.10 ± 0.09	0.05 ± 0.09	–0.04 ± 0.06	
c_s	0.09 ± 0.08	–0.06 ± 0.07	–0.05 ± 0.04	
RMSE (all)	2.58	2.72	2.12	
RMSE (training)	1.96	3.56	2.54	
RMSE (validation)	1.82	1.54	1.32	
RMSE (IA)	2.16	2.48	2.61	
RMSE (IC)	0.79[a]	3.11	2.22	
RMSE (IG)	4.69	1.05[a]	1.61	
RMSE (IT)	3.32	1.24	0.78[a]	
RMSE (IX)	3.09	1.99	1.71	
$\langle R^2 \rangle$	0.31	0.52	0.53	

RMSE values are listed for following sets of sequences: the whole set (all), training set of natural oligomers (training), validation set of natural oligomers (validation), sequences containing specific inosine pairs (IA, IC, IG, IT) and all inosine-containing sequences (IX).
[a] RMSE values optimised by the fitting procedure.

means that they include destabilising effects not covered by the following terms related to each type of interaction. The coefficients c may seem to be negligibly small, but these coefficients weight sums of interaction energies that are an order of magnitude larger than the total ΔG of unwinding. For example, we list all the terms in Model 1 for the average interaction energies (corresponding approximately to an octamer): $c_h * (E_h + \Delta G^{solv}) = -10.8$, $c_i * E_i = -4.4$ and $c_s * E_s = -18.9$ kcal/mol. It is clear from these numbers that intrastrand stacking is the most important interaction contributing to overall DNA stability, followed by hydrogen bonding, which is weakened by the unfavourable solvation Gibbs energy.

Inosine-Containing Oligomers

The parameters derived for oligomers containing only natural bases were applied without modifications to structures containing inosine. The calculated ΔG values are plotted against experimental data in Fig. 2. The RMSE for each type of inosine pairing (IA, IC, IG and IT) is shown in Table I. It is approximately 2 kcal/mol for all the pairs with the exception of IG, which is very wide and distorts the DNA most.

Since the quality of the fit is not as good as for DNA containing only natural bases, we thoroughly checked our results and the reliability of our model. We are aware that even the structures containing two inosine pairs consist mostly of natural base pairs, that are well described by our model, what suggests that the description of inosine pairs may be worse than the

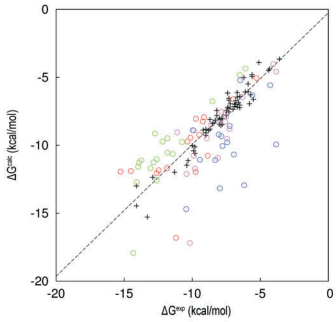

FIG. 2
Correlation between experimental and calculated Gibbs energies, including sequences containing inosine paired with adenine (red), cytosine (green), guanine (blue) and thymine (magenta). Parameters from the RI-DFT-D based Model 1 were used without modifications

overall results for the whole oligomer suggest. To quantify the hypothesis, the correlation between the experimental and calculated Gibbs energies was calculated for each set of four sequences which differ only in the base paired with inosine.

For eight sets of four oligomers differing only in the base paired with inosine, the correlation between the predicted stability and its experimental value was measured as correlation coefficient R^2. The values range from zero to 0.95, the average values for all the models are listed in the last row of Table I. These values clearly show that the description of unnatural base pair is inconsistent.

In an attempt to improve the agreement with experiment for inosine-containing oligomers, we also performed additional fits only for these structures (Model 2), for all available structures, mixing natural and inosine-containing DNA (Model 3) and for each type of inosine pairing (Models 4–7).

Model 2 can yield more accurate and more consistent results for inosine, but the description of natural oligomers is poor. Model 3 gives more balanced performance, but the improvement compared with Model 1 is not significant enough to justify the inclusion of inosine in the training set. Models 4–7 yield the best values for the base pair parametrised but fail completely for others.

From these results, especially from poor correlation of the sequences differing only in the base paired with inosine and from the difference between Models 1 and 2, it is obvious that some parameter(s) are missing in the model which would account for changes of stability in unnatural oligomers.

As the most critical parameter that can be directly calculated, we selected the backbone deformation energy. It is clearly evident that the formation of, e.g., IG pair should induce significant backbone deformation. It was intentionally omitted in the case of natural oligomers, because it is known that the conformation of the backbone is approximately retained in different sequences. On the other hand, when unnatural base pairs are introduced into the double helix, significant changes can occur.

The backbone deformation energy was determined at the MM level. A separately weighted parameter E_{BB} with a corresponding coefficient c_{BB} was introduced into Eq. (*7*). The results of the fits for Models 1–3 are listed in Table II.

Investigating the entries in Table II, we ascertained that the results are very similar to those where the backbone energy was not taken into consideration (Table I), mostly even slightly worse. The weighting coefficient for

this term is small, suggesting that the backbone energy, in the representation which we used, does not affect the overall stability.

There are two possible sources that might lead to observed failure of the model. One is the quality of presented calculations. The RI-DFT-D method proved itself to be very accurate and comparable to high-end calculations[8]. The other is the method we build on is the C-PCM calculation of solvation free energies. Although its absolute accuracy might not be perfect, we use it to evaluate relative differences between similar systems (different base pairs). In such application, large part of the error is cancelled. Also, the fact that our model works for natural DNA supports reliability of methods used.

Secondly, there are other terms missing in our model. We are convinced that the poor performance of the model for inosine-containing DNA oligomers is caused by some parameter that is needed to describe these systems, but cancels in natural DNA. The most obvious is absence of the entropy.

TABLE II
Fit results based on RI-DFT-D calculations with correction for the backbone deformation energy

Parameter	Model 1	Model 2	Model 3
K	–1.72 ± 2.16	–8.70 ± 4.55	0.52 ± 3.15
K_N	2.93 ± 0.38	1.55 ± 0.57	1.44 ± 0.41
c_n	0.07 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
c_i	0.19 ± 0.04	–0.07 ± 0.04	0.02 ± 0.03
c_s	0.19 ± 0.04	–0.08 ± 0.03	0.04 ± 0.03
c_{BB}	0.05 ± 0.02	0.07 ± 0.04	0.02 ± 0.03
RMSE (all)	1.79	1.88	1.45[a]
RMSE (training)	0.62[a]	2.46	1.20
RMSE (validation)	0.54	1.28	0.68
RMSE (IA)	2.11	1.73	1.71
RMSE (IC)	1.75	1.25	1.31
RMSE (IG)	3.48	1.51	2.17
RMSE (IT)	2.11	1.30	1.58
RMSE (IX)	2.44	1.35[a]	1.65

RMSE values are listed for following sets of sequences: the whole set (all), training set of natural oligomers (training), validation set of natural oligomers (validation), sequences containing specific inosine pairs (IA, IC, IG, IT) and all inosine-containing sequences (IX).
[a] RMSE values optimised by the fitting procedure.

Unfortunately, it is extremely difficult to calculate this variable, especially for solvated molecules. In our opinion, direct calculation of Gibbs energy from molecular dynamics simulations would be more feasible, at least for selected small DNA oligomer. Another approximation that could be questioned is the use of optimized geometries for all the calculations, while in reality DNA molecule is flexible. This applies particularly to single-stranded DNA. It is impossible to incorporate this behaviour into our static model, and the only proper treatment would be molecular dynamic simulation. This is, however, out of scope of the present paper, because it would require completely different approach to the problem.

AMBER Interaction Energies

In order to maximise the efficiency of our method, we fitted our model on MM interaction energies calculated using the AMBER ff99 forcefield. Only Model 1 for DNA containing natural bases was tested; the results of are presented in Table III.

The coefficients are slightly different when compared with the same model using RI-DFT-D energies. However, the fit quality and the reliability of coefficients are almost identical. The result shows that the forcefield calculation of interaction energies works well in this application. It is known that these interaction energies do not match the exact values perfectly, but the difference is systematic and as such is removed by the fitting procedure.

TABLE III
Fit results based on AMBER calculations

Parameter	Model 1
K	3.49 ± 0.73
K_N	2.85 ± 0.51
c_n	0.13 ± 0.01
c_i	0.16 ± 0.04
c_s	0.16 ± 0.03
RMSE (all)	1.77
RMSE (training)	0.65[a]
RMSE (validation)	0.49

[a] RMSE values optimised by the fitting procedure.

CONCLUSIONS

We have successfully extended our previous model for predicting DNA duplex stability to account for the variable length of oligomers.

The "Gibbs energy" term, which is proportional to intrastrand stacking interaction, is the most important contribution to the DNA duplex stability, followed by hydrogen bonding.

We attempted to apply the same method to oligomers containing inosine, an unnatural base that pairs with all four DNA nucleobases. The results were poor. It is clear that our model does not take into account some contributions to the total Gibbs energy which cancel in the case of regular natural DNA.

The inclusion of the backbone deformation energy calculated for each oligomer does not improve the model.

The model based on interaction energies from forcefield calculations yields results identical to the model based on accurate RI-DFT-D calculations, although the energies themselves are different. This error is systematic and cancels in the parametrisation.

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