Nucleic Acids Research, Vol. 20, No. 3 415–419

The role of a minor groove spine of hydration in stabilizing poly(dA)·poly(dT) against fluctuational interbase H-bond disruption in the premelting temperature regime

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Received November 15, 1991; Revised and Accepted January 7, 1992

ABSTRACT

Experimental estimates of the premelting Adenine-Thymine base pair opening probability for some B-DNA sequences are two orders of magnitude smaller than those of other B-DNA sequences. The AT pairs in the sequence with smaller open probability seem to be those that have a well defined spine of hydration in the minor groove. We show that this spine of hydration can significantly enhance the thermal stability of the base pairs to which they are attached. The effect of this spine of hydration coupled with the possible stabilization effect contributed from neighboring GC pairs can explain the the differences in the observed AT pair opening probability for different AT containing B-DNA sequences.

INTRODUCTION

In an earlier work[1] we employed the modified selfconsistent phonon approximation (MSPA) theory to evaluate premelting base pair opening probability as well as the probability of the disruption of the amino interbase H-bond of the B-conformation homopolymer Poly(dA)·Poly(dT). In that work and to a larger extent in a similar work on another B-DNA homopolymer Poly(dG)·Poly(dC)[2] we showed that the breakdown of the amino H-bonds can be associated with the 'open state' needed to facilitate amino proton exchange.[3,4] This association held for the two amino protons in a GC pair as well as for the one amino proton in an AT pair. We also showed that a base pair open configuration, i.e. that for which all interbase H-bonds are disrupted in a single base pair, could be associated with the 'open state' needed to facilitate imino proton exchange.[5–7] The agreement between our calculated probabilities and the experimentally determined open state probabilities was very good for GC base pairs. Our calculated open state probability was in agreement with some AT base pair observations but was too large for other sequence measurements.

That work however did not take into account effects associated with the well defined spine of hydration that exists in the minor groove of some DNA polymers. This spine of hydration is known to contribute substantially to the stabilization of the distinctive structure of Poly(dA)·Poly(dT) and its conservative behavior in various circumstances.[8–12] The disruption of this spine of hydration is also believed to be responsible for the observed premelting conformational transitions in Poly(dA)·Poly(dT).[10,13] To a large extent the AT systems our calculation seemed to fit were those considered not likely to have a well defined hydration spine. Those which appeared to have much lower opening probabilities are expected to have a well defined hydration spine. Because the spine of hydration plays such a significant role in the structural stability of DNA, we expect it to also be important in restricting the fluctuational bond disruption and base pair opening probabilities. The present paper is devoted to quantitatively carrying out a model calculation of a polymer with a spine of hydration to determine if such a spine can substantially affect the thermal stability of the interbase H-bonds of Poly(dA)·Poly(dT). We examine how this spine of hydration would influence the base pair opening probability and the disruption probability of the amino interbase H-bond at room temperature (293K). We show that the effect of this minor groove spine of hydration can be of the size needed to explain the differences in the estimated AT pair opening probability for different AT containing polymers.

We point out that our approach has to assume a model of the hydration spine, that is our methods do not allow us to easily solve for the structure of the hydration spine. We can however develop a theory of the disruption of the spine as well as the effect of the spine on the disruption of the interbase H-bonds. Those methods that can be adapted to solve for the hydration structure are not at all useful for studying the melting. From experimental observation base pair breakdown at premelting temperatures occurs on a millisecond time scale.[6,7] Molecular dynamic simulations, which are adapted to structure studies, can be run for times of tens of picoseconds for modest chunks of DNA. At present the simulation can't come withing eight orders of magnitude of observing the disruption behavior. Only theories based on statistical calculations of probability can deal with the slow process of the H-bond disruption. At the current level of development these different classes of theory will have to be used to study the separate problems of H-bond breaking and hydration spine structure.

To achieve results for bond disruption and melting we have to use statistical mechanics methods. The bonds that melt must have a bounded interaction potential, i.e. the interaction potential is finite, usually zero, at large distances. Such bounded potentials lead to infinite partition functions and cannot, in the absence of either limitations on the size of the available phase space, or the presence of a vapor pressure, lead to sensible predictions about bond breaking.[14] The MSPA approach overcomes this limitation

by defining a varying unbounded (harmonic) potential between atoms in the unmelted state. Statistical mechanics can then be performed and measures of a bond breaking achieved.[1,2] MSPA chooses the optimum such effective unbounded potential at each level of excitation of the system, i.e. at each temperature.

The existence of a spine of hydration in the DNA minor groove was first revealed in crystal structure analysis of the B-DNA dodecamer CGCGAATTCGCG.[15,16] Early simulations[10-12] indicate that such a spine of hydration can exist in any segment of a B-DNA sequence that contains A/T runs with AT steps but is less likely in the region that has TA steps. More recent studies[17-19] show that the existence of a minor groove hydration spine depends predominantly on groove width rather than on base sequence. However the exact conditions for the hydration spine are still studied and disputed. Nontheless experimental and theoretical studies to date seems to indicate that the location of water hydrogens and the network of hydrogen bonding depend on base sequence.[19] It is this network of hydrogen bonding that determines how the spine of hydration stabilize the base pairs.

Our model of the spine of hydration is such that the spine is formed by water molecules which zigzag from base pair to base pair along the helix axis in the minor groove. Between two adjacent base pairs a water molecule, which is usually reffered to as the first layer water molecule in the spine, forms a hydrogen bond with a thymine O2 atom in one base and an adenine N3 atom on the other strand in the adjacent base for this chain model. All along the helix water molecules are found joining neighboring AT base pairs. Then to complete the spine, these first layer water molecules are connected to each other by forming hydrogen bonds with a second layer of water molecules located between those which bond to the bases. In this model the spine does cross the gap between strands and should help to stabilize the double helix against strand separation. Aside from this distinct hydration spine, there are a number of other water molecules in and around the two grooves of the base pair. These water molecules however are much less organized compared with the minor groove hydration spine[15,16] and they have much smaller occupancy numbers.[20,21] Compared to the well defined minor groove spine of hydration the effect of these water molecules on the interbase H-bond thermal stability should be small. Therefore we will only examine the effect of the minor groove spine of hydration in this study.

Our analysis finds the point at which bonds can no longer retain bonded integrity in the face of thermal fluctuation. It is not a theory that determines when atoms move apart from each other. The agreement between our calculations and observed bond disruption and melting indicated the gross effects of melting such as atoms separating by large amounts follow shortly after bonds lose this bonded integrity. On the other hand this loss of integrity is a dynamic effect and should be relatively unaffected by outer layers of water. It is primarily a function of dynamics and thermal excitation.

METHOD OF CALCULATION

MSPA formalism

We have previously carried out a normal mode analysis for Poly(dA)·Poly(dT) with the presence of the spine of hydration. We found that our model could fit observed i.r. absorption data fairly well. The parameters used were consistent with the i.r. frequencies.[22] The coordinates of the DNA polymer are the latest version from the fiber study of Arnott and Chandrasekaran.[23] The coordinates of the spine of hydration are determined in accordance with the configuration given in the crystal analysis of Dickerson and coworkers.[15,16] The same structural model will be used in this work. In MSPA theory a DNA helix is represented by a secular equation with all interatomic interactions represented by realistic bounded potentials. These potentials are then replaced, at the atomic level of detail, by MSPA selfconsistent unbounded interactions i.e. effective harmonic force constants.[1,24] The thermal motion of each atom at a certain temperature is determined by a normal mode analysis of the matrix of these force constants. This in turn is used selfconsistently to redefine the effective force constants. The system is iterated to selfconsistency. The secular equation, which is derived by an effective harmonic Hamiltonian, is given by:

$$(\Phi - \omega^2 \mathbf{I})\mathbf{q} = 0 \qquad (1)$$

where Φ is the force constant matrix i.e. the matrix of temperature dependent spring constants, ω and $\mathbf{q}$ are eigenfrequencies and eigenfunctions in a mass weighted Cartesian coordinate system.[1,24] The DNA helical symmetry then allows one to factor this equation into block diagonal form; thus both ω and $\mathbf{q}$ are functions of a phase angle θ in the one dimensional Brillouin zone $-\pi < \theta \leq \pi$, and each block has a secular equation with reduced dimensionality of 123×123. The number 123 is 3 $\times$ the number of heavy atoms per base pair.

To introduce the spine of hydration into the minor groove we add two water molecules per base pair at the positions similar to but not exactly the same as that given in the crystal configuration of Kopka et al.[16] The difference in structure occurs because our helix model is constructed based on the fiber study of a standard B-DNA polymer Poly(dA)·Poly(dT). We do however place the spine water molecules at positions where the length of the water-water and water-base H-bonds are identical to those of Kopka et al. Since the essential dynamic elements arise from the strength of the coupling, this model should give a reasonable estimate of the effect of the spine of hydration not only for our structural model of a standard B-form AT pair but also for AT pairs with narrower minor groove and a slightly different configuration than the standard B conformation. The addition of this spine of hydration for Poly(dA)·Poly(dT) would then increase the dimensionality of the reduced secular equation from 123×123 to 129×129. The additional water-base atom and water-water H-bond stretch force constants are determined in the same way as the interbase H-bond force constants, except that these force constants are factorized by the occupancy number of the water molecules involved. These occupancy numbers are from Westhof.[20,21] The normalized occupancy number for a first layer water molecule is 0.96 and the occupancy number for a second layer water molecule is 0.76. At room temperature (293K) an initial value for these force constants are calculated from the Lippincott-Schroeder model[25] for the atoms and distances fixed by the x-ray structure. Table.1 gives the calculated spring force constants as well as the bond length and the dissociation energy of all the H-bonds in the spine of hydration per base pair. These spring force constants and hence the Morse parameters for the H-bond potentials are then fully determined by this data and are not arbitrary. The water-water H-bond force constants are much stronger than the water-base atom H-bond force constants, therefore the former should be more stable and they can be assumed to be independent of temperature. On the other hand the two water-base atom H-bond force constants as well as other interbase H-bond force constants are determined selfconsistently

Table 1. Force constant ϕ, bond length R and dissociation energy E of the H-bonds in the minor groove spine of hydration of of Poly(dA)·Poly(dT).

bond	ϕ kcal/mole·^{2}	R Å	E kcal/mole
N3-W1 (water-base)	11.66	2.886	1.877
O2-W1 (water-base)	14.98	2.829	2.276
W1-W2 (water-water)	73.08	2.639	6.124
W2-W1 (water-water)	59.34	2.661	5.461

W1 is the water molecule in the first layer in the spine. W2 is the water molecule in the second layer in the spine. The first water-water H-bond is the bond that connects the two water molecules in the same base pair and the second water-water H-bond is the bond that connects a water molecule to another water molecule in the neighboring base pair.

Table 2. Morse parameter and maximum bond stretch length of the water-base H-bonds and the interbase H-bonds of Poly(dA)·Poly(dT).

bond	a Å^{-1}	r_0 Å	V_0 kcal/mole	L Å
N3-H-O (water-base)	2.316	2.634	3.111	3.158
O2-H-O (water-base)	2.004	2.768	2.304	2.999
6-H-O4 (interbase)	2.356	2.758	2.492	3.188
1-H-N3 (interbase)	2.337	2.795	2.319	3.120

by MSPA formalism i.e. through a weighted average over H-bond stretch at each temperature by the following equation:

$$\phi_i = C_i \int_{-h_i}^{\infty} du e^{-u^2/2D_i} \frac{d^2}{du^2} V_i(R_i + u) \quad (2)$$

where i is the index for the two water-base atom H-bonds in the spine of hydration as well as for the interbase H-bonds. C_i is the normalization factor:

$$C_i^{-1} = \int_{-h_i}^{\infty} du e^{-u^2/2D_i} \quad (3)$$

D_i is the H-bond mean square stretch amplitude given by

$$D_i = \frac{1}{\pi} \sum_{\lambda} \int_0^{\pi} d\theta \frac{|S_i^{\lambda}(\theta)|^2}{2\omega_{\lambda}(\theta)} \coth \left[\frac{\omega_{\lambda}(\theta)}{2k_B T}\right] \quad (4)$$

i.e. is a thermal weighted incoherent sum over all the normal vibrational band mode (represented by the index λ) contributions projected onto the H-bond stretch squared motion. Here $\omega_{\lambda}(\theta)$ is the eigenfrequency and $S_i^{\lambda}(\theta)$ is the stretch of the i-th H-bond contributed from the λ-th band at a particular phase angle θ. h_i is the limiting inner turnaround point[1] and R_i is the mean equilibrium H-bond length found in MSPA as the centroid of the oscillatory motion between classical turnaround points. This is given by the condition:

$$V_i(R_i+\mu_i) = V_i(R_i-\mu_i) \quad (5)$$

where $\mu_i = 2\sqrt{2D_i \ln 2}$. The potential V_i in Eq.[2] and Eq.[5] is a Morse potential which we have chosen as a model of the hydrogen bonding interaction[1,24]:

$$V(r) = V_0 (1-e^{-a(r-r_0)})^2 - V_0 \quad (6)$$

Here V_0, a and r_0 are the parameters of the Morse potential fitted to room temperature data for each H-bond.[1,24] The Morse potential used in MSPA is an effective potential. Its parameters are determined by fitting to H-bond length, force constant and dissociation energy. Because of cooperative effects, the introduction of a minor groove spine of hydration results in a slightly different Morse parameters from those of earlier work.[1] Table.2 displays these parameters for all the water-base atom and interbase H-bonds of Poly(dA)·Poly(dT).

The inclusion of the spine of hydration also introduces the need for additional angle bending force constants between water molecules and base atoms. We approximate these angle bend force constants by taking the average of the stretch force constants of the bonds involved and then dividing by seven. The choice of $^1/_7$ of the magnitude of the stretch force constants is based on a comparison with other valence angle bend force constants and the relevant stretch force constants in DNA.[22] For those angle bend force constants that involve a water-base atom H-bond, we further scale these force constants by a factor that is proportional to the (weakest) stretch force constant of the water-base atom H-bond involved. This is done to allow for the possibility that the angle bend motion would disappear if one of the bonds involved is disrupted. Using these particular force constants and a large refined valence force field, plus a non-bonded interaction set, the force constant matrix of Eq.[1] is formed.[22] The normal modes are found, the force constants are reevaluated and the entire process is iterated until selfconsistent solution.

Base pair open state

Because of thermal fluctuation, there is a certain probability that a particular H-bond is disrupted at temperatures far below the melting temperature. The probability P_i for the disruption of the i-th H-bond as determined by the MSPA theory[1] is given by

$$P_i + C_i \int_{L_i}^{\infty} du \exp\left[-(u-R_i)^2/2D_i\right] \quad (7)$$

where L_i is the stretch at which the H-bond is broken. In MSPA it is the point at which the selfconsistent solution becomes unphysical.[1] The L_is result from MSPA calculation on the particular system studied. The values of L_is for all the water-base and interbase H-bonds of Poly(dA)·Poly(dT) are given in Table.1. Again we use a well defined procedure to determine the L_i parameters. They are not arbitrarily fitted to the resulting probabilities.

In earlier work[1,2] we also defined a base pair open state as an all interbase H-bond disrupted state. This open state is correlated with the open configuration needed for imino proton exchange. That definition, however, should only be valid for those base pairs that do not have a spine of hydration in the minor groove. For a base pair with a spine of hydration attached to its minor groove, one of the water-base H-bonds in the spine of hydration has to breakdown as well. The two water-base atom H-bonds connect the Adenine N3 atom of a base pair on one strand with the Thymine O2 atom of the neighboring base pair on the other strand. We therefore define the open state for a base pair with a spine of hydration as the state in which all of its interbase H-bonds and at least one of the water-base H-bonds in the spine of hydration are disrupted. The probability for this open state is then given by:

$$P_{op} = (P_{W-N3} + P_{W-O2} - P_{W-N3} \times P_{W-O2}) \prod_{interbase} P_i \quad (8)$$

where P_{W-N3} and P_{W-O2} are the probability of the disruption of each of the two water-base atom H-bonds in the spine of hydration, and the product runs over all the interbase H-bonds of a base pair.

Table 3. Calculated force constant ϕ, mean square stretching amplitude D, mean bond length R and bond breaking probability P for the water-base H-bonds and interbase H-bonds of Poly(dA)·Poly(dT).

bond	ϕ kcal/Å^2·mole	D Å^2	R Å	P
N3-H-O (water-base)	11.66	0.048	2.886	0.108
O2-H-O (water-base)	14.98	0.011	2.829	0.057
N6-H-O4 (interbase)	16.85	0.020	2.880	0.016
N1-H-N3 (interbase)	16.56	0.018	2.900	0.049

RESULTS AND DISCUSSION

The spine of hydration clearly crosses the gap between strands of the double helix and certainly adds additional interactions between the strands. It is no surprise that increased stability against separation results. The judgement as to the value of the current calculation then has to be based on the quantitative change in the predicted base opening probability and the relationship of that to details of the appropriateness of the parameters that went into the calculation. MSPA is a hybrid dynamic-statistical theory but it does use dynamic interatomic potentials as input rather than the generalized thermodynamic parameters that are used in, for example, the nearest neighbor helix-coil transition theory. The thermodynamic parameters are fit to data of base pair opening, the dynamic potential data are fit to spectroscopic data at room temperature. Opening probabilities determined by parameters fitted to opening measurements necessarily give order of magnitude fit, however results based on data not fit to the phenomena observed do not ac give such quantitative agreement. For this calculation the force field of the double helix was refined to Raman and i.r. data.[22,26-28] The force constants for the H-bonds for the connections in the spine and helix system were determined from the Lippincott-Schroeder model using observed x-ray distances. The Lippincott-Schroeder model is essentially a phenomenological model derived from a wealth of spectroscopic data.[25] As one can see from Eq.[7] the bond disruption probability varies exponentially with the mean square fluctuational amplitude D and the thermal expansion R. These parameters depend sensitively on the values of the interatomic potentials. The prediction of open bond probability is then a sensitive function of parameters which in this case are determined by observations not simply related to the quantity calculated. The quantitative agreement is then relevant as an indication of the significance of the approach.

Table.3 gives the calculated physical quantities of the water-base atom H-bonds and interbase H-bonds of Poly(dA)·Poly(dT) at 293K. Using the data given in Table.2 we find a base pair opening probability $P_{op} = 1.26\times10^{-4}$. This is compared to our earlier estimate of 6.76×10^{-3} for Poly(dA)·Poly(dT) without the presence of the spine of hydration.[1] Our calculation shows that the addition of the spine of hydration does stabilize the base pair substantially. The spine of hydration acts almost like an additional interbase H-bond bringing the P_{op} close to that which we find for GC pairs.[2]

An understanding of the effect of the spine of hydration could help to resolve a long standing puzzle regarding the value of the experimentally measured P_{op} for a B-DNA AT pair at premelting temperatures. Measurements from the formaldehyde-induced denaturation of Poly[d(A-T)][29] and from the imino proton exchange of Poly(rA)·Poly(rU)[6,7] gives a $P_{op} \sim 10^{-3}$. On the other hand measurement from the imino proton exchange of a B-DNA oligomer CGCGATCGCG[7,8] gives a $P_{op} \sim 10^{-5}$ for the AT pair. Much of the difference can arise from the effect of a minor groove spine of hydration, and to a certain extent the presence of neighboring GC pairs in the oligomer should also contribute to the stability. Although knowledge of when and where the spines exist is still an active area of research, some computer simulations[10-12] showed that a well defined minor groove spine of hydration can be formed on A/T (and the equivalent I/C) runs of a B-DNA sequence containing no TA (CI) steps. Studies of the hydration of several oligonucleotides[20,21] indicates that the water molecules of the spine have much higher occupancy numbers than those of non-spine water molecules. A recent work[30] on the crystal structure analysis of several B-conformation DNA oligomers CCAACGTTGG, CCAAGATTGG and CCAGGCCTGG revealed that regions of the minor groove of the AT pairs tend to be narrower than average and narrow regions of minor groove exhibit a zig-zag spine of hydration. Moreover the central region of an oligomer seems to have a narrow minor groove even if some of the base pairs involved are GC pairs. This is in general agreement with the results from several simulation studies.[18,19] Other analyses on the central AT regions of the B-DNA oligomer CGCGAATTCGCG[15,16] and CGTGAATTCACG[31,32] exhibited that the spine of hydration in the minor groove of the middle section AT pairs only extends to the neighboring GC pairs and begins to crumble past that base pair. According to the criterion drawn from these studies no well organized spine of hydration should exist in either Poly[d(A-T)] or Poly(rA)·Poly(rU). Indeed the estimated P_{op} for these polymers agrees with our calculation for Poly(dA)·Poly(dT) without the spine of hydration.[1] On the other hand the AT pairs in the middle section of the oligomer CGCGATCGCG assumes an AT step. Therefore a spine of hydration can be formed in the minor groove of these two AT pairs. Because of the stabilizing effect of this spine of hydration as well as that of neighboring GC pairs, the P_{op} for the AT pairs has to be substantially smaller than that of Poly[d(A-T)] or Poly(rA)·Poly(rU) as observed.

In addition to the stabilization resulting from the hydration spine as mentioned the AT pairs in CGCGATCGCG will be further stabilized by adjacent GC pairs. We have carried out calculations on a TATA insert in Poly[d(A-C)].[33] In that calculation the AT pairs were stabilized over the value found for poly[d(A-T)]. The P_{op} for these AT pairs were found to reduce to 41% of that for poly[d(A-T)]. AT pairs embedded in Poly[d(G-C)] would be stabilized to an even greater extent. The 0.41 factor when applied to our AT values with the hydration spine reduced the P_{op} to 5×10^{-5}. This 0.41 factor would be smaller for two AT pairs sandwiched in a number of GC pairs rather than the case for which it was calculated. Therefore the H-bond thermal stability of the AT pair in question should be stronger than described in this work due to these effects. These combined stabilization effects of further stabilizing the AT pairs for the oligomer CGCGATCGCG could well bring the P_{op} for the AT pairs down to 10^{-5}.

The helix and the spine of hydration is dynamically interconnected. The hydration spine is cooperative with the helix dynamics by altering the normal mode solution. The presence of the minor groove spine of hydration affects the motion of the interbase H-bonds. Consequently it affects the bond disruption probability of the interbase H-bonds. The disruption of the amino interbase H-bond can be measured by amino proton exchange.[1,2] For an AT pair there is one amino interbase H-bond (N6-H-O4

bond). In this work our calculated probability for the AT pair in this amino proton exchangeable state is P_{am} = 0.016. This is compared to the calculation of P_{am} = 0.063 of our earlier work where the spine of hydration is not considered. This result indicates that the P_{am} for the base pair with a minor groove spine of hydration is smaller than that of the base pair without such a spine of hydration.

ACKNOWLEDGEMENTS

This work supported in part by Indiana Elks Purdue Cancer Center Grant and ONR Grant N00014-89-K-0115.

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