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Thermal fluctuations of DNA enclosed by glycerol–water glassy matrices: an elastic neutron scattering investigation

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Abstract Through elastic neutron scattering measurements, we investigated the thermal fluctuations of DNA enclosed by glycerol–water glassy matrices, at different levels of hydration, over the wide temperature range from 20 to 300 K. For all the samples, the extracted hydrogen mean square displacements (MSD) show a purely vibrational harmonic trend at very low temperatures, and a first onset of anharmonic dynamics above $\sim$100 K. Such onset is consistent with the activation of DNA methyl group rotational motions. Then, at a certain temperature T_d, the MSD show a second onset of anharmonicity, which corresponds to the DNA dynamical transition. The T_d values vary as a function of the hydration degree of the environment. The crucial role of the solvent mobility to activate the DNA thermal fluctuations is proposed, together with a preferential hydration effect of the DNA phosphate groups. Finally, a comparison between the average mobility of homologous samples of DNA and the lysozyme protein is considered.

Keywords DNA · Glycerol · Glassy matrices · Dynamical transition · Picosecond fluctuations · Elastic neutron scattering

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Introduction

The accurate and detailed knowledge of the structural properties of key biological molecules such as proteins and DNA is capital to understand how they work in living organisms. However, this structural information is often incomplete without a reasonable picture of the processes that characterize the internal dynamics of these biomolecules. In fact, large-amplitude, slow, collective motions as well as low-amplitude, fast and local motions of atoms or groups of atoms are thought to be essential to biological activity (Zaccai 2000). In the case of DNA, the sequence-dependent variations in flexibility and relaxation rates, which may be modulated by thermal fluctuations around the equilibrium structure, have been supposed to be involved in gene recognition and DNA/protein interactions (Brauns et al. 1998). In addition, analogously to what happens in proteins, the rates of chemical reactions that take place within DNA is affected by fast internal dynamics, as it guarantees a prompt response of DNA to the approaching ligand molecule and a quick rearrangement to optimize the geometry in presence of the final products (Brauns et al. 1998). Thermal conformational fluctuations on the picosecond timescale modulate as well the electron transfer and transport processes along DNA (Voityuk et al. 2001; O'Neill and Barton 2004), as they may properly tune interbases coupling and regulate the localization of electronic wave functions. Quite interestingly, the relative localization length is temperature dependent, with a remarkable crossover at around 200–

250 K (Tran et al. 2000), just in correspondence to the dynamical transition emphasized by elastic neutron scattering (ENS) measurements on hydrated DNA (Cornicchi et al. 2007, Sokolov et al. 2001). This phenomenon, which marks the onset of the atomic mean square displacements over the low-temperature trend, is indeed the signature of the dynamical activation in the picosecond timescale.

There is a huge body of experimental evidences showing that fast structural fluctuations of biomolecules are strongly affected by the molecular environment surrounding their surface (Tarek and Tobias 2000; Vitkup et al. 2000; Paciaroni et al. 2002; Cornicchi et al. 2005, 2006). This is an important point as it implies that, on focusing the attention on DNA, one could modulate some fundamental functional properties such as the rates of biochemical reactions and electron transfer process, by simply choosing the suitable molecular environment. In the case of a simple model enzyme like lysozyme, we have recently found that both the temperature where the protein dynamical transition takes place and the extent of the subsequent anharmonic motions are modulated by the environment around the protein (Paciaroni et al. 2002; Cornicchi et al. 2006). We have studied the molecular mobility of lysozyme solvated with glycerol at different water contents, as a function of temperature, by means of ENS experiments (Paciaroni et al. 2002). Neutron scattering spectroscopy is a powerful technique particularly sensitive to the single-particle dynamics of hydrogen atoms (Bée 1988) in the nano- and picosecond time-window over the Angstrom spatial scale (Smith 1991). Such a technique gives invaluable information on the dynamical behaviour of biomolecules, where hydrogen atoms are almost homogeneously distributed. In particular we have pointed out that the protein internal mobility is profoundly altered when the character of its environment gradually switches from a stabilizer-like to a plasticizer-like nature. By increasing the hydration degree, the internal dynamics of lysozyme in glycerol appears to be more and more activated, and the dynamical transition temperature decreases by around 50 K. In these systems, the relevant plasticizing action of water suggested a role of the preferential hydration process (Timasheff 1995; Gekko and Timasheff 1981; Paciaroni et al. 2002), with the exclusion of glycerol molecules from the biomolecule surface.

Actually, as the properties of DNA related to hydration are quite different from those of lysozyme, due to the presence of phosphate charged groups and counterions, we may also imagine that its dynamical behaviour will be differently affected by the external molecular environment. On these grounds we have undertaken an ENS study on DNA solvated with glycerol, at various water contents, to study how and to what extent its thermal fluctuations are modulated by the embedding glassy matrix. The high concentration of glycerol in the investigated systems could simulate or replicate the physiological conditions of the binding of glycosylated drugs or glycoproteins to DNA, where very high local concentration of the polyol in proximity of the double helix is observed. In addition, the study of the interactions between the polyol matrix and the DNA is an intriguing subject, as the DNA-polyhydric complexation phenomena, which are still poorly understood, take part in a wide variety of processes in which DNA is involved. Indeed, polyhydric residues are components of some nuclear proteins and enzymes involved in transcription (Wang et al. 1993), while some powerful antibiotics and anti-cancer drugs contain carbohydrate moieties, which seem to exploit polyhydric residues as general binding elements for DNA grooves (Nicolaou et al. 1992; Paloma et al. 1994).

The results reported in this work suggest that the local fluctuations of DNA are strongly dependent on the water content of the glassy matrix surrounding the biomolecule and that a preferential hydration effect of phosphate groups seems to take place.

Materials and methods

Samples preparation

Lyophilised DNA from salmon testes was purchased from Sigma–Aldrich (St Louis, MO). In order to emphasize the incoherent signal from DNA non-exchangeable hydrogen atoms, the samples were prepared by using heavy water (Sigma–Aldrich) and deuterated glycerol (Sigma–Aldrich), as deuterium has a neutron scattering cross-section much lower than hydrogen (Bée 1988). DNA (about 500 mg) and an equal weight of deuterated glycerol were dissolved in D_2O to properly substitute all the exchangeable hydrogen atoms with deuterium (Printz and von Hippel 1965). In DNA, the exchangeable hydrogens belong to NH and NH_2 groups of the four nucleobases (adenine, guanine, cytosine and thymine) and to phosphate group. It is known that DNA forms and maintains the double-helix structure even in 99% glycerol (Bonner and Klibanov 1999). This has been also checked in our laboratory by differential scanning calorimetry and circular dichroism measurements.

The mixture was dehydrated under vacuum in presence of P_2O_5 in order to obtain the lowest achievable water content (about a few percent). The hydrated samples were prepared by putting the dry DNA + glycerol sample in presence of a KCl saturated solution of D_2O and properly varying the equilibration time. The water content w (w = g D_2O/g glycerol = g D_2O/g DNA) was determined by weighing the sample before and after the hydration process. In such a way samples at 0, 0.10, 0.17, 0.25, 0.32, 0.50, and

0.90 w have been obtained. After the preparation, they were held in a standard flat aluminium cell (inner thickness of 0.5 mm) and sealed with an indium wire to ensure a constant hydration level that was also checked by weighing the sample before and after the measurement.

Incoherent neutron scattering

Thermal neutrons scattering is a powerful technique to obtain precious information on the fast motions of biological samples, as it gives access to characteristic distances and times of the order of angstrom and pico- to nanoseconds respectively. Neutrons exchange with nuclei a momentum $\hbar\boldsymbol{Q}$ and an amount of energy $E = \hbar\omega$ with a probability given by the dynamical structure factor $S(\boldsymbol{Q},E)$, which is the experimentally measured quantity in a neutron scattering experiment. It can be shown (Lovesey 1988) that $S(\boldsymbol{Q},E)$ is the Fourier transform of the time-correlation function of the density fluctuation in the sample, thus providing information on both structure and dynamics of the investigated system through its $\boldsymbol{Q}$- and E-dependence. Since the samples are isotropic, the dynamical structure factor depends only on the modulus Q of the wave-vector transfer. The big hydrogen incoherent cross-section (which is an order of magnitude larger than that of any other atom present in our samples) allows us to describe the recorded elastic intensity within the following incoherent approximation (Bée 1988):

$$S(Q, E \approx 0) \approx \mathrm{e}^{-\langle u^2\rangle_G Q^2} A_0(Q) \tag{1}$$

In this equation, the first term is the so-called Debye–Waller factor $\mathrm{e}^{-\langle u^2\rangle_G Q^2}$, which takes into account for the Q-dependence of the elastic intensity originated by the vibrational atomic mean square displacements $\langle u^2\rangle_G$ of protons in the bottom of their potential wells. The term $A_0(Q)$ is the elastic incoherent structure factor (EISF), which represents the space-Fourier transform of the scatterers' distribution taken at infinite time and averaged over all the possible initial positions. Through its characteristic Q-dependence, information on geometry and type of motions of the scattering nuclei can be obtained.

We schematized the dynamics of DNA protons with the so-called double-well jump model. Such a model is based on an oversimplified schematic description of the complex energy landscape of biomolecules. Nevertheless, it has been successfully employed in describing the picosecond dynamics of dry and hydrated DNA powders (Cornicchi et al. 2007) and proteins embedded in different environments (Doster et al. 1989; Paciaroni et al. 2002, 2005; Cornicchi et al. 2006). Within this framework, the hydrogen atoms are considered dynamically equivalent and their motions are schematized as jumps between two distinct sites with different free energy. The corresponding EISF is (Bée 1988):

$$A_0(Q) = 1 - 2p_1p_2\left(1 - \frac{\sin(Qd)}{Qd}\right) \tag{2}$$

where d is the mean spatial distance between the two potential wells, and p_1 and p_2 are the occupation probabilities of the ground and the excited state respectively. From Eqs. (1) and (2) the total MSD of H atoms can be derived via the following relationship (Doster et al. 1989):

$$\langle u^2\rangle_{\mathrm{tot}} = -\left[\frac{d\ln S(Q, E \approx 0)}{d(Q^2)}\right]_{Q=0} = \langle u^2\rangle_{\mathrm{G}} + \frac{1}{3}p_1p_2d^2 \tag{3}$$

where the second term $p_1p_2d^2/3$ represents the conformational contribution to the total MSD (Fenimore et al. 2004; Paciaroni et al. 2005) and quantifies the proton mobility due to jumping between the two energetic sites.

Neutron scattering experiment

The measurements were performed at the high-resolution backscattering spectrometer IN13 (ILL, Grenoble). The energy resolution of 4.5 μeV (half-width at half-maximum) allowed us to resolve motions faster than about 150 ps. The data were collected in the wide Q-range 0.2–4.9 $\mathrm{Å}^{-1}$, with an average wave-vector transfer resolution of $\sim$0.2 $\mathrm{Å}^{-1}$. The sample holder was placed at an angle of 120° with respect to the incident beam direction. All the samples were investigated from 20 to 300 K. The acquired data were corrected in order to take into account for incident flux, cell scattering, self-shielding and detector responses. Then, the elastic intensity of each sample relative to a given temperature was normalized with respect to the corrected data acquired at the lowest measured temperature. Since an average transmission of about 90% was obtained, we neglected the contribution due to multiple scattering processes in the data treatment.

Results and discussion

In Fig. 1 we show the corrected elastic intensities as a function of Q^2 for DNA in glycerol at 0.32 w, at six different temperatures. The semi-logarithmic scale allows us to emphasize when the data depart from a purely linear trend i.e., the Gaussian-like factor in Eq. (1). We found that for temperatures higher than 100 K the elastic intensity exhibits a slightly non-linear behaviour. As temperature increases, the deviation from the Gaussian-like trend becomes more and more evident. Indeed, in the whole investigated T-range the data are well reproduced by using Eqs. (1) and (2), and the results shown in Fig. 1 are

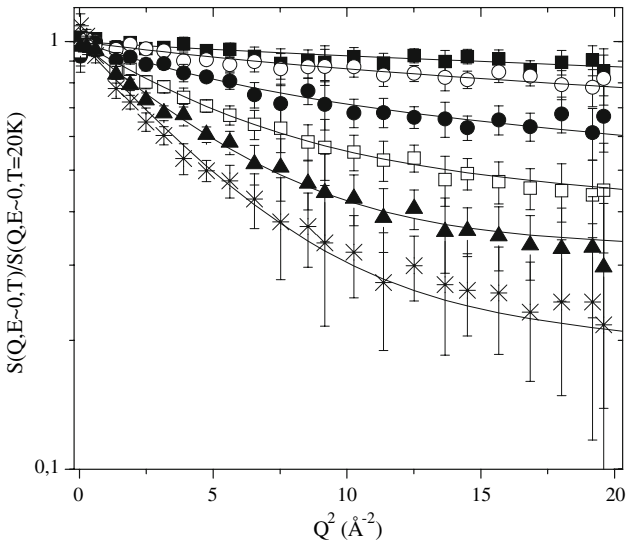

Fig. 1 Elastic neutron scattering intensities versus Q^2 for DNA in glycerol at 0.32 *w*, at $T = 100$, 150, 220, 260, 280 and 300 K (from *top* to *bottom*). *Lines* correspond to fitting with Eqs. (1)–(2)

representative of the general goodness of fit obtained for all the samples. Neutron scattering measurements on nucleic acids (Cornicchi et al. 2007; Caliskan et al. 2006) and protein powders (Doster et al. 1989; Roh et al. 2005; Paciaroni et al. 2006; Cornicchi et al. 2006) showed a non-negligible anharmonic deviation of the atomic MSD from the purely vibrational trend starting at a temperature around 100 K. This deviation has been principally ascribed to reorientation motions of methyl groups, where a significant fraction of non-exchangeable hydrogen atoms are located. Recently, this interpretation was also applied to explain the low-temperature MSD trend of DNA (Cornicchi et al. 2007) and tRNA (Caliskan et al. 2006) powders. In particular, Caliskan and co-workers (2006) found that in tRNA the low-temperature anharmonic onset is absent, consistently with the paucity of methyl groups in that biomolecule. In our samples, approximately 9% of DNA non-exchangeable hydrogen atoms belong to the methyl group located in thymine nucleobase (Chargaff et al. 1951). Thus we attributed the onset of the anharmonic dynamics above $\sim$100 K to the activation of methyl group motions. Within this picture, we estimated the Gaussian contribution $\langle u^2(T)\rangle_G$ by extrapolating the linear trend measured for temperatures up to approximately 100 K, where purely vibrational motions take place. The fit of the elastic intensities as a function of Q^2 to Eqs. (1) and (2) provides both d and the p_2/p_1 ratio at different temperatures. We found a value of $d = 1.0 \pm 0.2$ Å nearly constant with T and water content. We may regard d as a mean spatial length which takes into account for the methyl groups dynamics in the low-T range ($T < T_d$), while for $T > T_d$ it can be related to a larger variety of internal motions, involving displacements of phosphate group, sugar and nucleobase. Similar values of d have been reported from neutron scattering measurements on dry and hydrated DNA (Cornicchi et al. 2007) and proteins (Doster et al. 1989; Paciaroni et al. 2005) and for proteins embedded in glassy matrices of glycerol– (Paciaroni et al. 2002) and glucose–water (Cornicchi et al. 2006) systems.

The p_2/p_1 ratio as a function of T quantifies the dynamical response to temperature changes of those protons which are mobile within our experimental time-window, and provides information on their ability in overcoming the energy barrier of the parabolic potential well where they are trapped at low T. In Fig. 2 we report $ln(p_2/p_1)$ versus 1,000/T for DNA in glycerol at 0.10 and 0.50 *w*. This plot allows emphasizing an Arrhenius-like T-dependence, which is a signature of a thermally activated process. We found that the p_2/p_1 ratio can be described in a satisfactory way only with two distinct Arrhenius functions, which take into account for the low-T (LT) and the high-T (HT) behaviour, respectively $exp(-\Delta G_{LT}/RT)$ and $exp(-\Delta G_{HT}/RT)$. The first Arrhenius function reproduces the anharmonic motions of methyl groups that start above $\sim$100 K. The free energy change ΔG_{LT} due to proton jumps between the two energetic wells is related to the corresponding enthalpy and entropy change, ΔH_{LT} and ΔS_{LT}, through $\Delta G_{LT} = \Delta H_{LT} - T\Delta S_{LT}$. We found average values of $\Delta H_{LT} = 5.1 \pm 0.5$ kJ/mol and $\Delta S_{LT}/R = 2.3 \pm 0.5$ nearly constant with the water content. With this respect, the addition of water seems to not affect the T-dependence of methyl group motions, even at the highest hydration degree we investigated. Earlier neutron

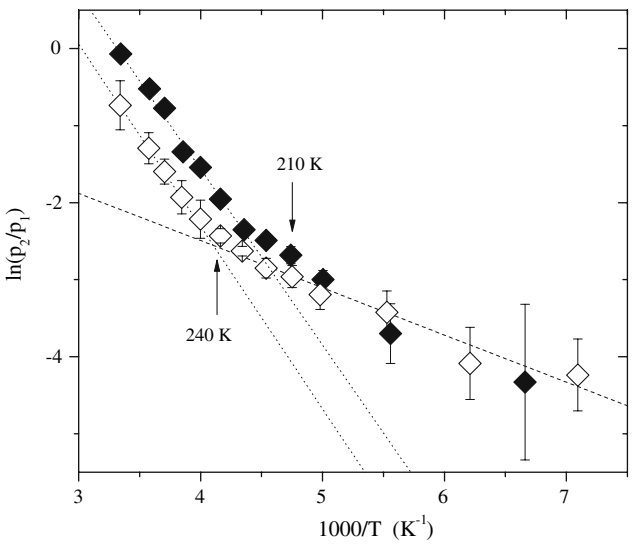

Fig. 2 Occupation probabilities ratio p_2/p_1 for DNA in glycerol at 0.10 *w* (*open rhomb*) and at 0.50 *w* (*filled rhomb*) with *solid* and *dotted lines* representing the Arrhenius-like trends fitted as described in the text

scattering and molecular dynamics studies indicate that the anharmonic methyl group rotation appears in proteins regardless of the hydration level (Roh et al. 2005) and the surrounding solvent (Paciaroni et al. 2006). Indeed, the CH_3 group has a hydrophobic character, and its dynamics is likely not sensible to the amount of water molecules surrounding the biomolecule.

At a certain temperature the p_2/p_1 values clearly depart from the low-T behaviour and an additional Arrhenius curve is needed in order to describe the trend up to room temperature. The thermodynamic parameters, which characterise this second Arrhenius function, are reported in Table 1. The enthalpy asymmetry ΔH_{HT} to be overcome in the nucleic acid fast fluctuations changes from 15 ± 2 kJ/mol, for DNA in neat glycerol, to an average of 20 ± 2 kJ/mol for the hydrated samples. The values of $\Delta S_{HT}/R$ exhibit a rather similar behaviour, going from 4 ± 1 at 0 w to 7.2 ± 0.1 at 0.10 w, and then slowly increasing up to 7.8 ± 0.1 for the sample with the highest water content. The estimated thermodynamic parameters ΔH_{HT} and $\Delta S_{HT}/R$ are definitively different from the low-T case, thus confirming that the corresponding dynamical process represents an additional anharmonic contribution well distinct from the methyl groups' activation. Moreover, unlike the low-T anharmonicity, the extent of the second dynamical process seems to be somehow affected by the environment. More in detail, both ΔH_{HT} and $\Delta S_{HT}/R$ show a significant increase with the addition of a few water molecules (about 3.5 per base pair for the 0.10 w sample). We may imagine that the water molecules give rise to the formation of hydrogen bonds which in their turns probably make more difficult the local side-groups motions, resulting in a growing up of the energetic barriers. At the same time, the excess of configurations accessible to H atoms, quantified by the $\Delta S_{HT}/R$ term, may increase due to the high H-bonds connectivity.

The second dynamical activation corresponds to the DNA dynamical transition. The deviation from the first Arrhenius trend of the p_2/p_1 values provides the temperatures T_d where the transition takes place. In Fig. 3, where we report the estimated T_d values as a function of water content, a rather constant trend around 235 K is visible up to 0.32 w. Then, for higher hydration degrees, T_d rapidly decreases toward a value of ~190 K. This behaviour indicates a critical role of the environment in affecting the structural fluctuations of DNA. To give an interpretation of such a peculiar result, it is useful to compare the T_d of DNA in glycerol–water glassy matrices with those of lysozyme in the same environment, which are reported as well in Fig. 3. Even if the dynamical transition of lysozyme and DNA takes place at the same temperature for very low and high hydration degrees, a clear difference between the two biomolecules is visible in the intermediate range. In fact, the T_d of lysozyme starts to decrease well before the DNA i.e., for water contents higher than 0.1 w. This suggests that the fast motions responsible for the dynamical transition appear to be activated at lower T in lysozyme than in the nucleic acid.

In the case of lysozyme, the T_d trend has been interpreted as ruled on the whole by the glassy properties (i.e., the critical temperature, see below) of the surrounding environment, even if a preferential hydration effect seems to determine the protein dynamics at very low water content (<0.10 w). This preferential hydration process can be explained in terms of both steric constrains exerted by the polyols molecule and interactions of water molecules with polar groups. With this respect, even if glycerol may give rise to a competitive process with water molecules in interacting with the biomolecule charged groups, a higher preferential interaction of such groups with water than with

Table 1 Parameters of the high-temperature anharmonic Arrhenius processe, Δ_{HT} and Δ_{HT}/R, for the different samples

Water content	ΔH_{HT} (kJ mol^{-1})	$\Delta S_{HT}/R$
0 w	15 ± 2	4 ± 1
0.10 w	20 ± 1	7.2 ± 0.1
0.17 w	18 ± 1	7.1 ± 0.1
0.25 w	20 ± 1	7.2 ± 0.1
0.32 w	18 ± 4	7.3 ± 0.1
0.50 w	20 ± 3	7.7 ± 0.1
0.90 w	19 ± 2	7.8 ± 0.1

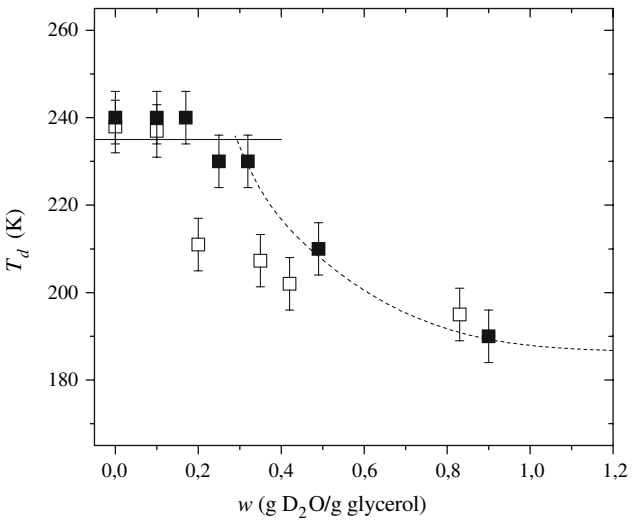

Fig. 3 Dynamical transition temperatures T_d for DNA embedded in glycerol–water matrices (*filled square*) and of lysozyme in glycerol–water matrices (*open square*) (Paciaroni et al. 2002) as a function of water content. The *solid line* corresponds to the experimental critical temperature T_c of glycerol (Franösc et al. 1997). The *dashed line* is a guide for the eye

glycerol seems to hold. Indeed, in lysozyme the sequential hydration of protein surface starts just with polar groups, whose coverage is reached at about 0.1 w, as revealed by infrared studies on protein films (Poole and Finney 1983).

In DNA the molecular charged groups are the phosphate groups (2 PO_4^- per base pair). Actually, water molecules show to have the highest affinity for the phosphate charged oxygens, followed by base oxygens and nitrogens. Theoretical analysis of the hydration patterns around DNA showed that both bases and phosphate groups have significantly organized hydration shells but the extent of hydration is larger around phosphates than bases (Schneider et al. 1998). The calculated water distribution around PO_4^- predicts that six molecules of water constitute the first hydration shell of a phosphate group (Pullman et al. 1975; Schneider et al. 1998). This complete hydration of phosphate groups, where each charged oxygen is hydrated separately by three water molecules in a tetrahedral arrangement called a "cone of hydration", corresponds to about 0.33 g of D_2O/g DNA, which is very close to the water content where the T_d trend shows a pronounced inflection. We may then suppose that up to $\sim$0.3 w the added water molecules are sequestrated by phosphate groups in such a way that the local composition of the environment near the mobile groups on DNA surface is almost invariant and very close to pure glycerol. In such a way, the onset of structural fluctuations, which are triggered by mobile groups, does not change up to about 0.32 w and takes place at around 235 K, which is remarkably close to the critical temperature T_c of neat glycerol (Franösc et al. 1997) [T_c is the temperature where, within the framework of the Mode Coupling theory, the dynamics of a glass-forming system changes from glass-like to liquid-like and relaxations are arrested (Götze 1991)]. Than, the T_d decreases of about 50 K from the dry to the most hydrated sample. In the case of lysozyme in glycerol–water systems (Paciaroni et al. 2002), it has been noted that, for $w > 0.1$, the T_d trend as a function of water content remarkably resembles that of the T_c of glycerol–water solutions. Analogously, the T_d estimated for DNA in the polyol-made matrices could reasonably follow a w-dependent behaviour similar to the T_c of the environment, providing that the effective composition is taken into account, as above described.

From these findings a conditioning role of the solvent in the activation of the nucleic acid fast dynamics, similar to that proposed for the protein, can be suggested. Actually, such scenario constitutes a further support to the hypothesis that the dynamical transition in biomolecules is solvent-induced (Fenimore et al. 2004; Paciaroni et al. 2002, 2005; Cornicchi et al. 2006). In particular, recent detailed neutron scattering experiments on protein and DNA powders embedded in the natural aqueous solvent (Paciaroni et al. 2005; Cornicchi et al. 2007) and of proteins in glassy matrices of bioprotectant systems as glycerol– (Paciaroni et al. 2002, 2006) and glucose– (Cornicchi et al. 2006) water mixtures, showed that the surrounding environment plays a conditioning role in the activation of the new anharmonic degrees of freedom beyond the mobility of methyl groups. It can also be supposed that the same solvent-driven mechanism is determinant for the observed dynamical activation in protein and nucleic acid, irrespective of the biomolecule. An analogous result has been recently found in the investigation of hydrated tRNA and lysozyme, which exhibit the same temperature of dynamical transition (Caliskan et al. 2006). The presence in DNA of a number of charged groups higher than that of lysozyme, could give insights on the reliability of a preferential hydration-based description of the biomolecule-solvent interaction. Indeed, our results suggest that it is the interaction of water with charged groups at the basis of the preferential hydration effect. As it happens for proteins in glycerol–water solutions (Gekko and Timasheff 1981), such a process promotes the exclusion of glycerol molecules from the DNA surface in order to minimize the free energy.

Further information about the role the environment plays in the biomolecule fast dynamics is provided by the total mean square displacements $\langle u^2 \rangle_{tot}$, calculated from Eq. (3) and shown in Fig. 4 as a function of temperature for all the measured samples. The low-T behaviour is actually independent on the water content, as already found for the p_2/p_1 ratio (see Fig. 2). At very low temperatures the total MSD increase linearly, so that the only relevant contribution comes from the purely vibrational term $\langle u^2 \rangle_G$

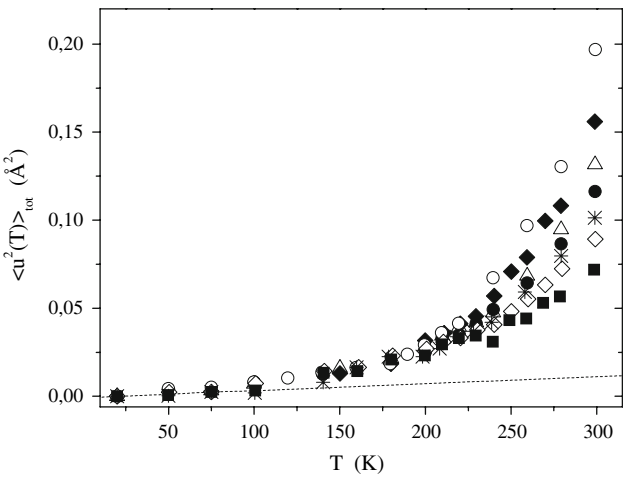

Fig. 4 Temperature dependence of the total mean square displacements for DNA at 0 w (*filled square*), 0.10 w (*open rhomb*), 0.17 w (*star*), 0.25 w (*filled circle*), 0.32 w (*open triangle*), 0.50 w (*filled rhomb*), and 0.90 w (*open circle*). The *dashed line* is the Gaussian contribution $\langle u^2 \rangle_G$ [see Eq. (3)] to the total MSD of the dry sample

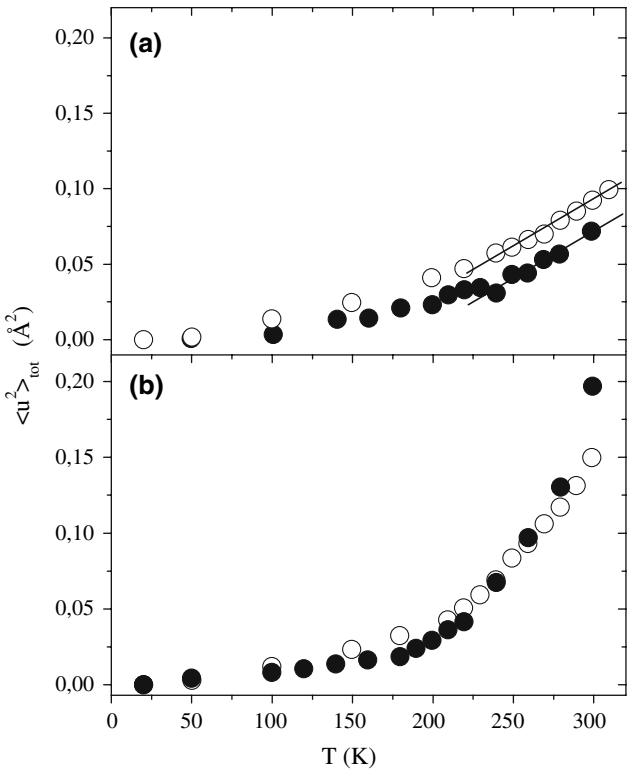

Fig. 5 Comparison between the $\langle u^2 \rangle_{\mathrm{tot}}$ of DNA in glycerol–water mixtures (*filled circle*) and of lysozyme in glycerol–water mixtures (*open circle*). *Panel* **a**: DNA in glycerol at 0 *w* and lysozyme in glycerol at 0 *w*. *Solid lines* are guide for the eye. *Panel* **b**: DNA in glycerol at 0.90 *w* and lysozyme in glycerol at 0.82 *w*

[see Eq. (3)]. Above $\sim$100 K, the MSD start to exhibit a anharmonic dynamics, which becomes more evident with increasing temperature. Such deviation from the harmonic trend is consistent with methyl group confined rotations, as previously discussed. This rather common trend is followed by the total MSD until the onset of the DNA dynamical transition occurs at the estimated temperature T_{d} (see Fig. 3). Then, above T_{d} the $\langle u^2 \rangle_{tot}$ show a different behaviour, according to water content. In particular, the atomic mobility increases with *w*, in such a way that the samples with the higher hydration level have the greater atomic amplitudes at any temperature. This trend can be regarded as a consequence of the well-known plasticizing role of the aqueous medium.

A comparison between the MSD of DNA and lysozyme (Paciaroni et al. 2006) in glycerol–water matrices gives new insights on the possible differences concerning the *T*-dependence of the fast dynamics when switching from lysozyme to DNA. In Fig. 5a, the comparison between DNA and lysozyme in glycerol shows that the atomic mobility of DNA is smaller than that of lysozyme at all the investigated temperatures. This difference, which arises at low *T*, can be explained with the lower percentage of methyl groups in DNA with respect to lysozyme, as mentioned above. For temperatures higher than T_{d}, the MSD of the two biomolecules have a remarkably similar slope, which could be explained by supposing that the same coupling with the environment dynamics holds. In Fig. 5b, we compare the MSD of DNA in glycerol at 0.90 *w* and lysozyme in glycerol at 0.82 *w*. After a slight difference in the low-*T* region, they almost superimpose. These comparisons together suggest that water confers a degree of flexibility, which can reasonably be independent on the biomolecule and on its three-dimensional conformation. In other words, the high degree of dynamical coupling between biomolecule and environment favoured by water molecules leads to a similar response in two different molecules such as protein and nucleic acid. Figure 5a and b seem to support MD simulation studies, which provided evidences that, in a biomolecule, intrinsic effects due to internal dynamics are prevalently important at low temperatures (Vitkup et al. 2000), while the activation of the solvent mobility determines the biomolecule structural fluctuations in the picosecond time-scale (Vitkup et al. 2000; Tarek and Tobias 2000).

The results shown in Figs. 3, 4 and 5, globally suggest that the solvent dynamics and composition could be regarded as fundamental parameters in determining the *T*-dependence of a biomolecule, regardless of the three-dimensional structure and chemical backbone. With this respect, it is worth noting that it was recently found that the temperature response of single- and double-strand DNA is the same, irrespective of the nucleic acid spatial structure (Cornicchi et al. 2007).

References

Bée M (1988) Quasielastic neutron scattering. In: Adam Hilger (ed) Bristol

Bonner G, Klibanov AM (1999) Structural stability of DNA in nonaqueous solvents. Biotechnol Bioeng 68:339–344

Brauns EB, Murphy CJ, Berg MA (1998) Local dynamics in DNA by temperature-dependent stokes shifts of an intercalated dye. J Am Chem Soc 120:2449–2456

Caliskan G, Briber RM, Thirumalai D, Garcia-Sakai V, Woodson SA, Sokolov AP (2006) Dynamic transition in tRNA is solvent induced. J Am Chem Soc 128:32–33

Chargaff E, Lipshitz R, Green C, Hodes ME (1951) The composition of the desoxyribonucleic acid of salmon sperm. J Biol Chem 192:223–230

Cornicchi E, Onori G, Paciaroni A (2005) Picosecond-time-scale fluctuations of proteins in glassy matrices: the role of viscosity. Phys Rev Lett 95:158104(1)–158104(4)

Cornicchi E, Marconi M, Onori G, Paciaroni A (2006) Controlling the protein dynamical transition with sugar-based bioprotectant matrices: a neutron scattering study. Biophys J 91:289–297

Cornicchi E, Capponi S, Marconi M, Onori G, Paciaroni A (2007) Temperature dependence of fast fluctuations in single- and double-stranded DNA molecules. A neutron scattering investigation. Phila Mag 87(3–5):509–515

Doster W, Cusack S, Petry W (1989) Dynamical transition of myoglobin revealed by inelastic neutron scattering. Nature 337:754–756

Fenimore PW, Frauenfelder H, McMahon BH, Young RD (2004) Bulk-solvent and hydration-shell fluctuations, similar to α- and β-fluctuations in glasses, control protein motions and functions. Proc Natl Acad Sci USA 101:14408–14413

Franösc T, Götze W, Mair M, Singh AP (1997) Evolution of structural relaxation spectra of glycerol within the gigahertz band. Phys Rev E 55:3183–3190

Gekko K, Timasheff S (1981) Thermodynamic and kinetic examination of protein stabilization by glycerol. Biochemistry 20:4677–4686

Götze W (1991) Liquids, freezing and the glass transition. North Holland, Amsterdam

Lovesey S (1988) Theory of neutron scattering from condensed matter. Oxford University Press, Oxford

Nicolaou KC, Tsay S-C, Suzuki T, Joyce GF (1992) DNA-carbohydrate interactions. specific binding of the calicheamicin y_1^I oligosaccharide with duplex DNA. J Am Chem Soc 114:7555–7557

O'Neill M A, Barton JK (2004) DNA-mediated charge transport requires conformational motion of the DNA bases: elimination of charge transport in rigid glasses at 77 K. J Am Chem Soc 126:13234–13235

Paloma LG, Smith JA, Chazin WJ, Nicolaou KC (1994) Interaction of calicheamicin with duplex DNA: role of the oligosaccharide domain and identification of multiple binding modes. J Am Chem Soc 116:3697–3708

Paciaroni A, Cinelli S, Onori G (2002) Effect of the environment on the protein dynamical transition: a neutron scattering study. Biophys J 83:1157–1164

Paciaroni A, Cinelli S, Cornicchi E, De Francesco A, Onori G (2005) Fast fluctuations in protein powders: the role of hydration. Chem Phys Lett 410:400–403

Paciaroni A, Cornicchi E, De Francesco A, Marconi M, Onori G (2006) Conditioning action of the environment on the protein dynamics studied through elastic neutron scattering. Eur Biophys Lett 35:591–599

Poole PL, Finney JL (1983) Sequential hydration of a dry globular protein. Biopolymers 22:255–260

Printz MP, von Hippel PH (1965) Hydrogen exchange studies of DNA structure. Proc Natl Acad Sci USA 53:363–370

Pullman B, Pullman A, Berthod H, Gresh N (1975) Quantummechanical studies of environmental effects on biomolecules. IV. Ab initio studies on the hydration scheme of the phosphate group. Theor Chim Acta 40:93–111

Roh JH, Novikov VN, Gregory RB, Curtis JE, Chowdhuri Z, Sokolov AP (2005) Onsets of anharmonicity in protein dynamics. Phys Rev Lett 95:038101(1)–038101(4)

Schneider B, Patel K, Berman H (1998) Hydration of the phosphate group in double-helical DNA. Biophys J 75:2422–2434

Smith JC (1991) Protein dynamics: comparison of simulations with inelastic neutron scattering experiments. Q Rev Biophys 24:1–65

Sokolov AP, Grimm H, Kisliuk A, Dianoux AJ (2001) Slow relaxation process in DNA. J Biol Phys 27:313–327

Tarek M, Tobias DJ (2000) The dynamics of protein hydration water: a quantitative comparison of molecular dynamics simulations and neutron-scattering experiments. Biophys J 74:3244–3257

Timasheff SN (1995) Preferential interactions of water and cosolvents with proteins In: Gregory R (ed) Protein–solvent interactions. Dekker, New York, pp 445–482

Tran P, Alavi B, Gruner G (2000) Charge transport along the λ-DNA double helix. Phys Rev Lett 85:1564–1567

Vitkup D, Ringe D, Petsko GA, Karplus M (2000) Solvent mobility and the protein 'glass' transition. Nat Struct Biol 7:34–38

Voityuk AA, Siriwong K, Rösch N (2001) Charge transfer in DNA. Sensitivity of electronic couplings to conformational changes. Phys Chem Chem Phys 3:5421–5425

Wang C, Altieri F, Ferraro A, Giartosio A, Turano C (1993) The effect of polyols on the stability of duplex DNA. Physiol Chem Phys Med NMR 25:273–280

Zaccai G (2000) How soft is a protein? A protein dynamics force constant measured by neutron scattering. Science 288:1604–1607