Changes in the Zero-Point Energy of the Protons as the Source of the Binding Energy of Water to *A*-Phase DNA

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The measured changes in the zero-point kinetic energy of the protons are entirely responsible for the binding energy of water molecules to *A* phase DNA at the concentration of 6 water molecules/base pair. The changes in kinetic energy can be expected to be a significant contribution to the energy balance in intracellular biological processes and the properties of nano-confined water. The shape of the momentum distribution in the dehydrated *A* phase is consistent with coherent delocalization of some of the protons in a double well potential, with a separation of the wells of 0.2 Å.

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Chemical interactions occurring in bulk water typically represent small changes in the energy of constituents compared to the energy sequestered in the zero-point motion of the protons in the water, primarily in that of the stretch mode. To the extent that this energy does not change in the interaction, it may be ignored, and usually is. Nearly all simulations of water in biological systems are done with models of water for which these changes cannot occur, or if they can, are not considered because of the additional expense of treating the protons quantum mechanically. The energy does change, however, as the structure of the hydrogen bond network changes. With the advent of Deep Inelastic Neutron Scattering measurements [1], it has become possible to observe the changes in zero-point kinetic energy directly. These changes can be large ($\geq kT$) and in either direction. Water confined in carbon nanotubes has a transition in which the kinetic energy of the protons in each water molecule is reduced by 6 kJ/mol [2], while water confined in the smallest pores of xerogel the kinetic energy increases by 14 kJ/mol [3]. We present here a system where zero-point energy changes play the dominant role in the transformation of DNA from the *A* to the *B* phase with the absorption of water.

DNA is hydrophylic, changing its structure as it incorporates water molecules, transforming continuously from the *A* phase to the biologically active *B* phase as the number of hydrated water molecules increases from 11 to about 20 [4]. The two structures are shown in Fig. 1, and are characterized by changes in the pitch of the helix, from 10 base pairs (bp) in *B* to 11 bp in A, the angle of the base pairs to the helix axis, from $-1.2°$ in *B* to $+19°$ in *A*, and changes in the diameter of the molecules, from 20 Å in *B* to 26 Å in A. More details can be found in Saenger [5], chapter 9. *A* recent x-ray study of the structure with different amounts of water of hydration is given in Egli *et al.* [6] Even before the DNA double helix structure had been resolved, Franklin and Gosling [7] recognized that the *B* phase only existed for natural DNA fibers, at relative humidities (r.h.) greater than 75%. Since then, careful studies have shown that the absorption of water and the changes in structure that accompany that absorption are reversible, and the number of water molecules/base pair (w/bp) has been determined accurately as a function of r.h. [8,9].

The energy input necessary to remove a molecule of water at any level of hydration and place it in bulk water can be measured with differential scanning calorimetry. It is on the order of 10 kJ/mol/water molecule, decreasing with increasing numbers of hydrating waters [10]. The attraction that leads to the hydrophylicity, and the source of the energy that produces the deformation of the *A*

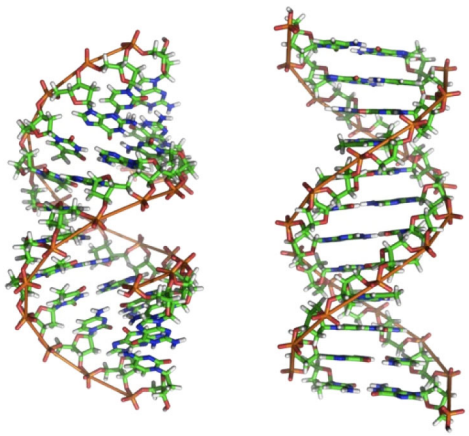

FIG. 1 (color online). *A* Phase (left) and *B* phase (right) of DNA. The *A* phase is the stable state in the absence any hydrating water molecules. It requires about 20 w/bp (water molecules not shown) to convert it to the *B* phase. Hydrogen is shown in white. Figure used by permission of Richard Wheeler, Sir William Dunne School of Pathology, Oxford University.

structure to become the B structure, is usually thought of as primarily electrostatic [11]. However, as the configuration of the surroundings of the water molecule change, the potential energy surface of the proton changes. As a consequence, the ground state wave function changes, and with that there is a concomitant change in the zero-point kinetic energy of the proton. It has recently become possible to measure accurately the kinetic energy of protons in water and other hydrogen bonded condensed matter systems [1]. This energy is almost entirely due to the zero-point motion of the protons. As the water of hydration is incorporated into DNA, the structure surrounding both the protons in the water and those in the DNA changes, and we would expect changes in the kinetic energy of the protons involved. We show here that reductions in the kinetic energy of the zero-point motion of the protons in weakly hydrated salmon DNA, containing 6 w/bp account for the entirety of the enthalpy of hydration of the molecules, at that level of hydration. There is also evidence, in the shape of the momentum distribution, that some of the hydrogen bonds in the dry DNA, have a double well character, as revealed by a characteristic oscillation in the momentum distribution. This property is lost upon hydration.

The kinetic energy of the protons in the DNA-water complex is readily measured using deep inelastic neutron scattering (DINS) [1]. At high energy transfers, the usual neutron scattering function $S(\vec{q}, \omega)$, which gives the differential cross section for scattering, is described by the impulse approximation limit, in which the target particle behaves as a free particle for the duration of the scattering process. It is given by

$$S(\vec{q}, \omega) = \int n(\vec{p}) \delta\left(\omega - \frac{\hbar q^2}{2M} - \vec{q} \cdot \frac{\vec{p}}{M}\right) d\vec{p}, \qquad (1)$$

where $n(\vec{p})$ is the momentum distribution of the protons, $\hbar\omega$ and $\hbar\vec{q}$ the transferred energy and momentum, respectively, and M the mass of the proton. $S(q, \omega)$ is thus the radon transform of $n(\vec{p})$, and as the transform is invertible, $n(\vec{p})$ can be obtained directly from the scattering data. This inversion is accomplished by a method [12] in which the momentum distribution is represented, for samples such as ours that are isotropic , as a series expansion

$$n(p) = \frac{e^{-(p^2/2\sigma^2)}}{(\sqrt{2\pi}\sigma)^3}\left[1 + \sum_{n=2}^{\infty} a_n (-1)^n L_n^{1/2}\left(\frac{p^2}{2\sigma^2}\right)\right], \qquad (2)$$

where $L_n^{1/2}(\frac{p^2}{2\sigma^2})$ are the associated Laguerre polynomials, and the parameter σ is related to the kinetic energy of the proton by $E_{\mathrm{kin}} = \frac{3\hbar^2}{M}\sigma^2$ [13]. The coefficients a_n are determined by a least squares fit of the data for $S(\vec{q}, \omega)$. The instrument used was Vesuvio, at ISIS, the pulsed neutron source at the Rutherford Laboratory in England. The instrument has 48 detectors, arranged with scattering angles from 35 to 75° on both sides of the beam. Since, in the impulse approximation limit, the data is determined entirely by the scaled variable $y = \frac{M}{q}(\omega - \frac{\hbar q^2}{2M})$, all detectors give the same information when the time of flight data is converted to the y variable, although the average value of q varies greatly with scattering angle of the detector. The parameters of the fit are obtained by simultaneously fitting the data from all the detectors. The raw data in the variable y collapses to give the Compton profile $J(y) = \frac{q}{M} S(q, \omega)$, shown in Fig. 2.

The measurements were made on fibrous salmon Na-DNA obtained from Sigma. The fibers were mechanically broken up into segments 2 mm in size to insure a random orientation in the sample. The total sample, approximately 5 g, was weighed and packed into an aluminum container. No special attempt had been made to control the humidity of the sample environment, which had been the local atmosphere for some weeks. The container was sealed with an indium seal, and placed in the neutron beam in an evacuated chamber. After obtaining the desired number of counts, the sample was removed from the container, weighed in air, and placed in a vacuum at 40 °C for 24 h. The sample was reweighed, again in air, and packed into the same container and resealed. The weighing took approximately 20 min. After counting for a day, the sample was removed and promptly reweighed. The difference between the weight upon removal from the dehydrating oven and upon removal from the sample holder was 0.12 g, presumably due to hydration during the period the sample was being packed into the sample holder or being weighed the second time, indicating that the protocol was

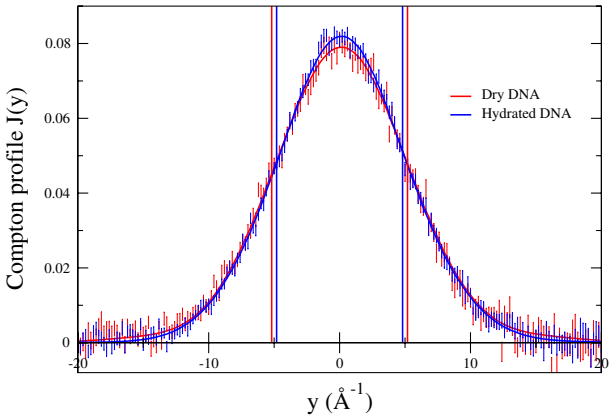

FIG. 2 (color online). The time of flight data from all 48 detectors, rebinned in the impulse approximation scaling variable y. The resolution function and the small corrections to the impulse approximation have not been corrected for here, leading to larger error bars than for the signal itself, as the uncorrected data does not scale precisely with y. The series expansion fit for $J(y)$, the solid lines, are obtained by simultaneously fitting the data sets for all 48 detectors, including the correction for the resolution function and deviation from the impulse approximation limit for each detector. The vertical lines give the values of σ for the two data sets. The statistical errors in $n(p)$ obtained from these fits are shown in Fig. 3.

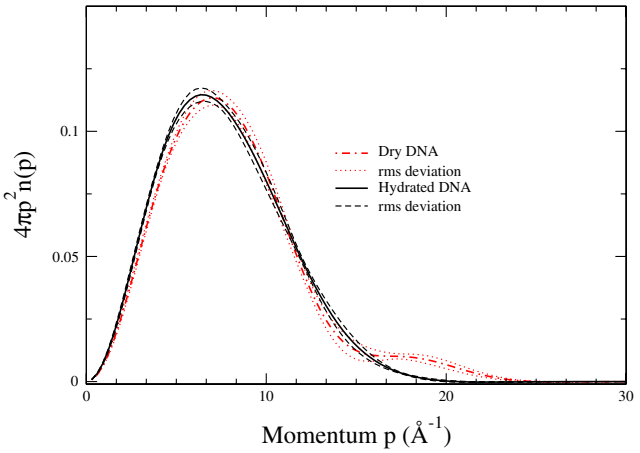

FIG. 3 (color online). Comparison of the average momentum distribution of the protons in hydrated salmon DNA with that in dry salmon DNA. The oscillation in the distribution of the dry sample indicates coherent delocalization of some of the protons in a double well. The position of the local minimum allows an estimation of the separation of the wells as 0.2 Å.

sufficiently accurate as to the weight of the sample for our purposes. The weight of the initial hydrated sample was 4.76 g, that of the dehydrated sample as removed from the sample holder 4.16 g. We will use these values to calculate the number of water molecules/nucleotide removed by the dehydration process. This is accomplished using the known stoichiometry of the Na-DNA salt. We used the value for the ratio of G-C to A-T of 0.436 [14,15]. Our results are very insensitive to this ratio, as the weight ratio of protons to heavier atoms in the G-C and A-T pairs is nearly the same. The number of water molecules removed is 6/nucleotide, which is sufficient to produce a substantial change in the structure of the DNA [9]. This is the number expected for the typical levels of relative humidity experienced by the sample [8,9].

A comparison of the radial momentum distributions, $4\pi p^2 n(p)$, of the protons in DNA with (hydrated) and without the hydrated water molecules (dry) is shown in Fig. 3. The values of σ and the significant parameters in the fits are shown in Table I for the hydrated and dry samples. The error bars shown in the figure are calculated from the least squares fitting procedure, and include the correlations between the parameters. We can see immediately that the dry sample has a larger second moment, as is confirmed by the values of σ in Table I. Moreover, $n(p)$ is not monotonic for large p. The appearance of an oscillation in the momentum distribution is characteristic of a population of protons that are coherently distributed in the ground state of a double well potential. The bimodal spatial wave function, when Fourier transformed to give a momentum wave function, produces oscillations with a wavelength in momentum space of π/d [12], where d is the separation of the wells. In this case, we obtain a value of d of 0.2 Å.

TABLE I. The fitting coefficients and their variances for the momentum distribution measured in dry and hydrated DNA.

Parameters of measured $n(p)$				
	Hydrated		Dry	
σ (Å^{-1})	4.81 ± 0.028		5.19 ± 0.067	
n	a_n	δa_n	a_n	δa_n
2	0	⋯	0.657	0.134
3	−0.259	0.075	0	⋯
4	0	⋯	−0.438	0.177
5	0	⋯	0	⋯
6	0.086	0.101	0.354	0.178

Evidently, the hydration of DNA, at least for the first 6 molecules of water, is accompanied by a reduction of the zero-point kinetic energy of the water-DNA system. That energy is available to produce a distortion of the A phase of the DNA towards the B phase. We wish to see how significant it is compared to the enthalpy change per water molecule upon removing the same number of water molecules from the DNA and placing them in bulk water. This has been measured, by Virnik *et al.* [10] and others [16,17], using differential scanning calorimetry. The value is 3.0 ± 0.15 kJ/mol for salmon DNA at this level of hydration. To do this, we compare the total kinetic energy of the dry DNA together with 6 water molecules in bulk water, with that of the hydrated DNA.

That is, if the number of protons in the dry sample is N_{DNA}, and the number of protons in the water that was removed from the hydrated sample is $N_{\mathrm{H_2O}}$, then in the hydrated sample the total kinetic energy is $(N_{\mathrm{H_2O}} + N_{\mathrm{DNA}})\frac{3\hbar^2}{2M}\sigma^2_{\mathrm{Wet}}$. The kinetic energy of the dry sample is $N_{\mathrm{DNA}}\frac{3\hbar^2}{2M}\sigma^2_{\mathrm{Dry}}$ to which must be added the kinetic energy of the water molecules in bulk water, $N_{\mathrm{H_2O}}\frac{3\hbar^2}{2M}\sigma^2_{\mathrm{H_2O}}$. The change in the kinetic energy/proton, averaged over all the protons, from the dry state to the hydrated state is therefore.

$$\Delta E_{\mathrm{kin}} = \frac{3\hbar^2}{2M}[N_{\mathrm{DNA}}\sigma^2_{\mathrm{Dry}} + N_{\mathrm{H_2O}}\sigma^2_{\mathrm{H_2O}} - (N_{\mathrm{DNA}} + N_{\mathrm{H_2O}})\sigma^2_{\mathrm{Wet}}]. \quad (3)$$

The change in the kinetic energy per proton is therefore

$$\Delta E_{\mathrm{kin}}/N_{\mathrm{tot}} = \frac{3\hbar^2}{2M}\left[\frac{N_{\mathrm{DNA}}}{N_{\mathrm{tot}}}(\sigma^2_{\mathrm{Dry}} - \sigma^2_{\mathrm{Wet}}) + \frac{N_{\mathrm{H_2O}}}{N_{\mathrm{tot}}}(\sigma^2_{\mathrm{H_2O}} - \sigma^2_{\mathrm{Wet}})\right], \quad (4)$$

where $N_{\mathrm{tot}} = N_{\mathrm{DNA}} + N_{\mathrm{H_2O}}$. The sigma parameter for bulk water was measured at the same time as the other experiments described here. The value of $\sigma = 4.76 \pm 0.035$ Å is consistent with earlier measurements [18]. With the ratio of protons in the dry DNA to that in the hydrated DNA as calculated from the stoichiometry as 0.696, and the values

for the σ parameters and their uncertainty given in Table I, we find the average change in the kinetic energy/water molecule, to be, since there are two protons in a water molecule

$$2\Delta E_{\mathrm{kin}}/N_{\mathrm{tot}} = 3.02 \pm 0.51 \text{ kJ/mol}. \quad (5)$$

That is, the change in kinetic energy of the protons in the combined water-DNA system, in going from the dry phase, with the water molecules in bulk water and the DNA unhydrated, to the hydrated phase, completely accounts for the measured enthalpy change. This does not mean that the electrostatic interactions are insignificant compared to the kinetic energy changes, only that the reduction in the electrostatic potential energy from the incorporation of the molecules of water in the DNA is nearly equal to the increase in the elastic potential energy of the *A* phase as it deforms into the *B* phase. The reduction in zero-point energy then provides the binding energy for the water molecules.

The extent to which this change in zero-point energy can be attributed to the protons in the hydrating water, rather than the hydrogen bonds in the DNA is unknown. Comparable changes in the kinetic energy of protons in water molecules have been seen in water confined in carbon nanotubes [2] and in xerogel [3], so one might think most of the change is due to changes in the environment of the protons in the water molecules. The shape of the momentum distribution for the dry phase, however, suggests changes in the base pair hydrogen bonds, as the potentials for the protons there are sensitive to the separation of the base pairs, and this is easily changed with a structural deformation. It is also the case that the measured changes in the stretch and bending modes vibrational frequencies for the hydrated water, which account for most of the zero-point energy, are far too small (at most 1.5%) to account for the changes in the kinetic energy if we assume the protons in the water remain in approximately harmonic potential wells [19,20]. In any case, our results show that a correct description of the energetics of hydration of DNA requires that the quantum delocalization of the protons in the system be accounted for. We expect this will be generically true of other systems in which the hydrogen bond network is strongly distorted, due for instance, to the confinement of the water in cells, or in the interstices of polymer electrolyte membranes such as Nafion.

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