Computer simulation results for the free-energy difference between B-DNA and Z-DNA

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Abstract.   The aim of the present work is to characterize by computer simulation the free-energy difference between B- and Z-DNA in saline solutions. We use a new scheme to rigorously calculate the electrostatic contribution and we use it to test the theoretical predictions for a so-called 'empty' DNA model (a set of charged hard spheres placed at the phosphate positions). The ions are considered as charged spheres in a continuum medium of dielectric constant equal to that of water. Also, we investigate the total free-energy difference for three different models differing exclusively in the degree of definition of the molecular shape. The comparison against experimental data shows that a precise shape is not required to give acceptable results. Finally, we use a simple grooved primitive model to study the effect of the ionic size and the ionic charges.

1. Introduction

The role of DNA as a repository of the genetic code generated the interest in its biochemical properties. Nevertheless, increasing attention is being paid to its physicochemical behaviour as we are becoming aware of the relationship between DNA structure and function [1, 2]. For instance, while DNA polymorphism is already an interesting issue from a physicochemical point of view, the transitions between different conformers (especially between B- and Z-DNA) seem to play an active biological role [1] and, thus, the investigation of the relative stabilities of the conformers becomes more relevant.

In solution, DNA is a negatively charged polyelectrolyte due to the complete ionization of the acidic phosphate groups [2]. As Z-DNA is thinner than B-DNA, its charged phosphates are closer to each other giving stronger repulsions among them, so B is, in principle, a more stable DNA form. The transition from B- to Z-DNA then requires an overstabilization of the Z form by means of extrinsic effects, the more interesting of which is a change in the solution ionic strength. Depending on the DNA sequence, mid-point concentrations of added NaCl ranging from 0.7 M to 5.4 M have been reported [3–5]. Also, an increase of the counterion (cation) charge results in a decrease of the salt concentration required to induce the transition [6]. The detailed variation of the relative stability of both DNA forms has only been reported for $d(C–G)_n \cdot d(C–G)_n$ oligomers in the highly monovalent salt region (i.e., 2–5 M NaCl) by Pohl [7]. The variation is linear in the logarithm of the salt concentration.

The theoretical treatment is far from trivial because the transition involves dramatic interconversions at the molecular level: the double-helix twist has opposite senses in both forms—right handed in B-DNA and left handed in Z-DNA—and the base pairs are flipped over in one form with respect to the other relative to the sugar–phosphate backbone [8]. Thus,

the theories proposed to tackle this problem are forced to use simplified models for the system components (polyion, water and ions) and the interactions between them. Despite the relative success of some treatments—in particular, that of the Soumpasis potential of mean force (SPMF) approach [9]—the theoretical progress has been slowed down due to the absence of a rigorous assessment of the approximations involved. On the other hand, it is not yet clear what the essentials of the transition are—i.e., the minimal structural features required to model it. In recent years we have undertaken a programme in order to clarify these points.

This paper presents the main results of our study. First, we describe a rigorous method for calculating, by computer simulation, the free-energy difference in conformational changes. Next, we check previous theoretical predictions against our computer simulation results for the same model. There follows an analysis of the ability of different models to account for the experimental data. Particular emphasis is given to the effect of the role played by the DNA shape in the stabilization of Z-DNA at high salt levels.

2. The calculation of the free-energy difference

The free-energy difference can be formally split into several contributions [10]:

$$\Delta G^{B \to Z} = \Delta G_{intra}^{B \to Z} + \Delta G_{hydr}^{B \to Z} + \Delta G_{electr}^{B \to Z} \tag{1}$$

where the subscript *intra* represents the intramolecular contributions except the electrostatic ones which are included in the electrostatic term. $\Delta G_{electr}^{B \to Z}$ is then independent of the molecular sequence, so simple models are suitable for its calculation. Finally, the hydration term accounts for the possible differences in the *specific* hydration of the conformers, i.e., those arising from the molecular nature of the solvent.

$\Delta G_{intra}^{B \to Z}$ has been estimated for the alternating guanine–cytosine hexamer [10] to be about $-0.1 \, k_B T$ per phosphate and almost independent of the salt concentration [10]. Despite the importance of DNA hydration, its influence for the B–Z transition is still controversial [11,12]. Notwithstanding the possible different hydration state of each conformer, it seems reasonable that the hydration term remains substantially invariant with changes in salt concentration. Because of this and also because the evaluation of $\Delta G_{hydr}^{B \to Z}$ by computer simulation is almost unaffordable, we neglect it in our calculations. It is important to stress that, if the neglected contributions are independent of the salt concentration, the calculated free energies should differ from experimental values by a constant factor but the *slope* should be the same. This is why we paid more attention to the dependence of the free energies on the ionic strength than to the precise determination of the transition mid-point.

Due to the dissimilarity of the two DNA conformers, the well known free-energy perturbation method is not useful for the computation of the electrostatic contribution. We have devised a new scheme [13] which is graphically displayed in figure 1. The computation for each conformer is split into two steps. The first one consists in the *set-up* of the *uncharged* conformer from uncharged particles within a solution at the ionic concentration of interest. Notice that, as long as the final systems are conformers, the starting solution with uncharged particles is common to the two states. Also, even though the conformers are structurally dissimilar, the set-up term is expected to give a small contribution. The other step corresponds to the *charging process*, in which the polyion sites are charged up while a number of counterions exactly compensate for its charge (see reference [15] for details). We will use the acronym SUCH to refer to the results obtained by computer simulation through the set-up and charge steps.
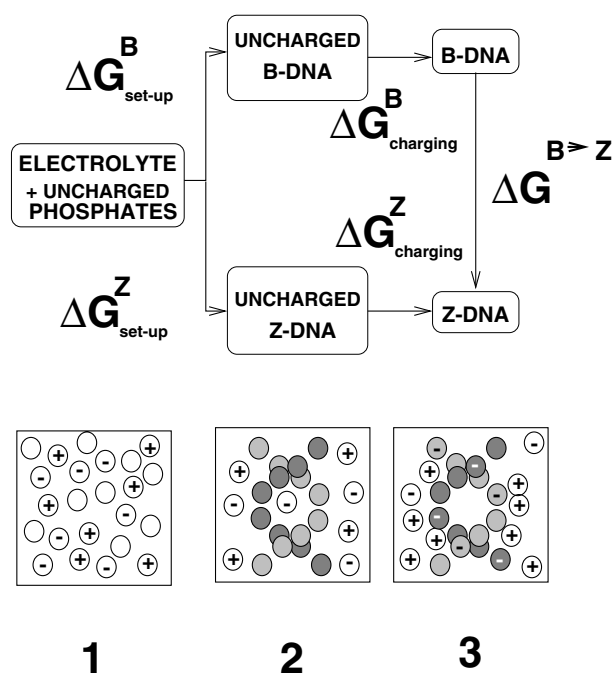
Figure 1. The thermodynamic path for the computation of the electrostatic free-energy difference. Top: the free-energy differences involved in each of the steps along the thermodynamic path. Bottom: a schematic representation of the actual system in every stage. From left to right, starting with uncharged particles dissolved in a simple electrolyte solution (1), the particles are placed at the positions of a DNA form (2) and, then, the uncharged DNA structure is charged up (3).

3. Results

3.1. Comparison with theoretical calculations

Several theories [9, 14] rely more or less explicitly on a DNA model which only considers the phosphate groups. These are modelled as solution anions placed at the canonical positions, neglecting completely the sugar and base atoms. The ions are charged spheres in a continuum medium of dielectric constant equal to that of water. The only contribution for this 'empty' DNA model is the electrostatic one and, thus, the theoretical predictions can be directly checked with the SUCH results. The comparison shows [15] that the SPMF approach reproduces quantitatively the electrostatic free-energy differences of the empty DNA model although the deviations increase with the salt concentration. The SPMF slope at the higher salt concentrations is 24% larger than the SUCH value. The polymer reference interaction site model (PRISM) theory [14, 16] gives poor results. It also predicts a negative slope of the free energy versus the logarithm of the salt concentration but its absolute value is far too large, about 20 times the SUCH value. Finally, the reported values calculated for $\Delta G^{B \to Z}$ using the counterion condensation (CC) theory [17] are always lower than the SUCH computed values but the overall shape is correct. The CC free-energy slope is -0.027, about half the SUCH value. Nevertheless, the CC calculations are based on the Debye–Hückel theory which considers point-like polyelectrolyte sites and ions. As we will show below, the repulsive forces have a strong effect on the slope of the free-energy difference, so the comparison with the CC theory should be treated with caution.

3.2. The role of the molecular shape

It is clear that the empty model provides an unrealistic description of the molecular core. Thus, it seems interesting to investigate whether the predictions for the empty model improve when it is supplemented with a soft cylindrical core [18]. The model is then made up of discrete charges embedded in a soft repulsive cylinder; hence the acronym DS. A further improvement in the description of the molecular core is given in the grooved primitive (GP) model [19]. As the name suggests, the model approximates the grooved DNA shape by means of simple geometric elements. Now, in addition to the phosphates of the empty model, a big sphere mimics the central DNA core while each nucleotide is completed by another uncharged sphere of the same size as the phosphate one. Notice that the three models differ exclusively in the definition of the repulsive forces. This allows us to investigate the role of the molecular shape in the conformational transition.

One important conclusion of the investigation of the empty model is that the set-up term gives only a very small contribution. Thus, we may omit the set-up contribution and retain only the charging and the intramolecular terms. The free-energy differences as a function of the salt concentration for 1:1 electrolytes are shown in figure 2. The DS model predicts a flat curve. It is clear that the repulsive cylinder is totally inappropriate for the thermodynamic description of the transition. In fact, the results do not differ essentially from the predictions of the PB equation for a homogeneously charged cylinder [20]. This indicates that a more detailed description of the charge distribution scarcely affects the free-energy difference of the repulsive cylinder.
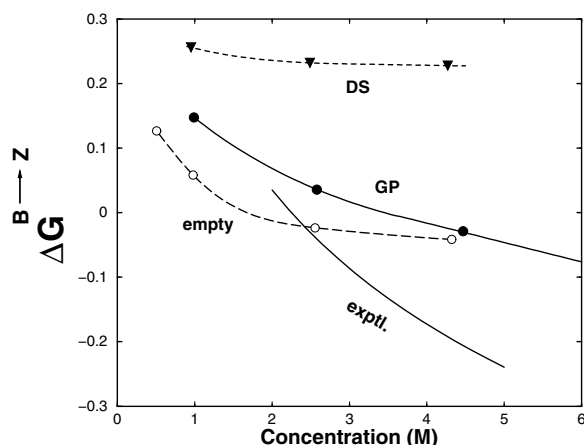

Figure 2. Transition free energies in $k_B T$ per phosphate in monovalent salt for different models. Lines are to guide the eye, except the experimental curve, which is the Pohl fit [7].

Surprisingly, the results for the empty model are quite close to the experimental data and to the calculations with the GP model. The effect of the molecular core seems a puzzle, but this can be readily solved when one realizes that the empty model and the GP model share an important property: both models allow the penetration of the ions within the grooves while the DS model does not. As the change in the relative stability of the conformers is induced by the counterions surrounding the DNA, the penetrability of the ions becomes an essential condition for the explanation of the transition free energy. It is worth noting that we speak of the role of the molecular shape, but the situation is a bit more complex. In fact, it is the coupling of the electrostatic charges placed along the ridge with the groove shape which 'pushes' the ions to

enter into the grooves.

On the other hand, the superiority of the GP model is manifested by the similarity of its slope to the experimental one. It seems that a full atomic description of the DNA shape (the van der Waals surface) should approach a little bit closer to the experiment and the computer simulation results. Nevertheless, the differences are important enough to make us wonder whether some of the discrepancies are not due to the neglect of the hydration term. As we will see below, the results are also quite sensitive to the ionic size, so the use of an effective value for the sizes of the hydrated cations may also contribute to the discrepancies between experimental and simulation results. But, considering the above definitions for the free-energy terms, such refinements should be just considered as part of the hydration contribution.

3.3. The effect of the ionic size and ionic charge

Once we had demonstrated that the computationally simple GP model gives satisfactory results for monovalent salts, we used it to investigate the effect of the ionic size and ionic charge [21]. The predictions for this model indicate that the slope of the curve increases with the ionic size. The dependence of the mid-point concentrations on the ionic size is in accordance with the experimental results for the alkaline cations.

Regarding the effect of the ionic charge, our findings indicate that the predicted transition mid-point is in excellent agreement with the experiment for 2:1 salts. Besides, the slope of the free-energy curve is coincident with an estimation of the experimental slope based on a wide set of experimental measurements [21]. We are not aware of any study of the transition in the presence of divalent anions and cations, but our investigations show that the curve is very close to that of 2:1 salts with a slight decrease of the slope. This means that, as should be expected, the anion charge has a negligible effect on the transition.

Acknowledgment

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