DNA Dynamics as studied by Dielectric Relaxation Spectroscopy (DRS) and Dynamic Mechanical Spectroscopy (DMS)

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ABSTRACT

An investigation was carried out on dynamics of Deoxynucleic acid (DNA) samples in solutions. The covered frequency range is from 10^{-2} to 10^{9} Hz within a wide temperature range. The mechanism of the observed relaxation processes was discussed and attributed to bound water of DNA and DNA itself. The effect of chain melting of DNA on dynamics will be discussed.

EXPERIMENTAL

Materials. Calf thymus and herring testes DNA were purchased from Sigma, and used without further purification. DNA solutions were made by dispersing DNA samples into pure deionized water and equilibrating overnight.

Techniques. Dynamics were investigated by dielectric relaxation spectroscopy (DRS) and dynamic mechanical spectroscopy (DMS). DRS facility combines a Novocontrol α high-resolution dielectric analyzer (3μHz-10MHz) and a Hewlett-Packard 4291B RF impedance analyzer (1MHz-1.8GHz). Both instruments are interfaced to computers equipped with heating/cooling controls, including Novocontrol's Novocool system custom-modified for measurements at low and high frequency. DMS facility is a Rheometrics Scientific Advanced Rheometric Expansion System (ARES) rheometer. Molecular weight of DNA were determined by DNA gelling and the concentration of DNA solutions were verified by Ultra-violet (UV) spectrophotometry.

RESULTS AND DISCUSSION

Introduction. DNA molecule possesses permanent dipole moment in its peptide unit, characterized by a dipolar vector of 3.7 Debye at an angle of 56° with C-N bond. The dipole moment in this way can be additive along the peptide chain, resulting in a cumulative dipole moment of individual peptide chain of the order of 1000 Debye. However, there is no net permanent dipole moment in DNA molecule when it takes up the helical configuration. The anti-parallel dipole moments in double-strand of DNA molecule cancel each other. The DRS response is believed to originate from an induced dipole moment due to counterion fluctuation rather than the orientation of a permanent dipole moment. DNA helix is formed from two strands, stabilized by a regular network of intramolecular hydrogen bonds involving the carbonyl C=O and amino N-H groups of the main chain. The purine adenine (A) pairs with the pyrimidine thymine (T) and the purine guanine (G) pairs with the pyrimidine cytosine (C), making DNA a precise genetic carrier for reproduction of various species. A-T pairs have two hydrogen bonds, whereas G-C pairs have three hydrogen bonds. These base pairs can be separated by breaking hydrogen bonds (e.g. heat, *p*H value or chemical agent) between them, transforming DNA from helical configuration (native state) to a random coil (denatured state).

Chain melting is caused by heating, and the melting point (T_m) of DNA is defined as the temperature at which 50% of the DNA has denatured, i.e. 50% of pairs (A-T pairs and C-G pairs) have opened up.

DRS spectroscopy. DNA solutions are conducting materials and relaxation processes are usually masked by high conductivity. There are two relaxation processes in frequency domain at extremely low temperature range from 173K to 213K, shown in Figure 1. The higher frequency dispersion (HFD) is Debye like. It is attributed to bound water and is independent of DNA concentration. The lower frequency dispersion (LFD) is related to DNA itself and it increases with decreasing DNA concentration.

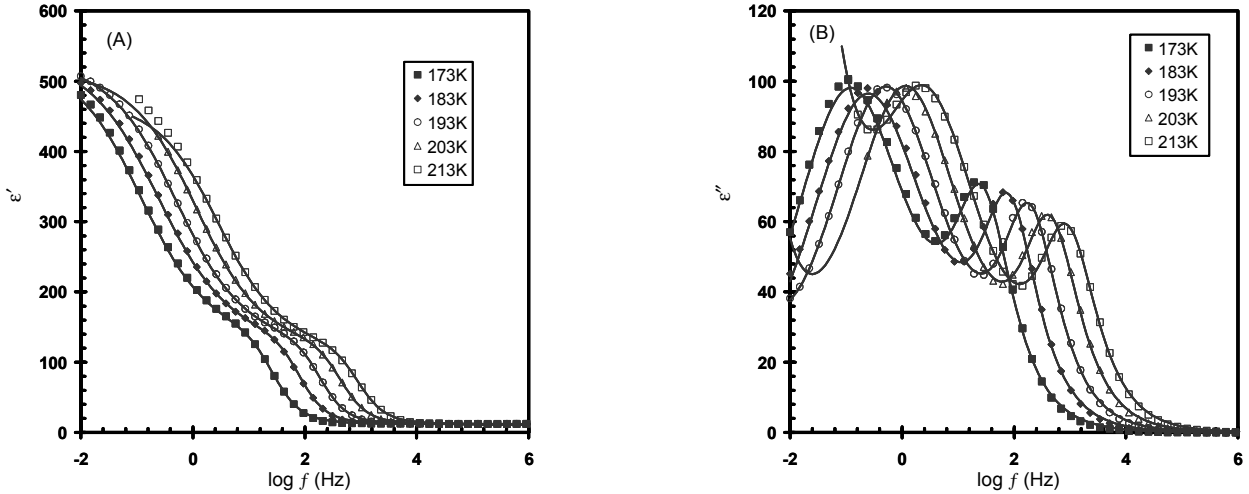

Figure 1. Dielectric permittivity (A) and loss (B) in the frequency domain for calf thymus DNA at concentration of 2mg/ml with temperature as a parameter.

High frequency measurements (above 1MHz) were conducted and presented in the dielectric modulus formalism for herring testes DNA at concentration of 2mg/ml, in Figure 2. The observed process in the MHz range is related to the counterion fluctuation along the DNA chain within a subunit length. The process shift to higher frequency with increasing temperature, but speeds up by dilution of DNA solutions. The chain melting results in a shorter characteristic time calculated from the peak frequency of dielectric modulus peak.

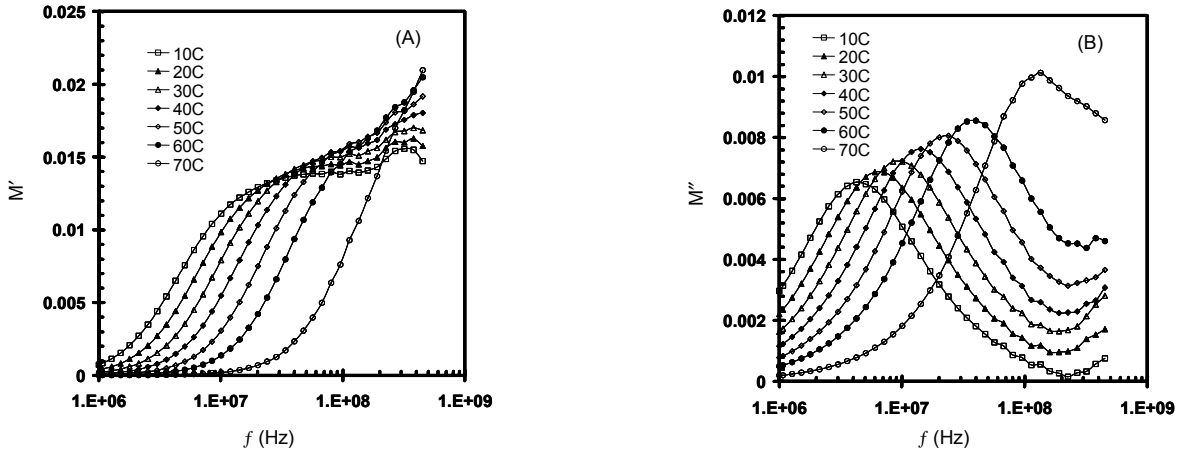

Figure 2. Dielectric modulus (real part in A and loss part in B) in the frequency domain for herring testes DNA with concentration of 2mg/ml with temperature as a parameter.

DMS spectroscopy. We present the DMS measurements of the linear viscoelastic storage (G′) and loss (G") modulus in the frequency domain for calf thymus DNA with DNA concentration as a parameter in Figure 3. Average molecular weight of calf thymus DNA was determined to be 803.4 Kg/mol, and the DNA solutions can only be measured by DMS at concentration over 1mg/ml.

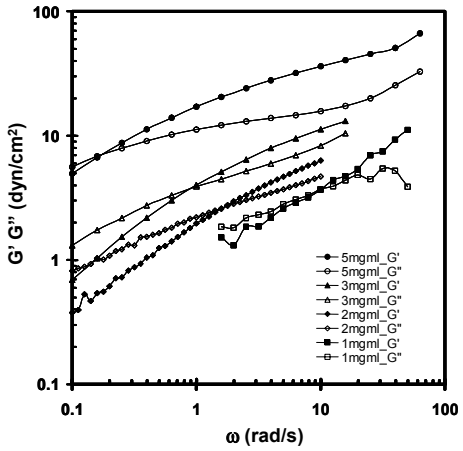

Figure 3. Storage and loss modulus of calf thymus DNA solutions in the frequency domain measured at 20°C with DNA concentration as a parameter.

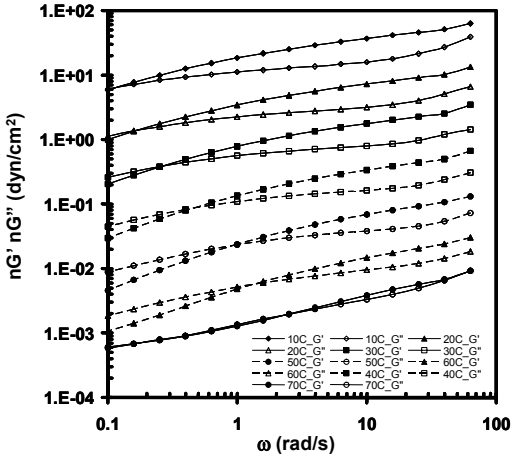

Figure 4. Storage and loss modulus of calf thymus DNA solutions (5mg/ml) in the frequency domain with temperature as a parameter.

Storage and loss modulus (G′ and G") have a crossover frequency (ω_C), that scales with power laws of $G' \propto \omega^{0.8}$ and $G'' \propto \omega^{0.5}$. The crossover frequency shifts to lower frequency with increasing DNA concentration. The scaling power of ω_C versus DNA concentration is $\omega_C \sim C_{DNA}^{-2.4}$, showing a good marking of semiflexible biopolymer solutions[1]. Figure 4 shows the temperature dependence of G′ and G" of calf thymus DNA solutions with concentration of 5mg/ml. A vertical shift was employed for clarity. Similar results were obtained for concentrations of 3 and 2 mg/ml; however, the higher temperature for lower concentration is not measurable due to the samples' low viscosity. It is clear that the crossover frequency shifts to higher frequency with an increasing temperature. We determined the relaxation time from $\tau = 1/\omega_C$. The relaxation time as a function of temperature is of Arrhenius type. The calculated activation energy is 32 kJ/mol for all three concentrations; however a deviation from the Arrhenius equation occurs above 50°C. It seems that the rigid rod-like DNA molecule in solution loses its rigidity as a whole at 50°C, much lower than the chain melting temperature (T_m = 87°C) of calf thymus DNA. This may be due to some "weak points" where the A-T pairs open up along the DNA molecules.

ACKNOWLEDGEMENT

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REFERENCE

1. Mason, T. G.; Dhople, A.; Wirtz, D. *Macromolecules* **1998,** 31, 3600.