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Short Note

Slow relaxation process in DNA at different levels of hydration

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Abstract. There are many speculations about the dynamic transition observed in hydrated bio- polymers at temperatures $T \sim 200 - 230$K being an important factor for enabling of their functions. The transition shows up as a sharp increase of atomic mean–squared displacements above this temperature. The nature of the dynamic transition is not yet clear. Using inelastic neutron scattering we show in this Note that the transition in DNA is related to the appearance of a slow relaxation process. Decrease in the hydration level suppresses the process and the dynamic transition. It is found that, in terms of dynamics, the decrease in water content is similar in effect to a decrease in temperature. The obtained results support the idea that the dynamic transition is mediated by the water of hydration since bulk water has a dynamic transition around the same temperature.

One key for understanding functions of bio-molecules is the analysis of their motion. Analysis of the kinetics of chemical reaction shows that many complicated relaxation processes are involved in enabling the functions [1, 2]. The relaxation processes correspond to conformational changes of the bio-molecules. Various experimental techniques demonstrate the presence of a dynamic transition in different bio-polymers at temperatures $T \sim 200 - 230$ K. In particular, it shows up as a change in the slope of the temperature dependence of the atomic mean–squared displacements $\langle x^2 \rangle$. This onset of anharmonicity appears around the temperature where functions of bio-polymers slow down significantly. These results lead to speculations about a relationship between functions and anharmonicity of molecular motion [2, 3, 4, 5]. However, it is not clear what types of anharmonic motions are responsible for the sharp rise of $\langle x^2 \rangle$ and how they can be related to biochemical activities of the molecules. The main goal of this Note is the analysis of the dynamic structure factor of DNA at different temperatures and levels of hydration in order to find out details of the dynamics that change around $T \sim 200 - 230$ K.

Oriented Li-DNA-fibers were obtained by the wet spinning method [6]. The samples were hydrated with D_2O to different levels by exposing them for several weeks to the vapor of saturated KBr ($\approx$ 81% r.h.), $NaClO_3$ ($\approx$ 75% r.h.) and $LiCl$ ($\approx$ 11% r.h., the "dry" sample) solutions in D_2O. The neutron scattering spectra were measured on IN5 time-of-flight spectrometer at ILL (Grenoble). Two wavelengths of incoming neutrons, 5 Å and 8 Å, were used in order to cover a sufficiently broad energy range. The data were corrected for the container contribution, for self absorption and multiple scattering in the isotropic

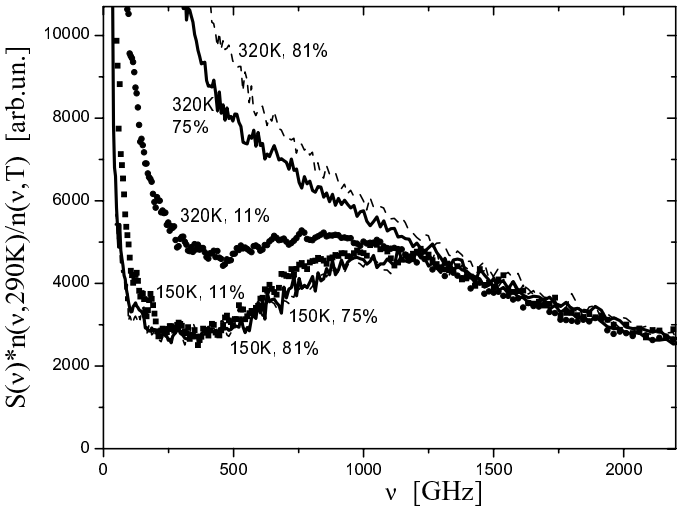

Figure 1. Dynamic structure factor of DNA at different temperatures for samples with 11%r.h. (symbols), 75% r.h. (solid lines) and 81% r.h. (dashed lines). The spectra are scaled by the Bose factor to 290 K and averaged over scattering $56^\circ < \phi < 126^\circ$ (corresponds Q-range $\sim 1-3$ Å).

approximation. As a consequence the corrected spectra essentially coincided in the high frequency region and continuity between the 5 Å and 8Å measurements was achieved.

Due to the extremely high incoherent cross-section of hydrogen atom, the neutron spectra reflect essentially the motion of hydrogens. Substitution of the *H*-atom by deuterium decreases the scattering contribution by roughly a factor of 40. Thus the neutron scattering spectra of DNA/D_2O reflect the motion of hydrogen atoms of the DNA molecule [7]. The frequency range of our interest ($\nu < 1$ THz) is two orders of magnitude lower than the frequency of a local hydrogen atom vibrations. Thus the motion of H-atoms at these frequencies corresponds to cooperative motion of large parts of the molecule.

Figure 1 presents neutron scattering spectra of the three samples at a few temperatures. The spectra are scaled by the Bose factor, $n(\nu,T) = [exp(h\nu/kT) - 1[^{-1}$, to $T = 290$ K in order to simplify for the trivial temperature dependence. At frequencies above ~ 1 THz the scaled spectra do not show significant temperature variations. This corresponds to harmonic behavior of vibrations at these frequencies. The strong increase of the quasielastic scattering at frequencies below ≈ 1 THz with temperature is a quite general for soft matter and corresponds to an activation of a relaxation–like motion. This anharmonic contribution is evident in the spectra of all samples, but its increase with temperature is much more pronounced for the samples with high humidity level. Figure 2 shows the temperature dependence of the integrated quasielastic intensity that can be related to the mean-squared displacement scaled by kT, $\langle x^2\rangle/kT$. The data in Figure 2 demonstrate a strong increase at T above ~ 200 K for samples with high humidity level and a gradual increase for the "dry" sample. These results are similar to many previous observations obtained on bacteriorhodopsin [8], myoglobin [5] and other bio-polymers. Thus they reflect a general

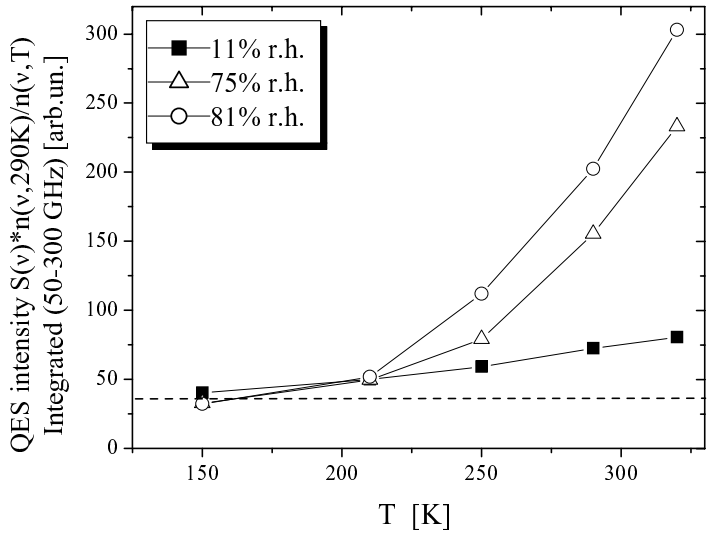

Figure 2. Temperature variations of the normalized quasielastic intensity integrated in the frequency range 50-300 GHz. The dashed line shows expected harmonic behavior.

trend in the dependence of low frequency dynamics of bio-polymers on temperature and humidity.

In order to emphasize details of the quasielastic (relaxation-like) spectra, Figure 3 presents them in terms of the imaginary part of the susceptibility. The susceptibility presentation, $\chi''(q,\nu) \propto S(q,\nu)/n(\nu)$, has advantages in comparison with the traditionally used dynamic structure factor $S(q,\nu)$: relaxation process appears as a peak at $\nu = (2\pi\tau)^{-1}$ (τ is an average relaxation time). As a result the processes strongly separated in time (or frequency) appear as separated peaks. A relaxation process with a single exponential decay will have $\chi''(\nu) \propto \nu$ at low frequency ($2\pi\nu\tau \ll 1$) and $\chi''(\nu) \propto \nu^{-1}$ at high frequency ($2\pi\nu\tau \gg 1$).

Analysis of the spectra presented in Figure 3 clearly demonstrates the presence of two processes: the "fast" one that dominates the spectra at $\nu > 100$ GHz and the "slow" one that dominates at $\nu < 100$ GHz. The latter shows strong dependence on both T and relative humidity. The resolution of our spectrometer is not sufficient to resolve the process and only the high frequency tail is observed. The tail is strongly stretched, $\chi''(\nu) \propto \nu^{-b}$, with $b \sim 0.25$ being rather temperature independent. That corresponds either to a complex non-exponential relaxation process or to a very broad distribution of relaxation times.

Only the fast process is present at the lowest temperature and the quasielastic contribution to the spectrum in dry sample is stronger than in the samples with higher humidity (Figures 1,3). The result suggests that decrease in water content increases flexibility of the DNA molecule at lower temperatures.

The spectra of DNA show weak dependence on level of hydration up to $T \sim 210$ K (Figure 2). However, the situation changes at higher temperatures. Distinct differences develop at $T \sim 250$K, e.g. just above the dynamic transition. The result of the presented

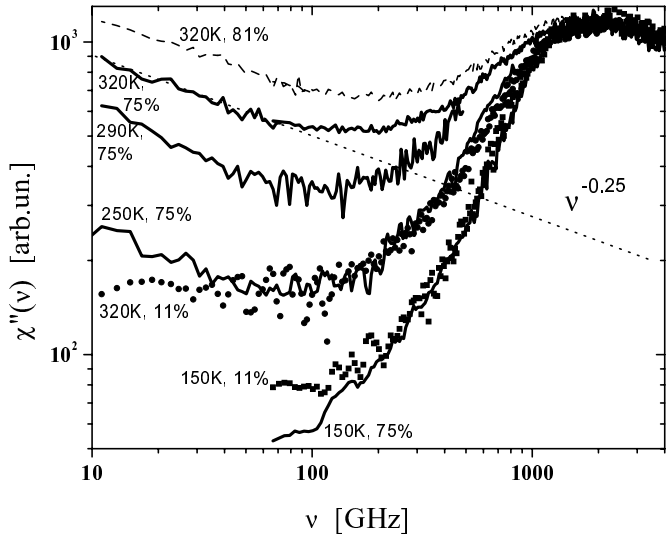

Figure 3. Susceptibility spectra of DNA samples for Q at 11% r.h. (symbols), 75% r.h. (solid lines) and 81% r.h. (dashed line). The dotted line shows the slope of the high frequency tail of the slow process, $\chi''(\nu) \propto \nu^{-0.25}$.

analysis (Figure 3 demonstrates that at high temperatures, $T > 250$ K, a decrease in the humidity level decreases the flexibility of DNA and is similar to a decrease in temperature. This conclusion agrees with the analysis of thermodynamic data [9]: it was demonstrated from enthalpy relaxation measurements that a decrease in the water content accomplishes the same change in DNA kinetics as a decrease in the annealing temperature at $T < 190$ K and has a reverse effect at temperatures above 190 K.

The most important observation in Figure 3 is the absence of the slow process in the spectra of the "dry" sample. Even at the highest temperatures, $T \sim 320$ K, only the tail of the fast process is evident at $\nu < 100$ GHz. The fast process gives anharmonic contribution to $\langle x^2 \rangle$ observed as an increase of the quasielastic intensity (Figures 1-3) even in the dry sample. However, this anharmonicity is weak. This result suggests that the sharp rise of $\langle x^2 \rangle$ at temperatures above ~ 210 K for the two "wet" samples is related to the slow process. Thus the decrease of the humidity level does not suppress anharmonicity in general but suppresses the particular slow process. The nature of the slow process in bio-polymers is not known. The authors [10] relate the process to particular jumps of water molecules on surface of bio-polymers. Their conclusion is based on analysis of dielectric relaxation spectra that are dominated by contributions from water molecules (due to their large dipole moment). Strong increase of acoustic modes attenuation with increase in humidity level was observed in DNA fibers [11]. The attenuation was ascribed to relaxation mode of water. However, our data clearly indicate that the process is related to an intrinsic relaxational motion of DNA molecule (the contribution of D_2O to our spectra is negligible in comparison with the contribution of DNA protons). This conclusion agrees with results of NMR investigations that also found the slow process in relaxation spectra of P-atoms of

DNA [12]. We ascribe that process to cooperative motion of many DNA monomers where all parts of the molecule, backbone and base-pairs, are involved. Molecules of water of hydration follow the motion of the DNA surface and in that way the process appears in the dielectric relaxation spectrum.

The observed dependence of the dynamics upon the level of hydration supports the idea of strong influence of the solvent on bio-polymer dynamics. In particular, it supports our previous speculations [13] that the dynamic transition observed around 200 – 230 K can be related to the dynamic transition in bulk water observed at the same temperature range [14]. The dynamic transition in water of hydration makes the DNA molecule more flexible and enable slow conformational variations. That can be important for opening of base-pairs. Thus our results suggest a possible explanation for suppression of bio-chemical activities by changes in the water content: the slow process appears to be strongly suppressed by decrease in water content and the variations in dynamics appear to be similar to variations caused by decrease in temperature.

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References

1. Nienhaus, G.U., Mourant, J.R., Frauenfelder, H.: Spectroscopic evidence for conformational relaxation in myoglobin, *Proc.Natl.Acad.Sci.USA* **89** (1992), 2902-2906.
2. Post, F., Doster, W., Karvounis G., Settles, M.: Structural relaxation and nonexponetial kinetics of CO-binding to horse myoglobin, *Biophys.J.* **64** (1993), 1833-1842.
3. Rasmussen B.F., Stock, A.M., Ringe, D., Petsko, G.A.: Crystalline ribonuclease-A loses function below the dynamic transition at 220-K, *Nature* **357** (1992), 423-424.
4. Parak, F., et al.: Evidence for a correlation between the photoinduced electron-transfer and dynamic properties of the chromophore membranes from rhodospirillum-rubrum, *FEBS Lett.* **117** (1980), 368-372.
5. Doster, W., Cusak, S., Petry, W.: Dynamical transition of myoglobin revealed by inelastic neutron scattering, *Nature* **337** (1989), 754-756.
6. Rupprecht, A.: Wet spinning apparatus and auxiliary equipment suitable for preparing samples of oriented DNA. *Biotechnol. Bioeng.* **12** (1970), 93-121.
7. Grimm, H., Rupprecht, A.: Low frequency dynamics of DNA, *Physica B* **234-236** (1997), 183-187.
8. Ferrand, M., Dianoux, A.J., Petry, W., Zaccai, G.: Thermal motions and functions of bacteriorhodopsin in purple membranes – effects of temperature and hydration studied by neutron scattering, *Proc. Natl. Acad. Sci. USA* **90** (1993), 9668-9672.
9. Rudisser, S., et al.: Enthalpy, Entropy, and Structural Relaxation Behaviors of A- and B- DNA in their Vitrified States and the Effect of Water on the Dynamics of B-DNA, *J. Phys. Chem.* **101** (1997), 266-277.
10. Mashimo, S., et al.: Dielectric study on dynamics and structure of water bound to DNA using a frequency range 107-1010 Hz, *J. Phys. Chem.* **93**, 4963-4967 (1989).
11. Lee, S.A., et al.: Brillouin-scattering study of the hydration of Li-DNA and Na-DNA films, *Biopolymers*, **26** (1987), 1637-1665.
12. Hogan, M.E., Jardetzky, O.: Internal motions in DNA, *Proc. Natl. Acad. Sci. USA* **76** (1979), 6341-6345.
13. Sokolov, A.P., Grimm, H., Kahn, R.: Glassy dynamics in DNA: Ruled by water of hydration? *J. Chem. Phys.* **110** (1999), 7053-7057.
14. Sokolov, A.P., Hurst, J., Quitmann, D.: Dynamics of supercooled water: Mode-coupling theory approach, *Phys. Rev. B* **51** (1995), 12865-12868.