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A method for the computer simulation of the free-energy difference in conformational changes of polyelectrolytes. Application to the B- to Z-DNA transition

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Abstract. – A method for calculating the free-energy difference between two dissimilar conformational states of a nucleic acid is proposed. The procedure is especially suited for computer simulation. The calculation of the free energy of each conformer is split into two steps; the first one consists in the *build-up* of the *uncharged* conformers while the other one —which leads to the more important contribution— corresponds to the *charging process* of such structures. We report preliminary results on the application of the method to evaluate the free-energy difference for the salt-induced conformational change between the B and Z forms of DNA. This is the first rigorous evaluation of the $\Delta G^{\mathrm{B}\to\mathrm{Z}}$ dependence on the added salt concentration.

Introduction. – The equilibrium state in a biomolecular system is often determined by a subtle interplay of intermolecular forces which can cause large conformational changes. In some cases where the structures in equilibrium are similar, perturbation treatments are adequate for calculating the free-energy difference. In other cases the structures in equilibrium are strongly different and the passing from one conformer to another one is not possible through a simple path. Typical examples are the significant structural modifications in the interconversion between different DNA conformational variants. But despite the structural dissimilarity, the conformers have several features which can be exploited in order to devise a well-founded method for the calculation of free-energy differences. Firstly, the chemical definition of conformers implies that the structures in equilibrium are *built from the same chemical groups* arranged in a different manner. This provides the basis for a common reference state. Besides, for the conformational change to occur, there must be a *different response* of the conformers to an *external perturbation.* In this way, it is advisable to separate the contributions depending on the agent responsible for the change from those which are not (or in a lesser degree). In this letter, we formulate a method to calculate the free-energy difference when the conformers are polyions showing a different electrostatic response. The method is well

suited for evaluation using computer simulation. Our target (and motivating) application is the B → Z transition.

The interconversion between the B and Z forms of DNA was discovered in 1972 by Pohl and Jovin [1]. Although there is no transition in the strict physical sense, it is customary in molecular biology and biophysics to refer to it as the B- to Z-DNA transition; we freely adopt such terminology. The transition involves dramatic modifications at the molecular level, the most striking feature being that the double-helix twist has opposite senses in both forms, right-handed in B-DNA and left-handed in Z-DNA [2]. Z-DNA being thinner than B-DNA [3], its charged phosphates are closer to each other giving stronger repulsions among them so that B is, in principle, the most stable DNA form. The transition from B- to Z-DNA then requires an overstabilization of the Z with respect to the B form by means of extrinsic effects, the more interesting of which is a change in the solution ionic strength.

Few theories have been applied to the relative stability of the B and Z forms of DNA [4], [5]. Besides, they have been 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 [4]— 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 are the essentials of the transition, *i.e.* the minimal structural features required to model it. Although it seems obvious that the conformational change is driven by the polyion-solution ions electrostatic forces affecting differently to both conformers, the difference in the charge density between B- and Z-DNA does not account for the transition. This is what the counterion condensation theory calculations indicate [6], though the problem could also arise from theoretical inadequacies. Thus, there is a clear need for "exact" results which can only be provided by computer simulation. Unfortunately, the free-energy perturbation scheme to compute free-energy differences is not applicable to our case as the interconversion between those DNA conformers is not feasible through simple steps. The goal of this letter is then double: to develop a method for calculating the free-energy difference of nucleic-acids conformational changes and to show its usefulness for the case of the B → Z transition.

The method. – We deal with a rigid polyion made of N repeating units. The polyion presents two dissimilar conformers (which will be called B and Z for simplicity) in solution. We start from a reference state, common to both conformers, consisting in a collection of uncharged spheres immersed in an electrolyte solution. In a subsequent step, the spheres are placed at the appropriate positions to build-up the uncharged B and Z forms. As the conformers are made of the same chemical groups, it is natural (and convenient for making further approximations) that they are set up from the same number of sites, $N_{\rm s}$. Finally, N_q atomic groups are charged up. In our pathway, the free-energy difference between the conformers is

$$\Delta G^{\mathrm{B}\to\mathrm{Z}} = \Delta G^{\mathrm{Z}}_{\text{set-up}} + \Delta G^{\mathrm{Z}}_{\text{charging}} - \Delta G^{\mathrm{B}}_{\text{set-up}} - \Delta G^{\mathrm{B}}_{\text{charging}}, \tag{1}$$

where the subscripts "set-up" and "charging" refer to the building of the neutral polyion structure and its charging process, respectively. Notice that we do not establish how detailed the polyion and the solution components should be. For instance, the repeating unit may be made of many sites each of them with a different charge. On the other hand, the polyion might consist in a single charged site replicated along a given pattern. Thus, the method can deal with systems ranging from full atomic DNA description down to a simple charged hard-spheres DNA model. It is only required that both conformers are described at the same level of detail.

The total charging free energy per repeating unit is given by the usual charge integral

$$\Delta G^{\Omega}_{\text{charging}} = \frac{1}{N}\int_0^1 \sum_{i=1}^{N_q} \lambda q_i \varphi_i(\lambda) \mathrm{d}\lambda, \tag{2}$$

where the sum extends over polyelectrolyte sites i with charge λq_i, φ_i is the total electrostatic potential at those points and Ω refers to either the B or Z conformer. The set-up free energy per repeating unit can be calculated through the (multiparticle) potential of mean force which depends on the set of positions of the particles in the Ω form

$$\Delta G^{\Omega}_{\text{set-up}} = \frac{1}{N} W^{(N_\text{s})}(\mathbf{r}^{\Omega}_1,\ \ldots,\ \mathbf{r}^{\Omega}_{N_\text{s}}). \tag{3}$$

The $\Delta G^{\Omega}_{\text{charging}}$ terms may be "exactly" calculated by computer simulation. The difficulties have been transferred to the evaluation of $\Delta G^{\Omega}_{\text{set-up}}$. It is expected that the free-energy difference between the conformers is much lower for the uncharged structures than for the charged polyelectrolytes. In fact, the calculation along a path —from one uncharged form to the other one— seems a waste of computing resources as long as approximate methods should be accurate enough in these conditions. It has recently been shown that the Kirkwood superposition approximation (KSA) predicts acceptable results in inhomogeneous uncharged media [7]. It is then expected that, when the macromolecule sites are not charged, the multiparticle potential of mean force may be safely substituted by a sum over pair potentials using the KSA so that eq. (3) transforms into

$$\Delta G^{\Omega}_{\text{set-up}} = \frac{1}{N} \sum_{m=1}^{N_\text{s}} \sum_{n>m}^{N_\text{s}} W^{(2)}(r^{\Omega}_{mn}). \tag{4}$$

In the above expressions, r^{Ω}_{mn} is the distance between the m and n sites in the Ω form and $W^{(2)}$ is the potential of mean force between those particles dissolved in the electrolyte which is given by

$$W^{(2)}(r_{mn}) = \mu(r_{mn}) - \mu_m(\infty), \tag{5}$$

where $\mu_m(\infty)$ is the chemical potential of the m-th particle in a bulk electrolyte and $\mu(r_{mn})$ is the same quantity when the n-th site is present at a distance r_{mn}.

Application to the B- *to* Z-DNA *transition.* – We have applied the method to the conformational change between simple "empty" B- and Z-DNA models in which the polyion consist solely in charged spheres placed at the phosphate groups ($N_\text{s} = N_q$). The polyion is immersed in an electrolyte solution —modelled at the McMillan-Mayer level— containing the necessary counterions to compensate the DNA charge and additional cations and anions to give the desired salt bulk concentration. The calculations, even for this simple system, are far from trivial as they involve inhomogeneous long-ranged systems. The methodology for the treatment of boundary conditions and long-range forces in the simulations has been described in ref. [8]-[10]. Besides, the comparison with theoretical results is not immediate as long as the theories use different reference states, so additional terms are needed. A full report of the implementation of the method and results will be given in forthcoming publications [11]. A brief summary follows.

The coordinates for the B and Z forms have been taken from Arnott and Hukins [12] and Wang *et al.* [13], respectively. The same potential and parameters —a soft repulsive interaction plus a Coulombic potential— are used for the interactions between all the system particles.

The only difference between the polyion sites and the ions in the solution is that the former are fixed and negatively charged while the latter are mobile and carry positive or negative charges. For the computation of $\Delta G^{\Omega}_{\text{charging}}$, Monte Carlo simulations have been performed for the B and Z polyelectrolytes with the polyion charges switched to several λ values.

The simulation of systems with Coulombic interactions is notoriously tricky. We have used separate treatments for the forces along the radial and axial directions. The modulated bulk as a fuzzy boundary (MBFB) method [8] has been used for the interaction along the radial coordinate. This procedure enables the existence of a bulk region around the inhomogeneous region. With regard to the axial direction, we have used an exact summation formula [10]. As B-DNA has 10 phosphorus atoms per helix turn in each of the strands, there are 20 charged sites per turn in the case of our helical model. Each of the charged sites generates an array of charges when the basic cell is infinitely replicated along the axial direction. The total potential between the charges in an infinite DNA helix and a solution ion is

$$U_{ip}^{\text{helix,inf}} = \sum_{\nu} U^{\text{array,inf}}(\rho_\nu, \Delta z_\nu), \tag{6}$$

where the subscript ν refers to any of the 20 arrays of charge and thus $\xi_\nu = \xi/20$, and $U^{\text{array,inf}}(\rho, \Delta z)$ is the interaction of a charge with an infinitely long array of charges spaced out, which is given by

$$U^{\text{array,inf}}(\rho, \Delta z) = 2 z_i \xi_\nu \beta^{-1} \Big[\log \rho - 2 \sum_{j=1}^{\infty} K_0(\kappa_j \rho) \cos(2\pi j \Delta z / d) \Big], \tag{7}$$

where ρ is the distance from the ion to the array, z_i the electrovalence of the charge, ξ_ν the reduced axial charge density of the array, Δz the axial coordinate of the ion with respect to the closest charged site, d the distance between two consecutive charges along the array, and K_0 is the modified Bessel function of order zero and second kind.

By integrating over the states with $\lambda = 0$, 0.5 and 1 we obtained essentially the same result as using five λ points. The *charging* free energy of the B form is always negative, its absolute value strongly increasing with the concentration of added salt (table I). For Z-DNA this term is positive at moderate ionic concentrations: the electrostatic interactions make this form unstable with respect to the uncharged structure due to the dominant effect of the repulsion among phosphates. At higher concentrations of added salt, the *charging* free energy of Z-DNA becomes negative and shows even a stronger dependence on concentration than the B-form does. In this way, at very high concentrations, the value for both forms becomes closer. This is due to the strong counterion condensation which screens the polyelectrolytes charges and makes the differences between the B and Z charge arrangements not significant. Thus, the *charging* contribution to the free-energy difference is always positive in the conditions studied, its value decreasing with concentration. In other words, the *charging* term clearly favours the B form in dilute saline media but with increasing salt concentrations the difference becomes much less significant.

The *set-up* chemical potential has been computed by Widom's method [14]. Electrolyte solutions at the desired ionic concentrations (but with a neutral particle added) have been simulated. Ghost uncharged-particle insertions are attempted at distances from the reference one covering the whole set of interparticle separations between the polyion sites in the B and Z forms. In this way, all the necessary $\mu(r^{\Omega}_{mn})$ are readily obtained, the asymptotic behaviour at large distances furnishing $\mu(\infty)$. By using eq. (5), the potential of mean force can be evaluated from which the *set-up* term —eq. (4)— is computed. As μ_∞ is a common reference state for both conformers, it does not contribute to the free-energy difference. The *set-up* contribution is always negative, and, in accordance with the assumptions made, its value is *much smaller* than

TABLE I. – *Contributions to the free-energy difference* (*in* k_BT *units per phosphate*) *for the* B- → Z-DNA *conformational change at* 298 K.

Concentration	$\Delta G^{B}_{charging}$	$\Delta G^{B}_{set\text{-}up}$	$\Delta G^{Z}_{charging}$	$\Delta G^{Z}_{set\text{-}up}$	$\Delta G^{B\to Z}_{charging}$	$\Delta G^{B\to Z}_{set\text{-}up}$	$\Delta G^{B\to Z}$
0.5	−0.087	−0.003	0.075	−0.004	0.162	−0.001	0.161
1	−0.205	−0.008	−0.104	−0.013	0.101	−0.005	0.096
2.5	−0.438	−0.014	−0.392	−0.031	0.046	−0.017	0.029
4.3	−0.570	−0.002	−0.536	−0.028	0.034	−0.026	0.008

the *charging* term. On the other hand, we have observed that the computer simulation results for the pair potential of mean force, $W^{(2)}$, are essentially coincident with hypernetted chain integral-equation (HNC) calculations (for more details, see ref. [11]). Besides, the latter have the additional advantage that they are not noisy, and then, their concentration dependence is more reliable with a lower computing cost. For this reason, in table I we present the *set-up* contributions at different concentrations obtained using the HNC equation. The contribution for both forms is always negative, the absolute value slightly increasing with concentration with a greater slope for the Z-DNA. The free-energy difference $\Delta G^{B\to Z}_{set\text{-}up}$ is small and favours the Z-DNA at all concentrations.

The results for the total free-energy difference show that the B conformer is much more stable at low to moderately high concentrations of added salt, the electrostatic interactions with the polyion charges being responsible for this. As the slope of the *charging* and *set-up* terms are negative, both contributions increase the relative stability of the Z form with higher ionic concentrations. The relative importance of each of the terms is manifested by the fact that, from the total change in the free-energy difference in going from 0.5 M to 4.3 M ($0.153\,k_BT$ per phosphate), the *charging* term accounts for about 81% ($0.128\,k_BT$) while only the remainder 19% ($0.025\,k_BT$) is due to the *set-up* term. As we are dealing with a chemical equilibrium, a small value of $\Delta G^{B\to Z}$ (negative or positive) simply indicates that the relative population of the corresponding form (Z or B) is marginally larger. In summary, at low concentrations the population consists almost exclusively of B-DNA while at concentrations at which the transition takes place the population of both conformers is approximately the same. The main effect of the *set-up* term would be to modify the concentration at which the population of both forms is the same, the so-called *transition midpoint*. It is likely that other contributions not included in our model (especially chemical bonding and hydration) would produce larger shifts in the transition midpoint than that due to the *set-up* term.

The method presented here has been used by us to investigate what are the model requirements to give rise to the transition (not any model does it clearly) and the ionic structural changes involved. Other addressed questions which will be the subject of forthcoming reports are a check of the SPMF theory and the dependence of the transition midpoint with the ionic parameters (charge and size specifically).

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