

# Glass transition in DNA from molecular dynamics simulations

(oligonucleotide duplex/mean square atomic fluctuation/hydrogen bonds/water)

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**ABSTRACT** Molecular dynamics simulations of the oligonucleotide duplex d(CGCGCG)<sub>2</sub> in aqueous solution are used to investigate the glass transition phenomenon. The simulations were performed at temperatures in the 20 K to 340 K range. The mean square atomic fluctuations showed that the behavior of the oligonucleotide duplex was harmonic at low temperatures. A glass transition temperature at 223 K to 234 K was inferred for the oligonucleotide duplex, which is in agreement with experimental observations. The largest number of hydrogen bonds between the polar atoms of the oligonucleotide duplex and the water molecules was obtained at the glass transition temperature. With increasing temperature we observed a decrease in the average lifetime of the hydrogen bonds to water molecules.

Dynamical aspects of nucleic acids are of great interest as a means to increase the understanding of the DNA double helix. Nuclear magnetic resonance experiments and molecular dynamics (MD) simulations have been used to gain insight into the internal motion of oligonucleotide duplexes (1, 2).

The glass transition phenomenon, which has recently been reviewed (3), has so far mainly been examined for proteins using neutron scattering experiments (4, 5), x-ray diffraction (6), Mössbauer (7) and MD simulations (8–11). In pure water, the number of different hydrogen bonds was observed to increase with increasing temperature (8). In MD studies of myoglobin, the glass transition was in part due to torsional jumping (9), and a significant increase was found in the anharmonic component of atomic positional fluctuations above the glass transition temperature upon hydration (10). Protein dynamics from MD simulations at low temperatures was observed to depend on the way the low temperature state is achieved (11). A previous experiment (12) indicated that the glass transition of a protein was induced by the surrounding solvent. Indications of a glass transition in solid calf thymus DNA, with around 40% water content, have been given by thermally stimulated depolarization current measurements (13, 14). The origin of the glass transition was interpreted as the melting of hydrogen bonds of bound water molecules on the surface of the DNA (13). At low temperatures part of the bound water, especially in the first hydration shell, of DNA was in the glass form (14). From dielectric relaxation spectroscopy on solid calf thymus DNA at water content up to 32% the glass transition was estimated to 238 K (15).

We present MD simulations of the oligonucleotide duplex d(CGCGCG)<sub>2</sub> in aqueous solution. Simulations were performed at 12 different temperatures between 20 K and 340 K. The mean square atomic fluctuations were partitioned into contributions from the different constituents, bases, sugar riboses, and phosphate groups of DNA. The influence of solvent was examined by calculating the hydrogen bonds between the polar atoms of the oligonucleotide duplex and the water molecules. The analysis of the MD simulations permits

us to interpret the dynamical phenomenon that underlies the experimental observations of a glass transition in DNA.

## METHODS

The hexamer oligonucleotide d(CGCGCG)<sub>2</sub> duplex studied here was generated as a right-handed double-stranded standard B-DNA conformation from x-ray fiber diffraction data (16). To make the oligonucleotide duplex electrically neutral, 10 sodium counterions were placed on the bisector of the phosphate oxygens, 4.7 Å from the phosphate atom. The phosphates were treated with full charges. The oligonucleotide duplex was immersed in a sphere built of TIP3P water molecules (17) and with a radius of 23.0 Å. To remove bad contacts in the systems, the water molecules were energy minimized 200 cycles of steepest descent, whereas the duplex was constrained using a harmonic potential with a force constant of 20.0 kcal·mol<sup>-1</sup>·Å<sup>-2</sup>. Stochastic boundary conditions (18) were applied during the simulations and water molecules in the layer outside a radius of 20 Å were propagated with Langevin dynamics using a friction constant of 50.0 ps<sup>-1</sup> for the oxygen atoms, whereas all atoms inside this radius were treated with the Verlet algorithm (19). To allow for a time step of 0.002 ps, all hydrogen atom-heavy atom bond lengths were constrained using the SHAKE algorithm (20). A relative dielectric constant of 1.0 was used and the nonbonded interactions were smoothly shifted to zero at a cutoff of 11.5 Å using the atom based force-shift method (21). The nonbonded interactions were updated every 20 steps and the coordinates were saved every 0.2 ps. No constraints for the hydrogen bonds or for the terminal ends were used during the MD simulations. Each system was first heated from 0 K to the desired temperature during 30 ps and then equilibrated for 70 ps; the systems were judged to be well equilibrated, as there was no drift in the root mean square atomic displacement with time (data not shown) after the equilibration period. Thereafter, the MD simulations were continued for another 50 ps and this period of each trajectory was further used for the analysis. The energy minimizations and MD simulations were performed using the CHARMM program (22) with all atom parameters (23) on DEC AXP 3000/400 workstations. The MD simulations were carried out on the oligonucleotide d(CGCGCG)<sub>2</sub> duplex at the following 12 temperatures 20, 60, 100, 140, 160, 190, 220, 240, 250, 280, 300, and 340 K. The setup and MD simulations were carried out in an identical manner at each temperature.

## RESULTS

The mean square atomic fluctuation ( $\Delta r$ )<sup>2</sup> of all the atoms of the oligonucleotide duplex were calculated at every temperature. The mean square atomic fluctuation can be partitioned into harmonic and anharmonic contributions. The harmonic contribution to the fluctuations was determined by linear

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Abbreviation: MD, molecular dynamics.

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regression of the data points obtained from the low temperatures between 20 K and 190 K. For the regression line a correlation coefficient of 0.990 and a variance of fit of  $6.01 \cdot 10^{-5}$  were obtained. The regression line was extrapolated to the temperature of 340 K and this line indicates the contribution of harmonic behavior at the higher temperatures. At room temperature, about 300 K, the mean square atomic fluctuation was  $0.60 \text{ \AA}^2$ . From this we determined the harmonic limit behavior to be 38% and the anharmonic behavior 62%. A similar amount of harmonic behavior (41%) was observed in an MD study (9) of myoglobin at room temperature. The anharmonic contribution as displayed in Fig. 1 was obtained by subtracting the harmonic part from the mean square atomic fluctuation. The temperature dependence of the mean square fluctuations of the oligonucleotide duplex was very linear to 190 K, but at 220 K the deviation from linearity started. To determine the glass transition temperature we used linear regression of the values from 240 K to 340 K and extrapolated to lower temperatures. The intersection with the straight line from the harmonic behavior was obtained at 234 K and this value was therefore defined as the glass transition temperature. The correlation coefficient for the regression line was 0.998 and the variance of fit was  $3.0 \cdot 10^{-4}$ . If we included the data point at 220 K in the linear regression analysis we obtained a glass transition of 223 K and a slightly worse correlation coefficient of 0.974. From this we concluded the glass transition temperature to be at 223 K to 234 K. The oligonucleotide duplex behaved like a glass at low temperatures and was more liquid-like above the glass transition temperature. The glass transition temperature obtained here agrees well with experimental observations (15).

To understand the mobility of various parts of the oligonucleotide duplex we calculated the mean square atomic fluctuation of the cytosine and guanine bases, sugar riboses, and phosphate groups. The terminal 5' and 3' hydrogen atoms were not included in the sugar riboses. The phosphate groups contained the phosphate atom and the two oxygen atoms, which were not included in the ester bonds. The mean square atomic fluctuation of these parts together with the regression line for all atoms of the oligonucleotide duplex are shown in Fig. 2 *a* and *b*. At very low temperatures the cytosine and guanine bases were observed to show mean square atomic fluctuations of the same magnitude, but already at 100 K we found significant differences in the thermal mobility between the larger guanine bases and the smaller cytosine bases. The mean square atomic fluctuations of the cytosine bases were observed to be higher than for the guanine bases at every temperature above 60 K.

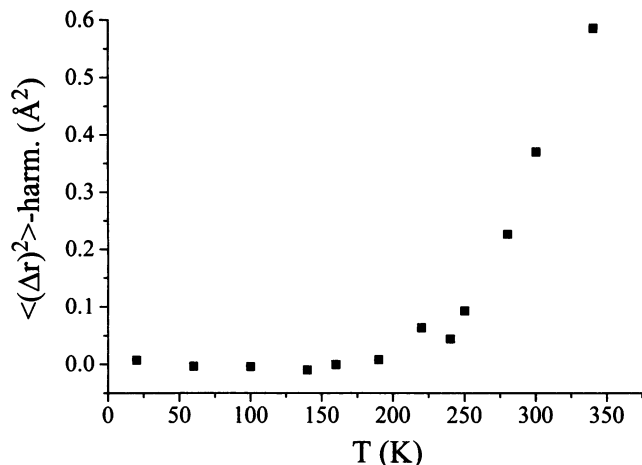


FIG. 1. Anharmonic contribution obtained by subtraction of the harmonic contribution from the mean square fluctuations averaged over all atoms of the oligonucleotide duplex.

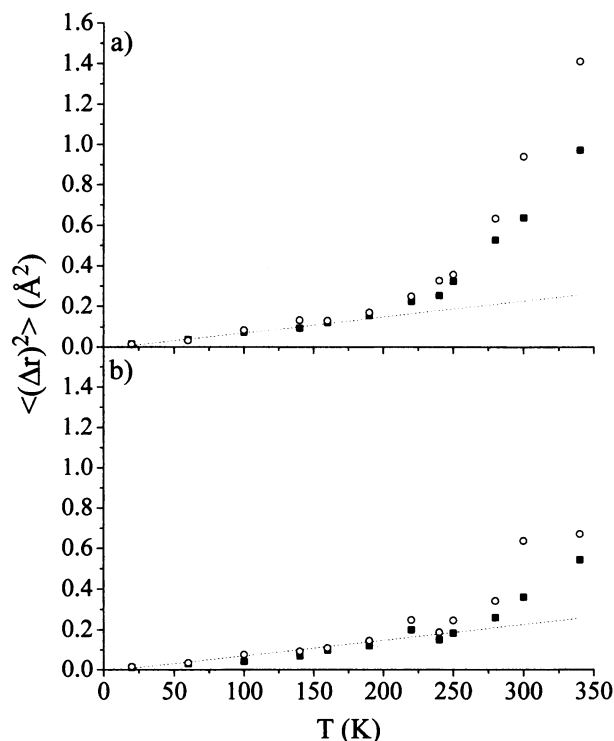


FIG. 2. Mean square atomic fluctuation averaged over the (a) phosphate groups ( $\circ$ ) and sugar riboses ( $\blacksquare$ ) and over the (b) cytosine ( $\circ$ ) and guanine ( $\blacksquare$ ) bases.

We obtained larger mean square atomic fluctuations for the phosphate groups than for the sugar riboses, except at 20 K and 60 K. From 100 K and higher temperatures we observed that the sugar riboses and the phosphate groups showed equal or larger mean square atomic fluctuations than for all atoms of the oligonucleotide duplex. The mean square atomic fluctuations decreased in the following order: phosphate groups > sugar riboses > cytosine bases > guanine bases. This is the same order as previously determined from x-ray crystallographic temperature factors,  $B_i = 8\pi^2 \langle \Delta r_i^2 \rangle / 3$ , of a DNA dodecamer (24): phosphates ( $1.90 \text{ \AA}^2$ ) > sugars ( $1.56 \text{ \AA}^2$ ) > bases ( $1.02 \text{ \AA}^2$ ). In principle it would be possible to determine separate glass transition temperatures for the various parts of the oligonucleotide duplex.

An experimental study of a protein (12) has found indications that the glass transition was induced by the surrounding solvent and this was also observed in an MD study (8). We calculated the number of hydrogen bonds between the polar atoms of the oligonucleotide duplex and surrounding water molecules (Fig. 3). A hydrogen bond was assumed to exist if  $H \cdots X \leq 2.4 \text{ \AA}$  between an acceptor (donor) of the oligonucleotide duplex and a donor (acceptor) of the water molecules. The largest number of hydrogen bonds to water was obtained for the phosphate oxygens at every temperature. The number of hydrogen bonds to water was about 104 at 20 K and increased with increasing temperature to 220 K. By further increasing the temperature from 220 K, where the maximum number of hydrogen bonds to water was obtained, we found a decrease of hydrogen bonds. We interpreted this behavior in the following way: When the oligonucleotide duplex is in the glass state under the glass transition temperature of around 234 K, a limited number of hydrogen bonds can be formed due to the low mobility of both the oligonucleotide duplex and the surrounding water molecules. At low temperatures all water molecules do not occupy favorable positions to form hydrogen bonds. By increasing the temperature above 220 K we observed larger thermal mobility, which caused larger disorder or higher

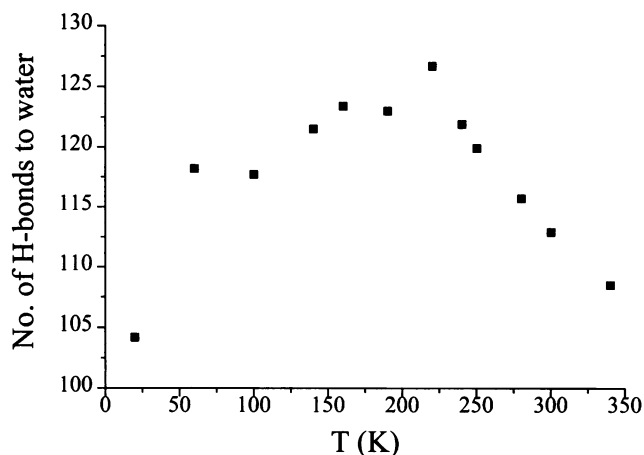


FIG. 3. Average number of hydrogen bonds between the oligonucleotide duplex and water molecules versus temperature.

flexibility of the water molecules. The water molecules can therefore occupy more favorable positions and we observe a larger number of hydrogen bonds between the oligonucleotide duplex and the water molecules. From 20 K to 220 K the number of hydrogen bonds to water increased by 22%. With the glass transition temperature at around 234 K, the oligonucleotide duplex passes over to a more liquid-like state and exhibits larger flexibility. For the water molecules the same phenomenon was found. The water molecules occupy less favorable positions for shorter periods and this is simultaneously observed for the polar atoms of the oligonucleotide duplex. This larger thermal mobility of both the oligonucleotide duplex and the water molecules leads to fewer hydrogen bonds between the oligonucleotide duplex and the water molecules could be formed. Accordingly, by increasing the temperature from 220 K to 340 K, a decrease of the number of hydrogen bonds to water was found.

The average lifetime of the hydrogen bonds between the polar atoms of the oligonucleotide duplex and the water molecules were calculated by

$$\tau = \frac{1}{N} \sum_i n_i \tau_i$$

where  $n_i$  is the average number of hydrogen bonds between a specific polar atom of the oligonucleotide duplex and the water molecules,  $N$  is the total number of hydrogen bonds between the oligonucleotide duplex and the water molecules and  $\tau_i$  is the average lifetime of the hydrogen bonds between a specific polar atom of the oligonucleotide duplex and the water molecules. The average lifetime of the hydrogen bonds to water was observed to decrease with increasing temperature as displayed in Fig. 4. At low temperatures the average lifetime of the hydrogen bonds to water molecules was about 30 ps, because both the oligonucleotide duplex and the water molecules were in the glass state where they exhibit very little thermal mobility. A favorable position of a hydrogen acceptor or donor could be preserved for a long time. At higher temperatures these favorable positions were occupied for shorter periods and therefore the number of hydrogen bonds between the oligonucleotide duplex and the water molecules decreased.

## CONCLUSIONS

Molecular dynamics simulations have provided new insights into the microscopic mechanism of the glass transition in the aqueous solvated oligonucleotide duplex  $d(\text{CGCGCG})_2$ . From the mean square atomic fluctuation we determined the harmonic limit behavior to be 38% at 300 K. We predict the glass

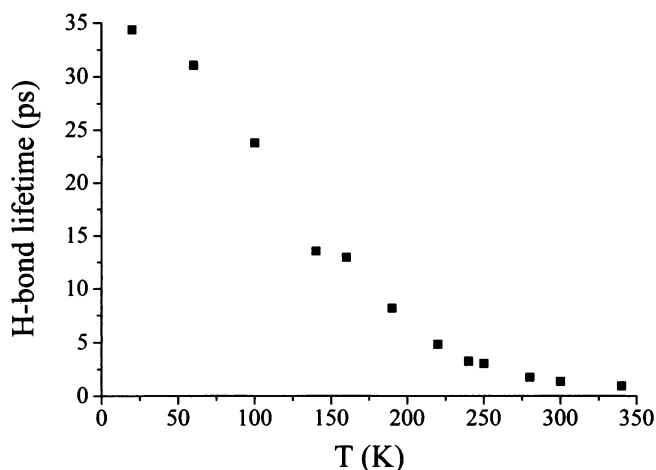


FIG. 4. Average lifetime for the hydrogen bonds between the polar atoms of the oligonucleotide duplex and the water molecules versus temperature.

transition temperature to be at 223 K to 234 K, in accord with experimental observations (15). The various components of DNA have different mobilities and we find the following order of the mean square atomic fluctuations, which is in agreement with crystallographic temperature factors (24): phosphate groups > sugar riboses > cytosine bases > guanine bases. The influence from solvent is supposed to be important for the origin of the glass transition (12, 13), and we found a close relation between the solvent and DNA behavior in that the maximum number of hydrogen bonds from the DNA to water molecules was found at the glass transition temperature. The average lifetime of the hydrogen bonds to the water molecules decreased with increasing temperature.

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