Thermochimica Acta, 194 (1992) 67–75
Elsevier Science Publishers B.V., Amsterdam

A new method of investigation of the DNA melting process — the thermal conductivity method

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(Received 25 March 1991)

Abstract

In this paper, a new method of investigation of the conformational changes of macromolecules in solutions is presented. This method, based on thermal conductivity measurements, has been employed in the study of thermally induced conformational changes in the NaDNA macromolecule. The results are presented in the form of temperature dependences of the thermal conductivity coefficient of NaDNA solutions in 0.01 M NaCl and in 0.005 M $MgCl_2$ aqueous solution. On the basis of the performed measurements, the melting temperatures T_M and the width of the helix–coil transition ΔT_M were determined.

INTRODUCTION

Investigations concerning changes to biopolymer macromolecules have been performed by many measurement techniques. According to the technique employed one can obtain different information about the medium under study [1].

It is well known that structural changes in the medium under study or in its molecules are accompanied by changes of the macroscopic thermophysical quantities such as specific heat (C_p), thermal conductivity coefficient (λ) or thermal diffusivity coefficient (κ). These quantities are interrelated by the formula

$$\lambda = \rho \kappa C_p \tag{1}$$

where ρ is the density of the medium.

In the investigation of structural changes of macromolecules in solution, microcalorimetric methods (DSC) are generally used [2–5] to determine temperature dependences of C_p.

Until now there has been a lack of literature data concerning the application of thermal conductivity coefficient measurements in investigation of these phenomena. In solid state physics, measurements of the thermal conductivity coefficient λ and of the thermal diffusivity coefficient κ have been used in the study of phase transitions in crystals [6–8] and also

in the investigation of synthetic polymers [9]. The studies of thermal conductivity of synthetic polymers proved the dependence of the thermal conductivity coefficient on the degree of crystallinity of the sample and its spatial cross-linking [9,10].

The spatial structure of biopolymers in solutions is strongly dependent on solution properties, and undergoes essential changes under the influence of temperature [11–14]. The temperature induced changes in spatially ordered native biopolymer conformation from the helix form to the random coil form are referred to as melting or thermal denaturation.

The main aim of this work is to find out whether the thermal conductivity phenomenon is sensitive to the melting process of the DNA molecule. We have chosen DNA because its structure is already well recognized [1,12], and because the influence of many external factors and the structure of its molecule on the melting process (helix–coil transition) have been the subject of much research work [15–22].

The results obtained have proved the possibility of adopting the thermal conductivity method for investigation of the melting process of DNA macromolecules.

MATERIALS

Highly polymerized calf thymus NaDNA samples were obtained from the Research Center of the Medical Academy in Łódź (Poland). The mean molar weight was about 1×10^7.

The measurements were performed in two kinds of solution: (a) NaDNA in 0.01 M NaCl and (b) NaDNA in 0.005 M $MgCl_2$, for three different concentrations of NaDNA.

The weighed samples of dry NaDNA were dissolved in the volume required for the measurement, i.e. 60 ml, and the solution was dialysed at 4 °C against a 20-fold volume of pure aqueous solution of 0.01 M NaCl or 0.005 M $MgCl_2$ appropriately, with three changes of these solutions. After the dialysis (lasting for 18 to 20 h) the solution was degassed under vacuum for 10 min.

The concentration of NaDNA was determined by weighing the dry sample of NaDNA, and is expressed in grams per liter. To determine the purity of the used specimen we measured the value of the absorption coefficients at 260 and 280 nm. Their ratio was found to be 2.0.

THERMAL CONDUCTIVITY METHOD

The thermal conductivity of NaDNA solutions was determined by the transient hot-wire (THW) method. This method [23–26] is the most accurate and rapid among all thermal conductivity measurement methods for liquids.

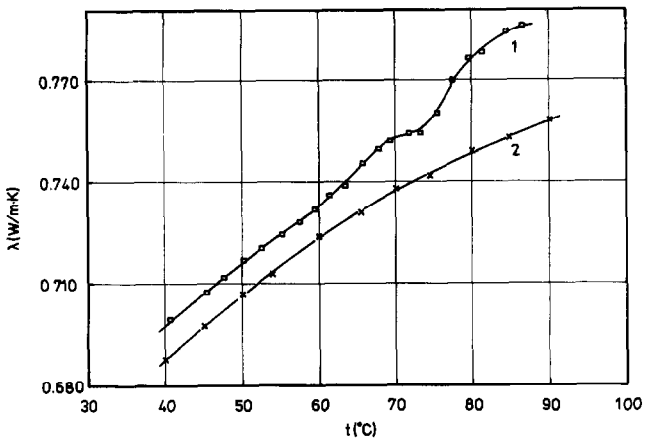

Fig. 1. Thermal conductivity λ of NaDNA solution in 0.01 M NaCl (curve 1) and of pure 0.01 M NaCl aqueous solution (curve 2) as a function of temperature.

Because the solutions under study were dielectrics (aqueous solution) and electrolytes (the salt), the alternating current (a.c.) version of the THW method [27–29] was employed to minimize systematic errors.

First we found (using the above mentioned a.c. version) the temperature dependence of thermal conductivity λ_S for a pure 0.01 M NaCl solution. Then the temperature dependences of thermal conductivity $\lambda_{DNA/S}$ of NaDNA solutions in 0.01 M NaCl with various concentrations of NaDNA were determined. A similar procedure was applied for the solutions of NaDNA in 0.005 M $MgCl_2$. Figures 1 and 2 show illustrative curves $\lambda_S = f(t)$ and $\lambda_{DNA/S} = f(t)$ for solutions in 0.01 M NaCl and 0.005 $MgCl_2$,

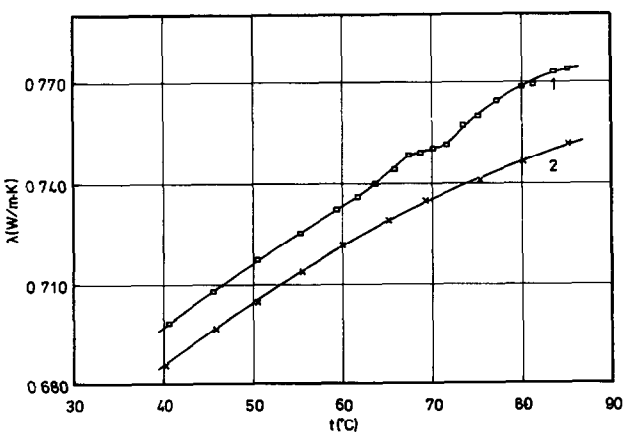

Fig. 2. Thermal conductivity λ of NaDNA solution in 0.005 M $MgCl_2$ (curve 1) and of pure 0.005 M $MgCl_2$ aqueous solution (curve 2) as a function of temperature.

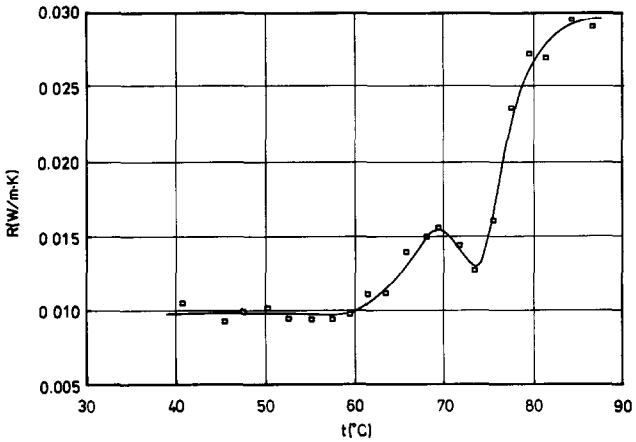

Fig. 3. Illustrative temperature dependence of R for NaDNA solution in 0.01 M NaCl.

respectively. On the basis of these measurements we introduced a parameter R, which is the difference between $\lambda_{DNA/S}$ and λ_S

$$R = \lambda_{DNA/S} - \lambda_S \tag{2}$$

The temperature dependences of R [$R = f(t)$] for various concentrations of NaDNA in the studied solutions (0.5 g l^{-1}, 1.0 g l^{-1}, 2.0 g l^{-1}) were analyzed. Figures 1 and 2 show curves for $\lambda = f(t)$, whereas Figs. 3 and 4 show examples of curves illustrating the dependences $R = f(t)$. The analysis and discussion are presented in the next section.

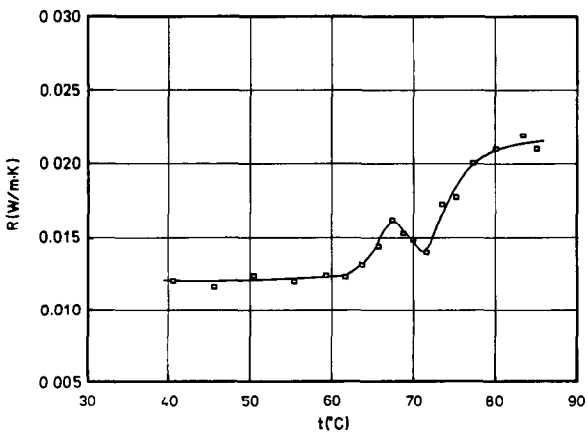

Fig. 4. Illustrative temperature dependence of R for NaDNA solution in 0.005 M $MgCl_2$.

RESULTS AND DISCUSSION

Typical DNA melting curves obtained by the use of the light absorption method, the viscosimetric method or by measurements of the electrical conductivity have the shape of a letter S or its mirror reflection. The melting process is accompanied by a division of the molecule into alternate native regions (with unchanged helical structure) and denatured regions (coil) [30]. The temperature at which the number N_1 of the base pairs in the denatured regions is equal to the number N_2 of base pairs in the helical regions is referred to as a melting temperature, T_M. The degree of denaturation is measured by the parameter θ which is defined by the formula

$$\theta = 1 - \frac{N_1}{N_1 + N_2} \tag{3}$$

or

$$1 - \theta = \frac{N_1}{N_1 + N_2} \tag{4}$$

The melting temperature T_M is assumed to be the one at which $1 - \theta$ (or θ) is equal to 0.5 [17,19,31]. The value of θ in eqn. (3) is usually obtained from absorptiometric or viscosimetric measurements. In the absorptiometric methods, θ is found from

$$\theta = \frac{A_{max} - A_t}{A_{max} - A_{min}} \tag{5}$$

where A_{max} and A_{min} stand for the maximum and minimum value of the absorption coefficient, respectively, and A_t is the absorption coefficient at a given temperature t, obtained at wavelength $\lambda = 260$ nm. On the basis of viscosimetric measurements, θ can be found from

$$\theta = \frac{[\eta_{max}] - [\eta_t]}{[\eta_{max}] - [\eta_{min}]} \tag{6}$$

where $[\eta_{max}]$ and $[\eta_{min}]$ denote the maximum and the minimum value of the intrinsic viscosity and $[\eta_t]$ is the intrinsic viscosity at a temperature t.

In analogy to eqns. (5) and (6), in order to determine the melting temperature T_M from thermal conductivity measurements, we introduce, for the first time ever, the following parameter as a measure of degree of denaturation

$$\theta = \frac{R_{max} - R_t}{R_{max} - R_{min}} \tag{7}$$

where R_{max} and R_{min} stand for the maximum and minimum values of the

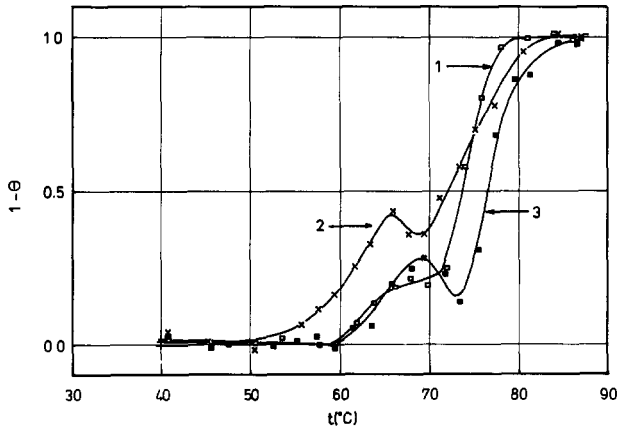

Fig. 5. Temperature dependences of $(1-\theta)$ for different concentrations c of NaDNA in 0.01 M NaCl; 1: $c = 0.5$ g l^{-1}; 2: $c = 1$ g l^{-1}; 3: $c = 2$ g l^{-1}.

difference R, and R_t is the value of R at the given temperature t. Figures 5 and 6 show the plots of $(1-\theta) = f(t)$ dependences for the solutions of NaDNA in 0.01 M NaCl and NaDNA in 0.005 M $MgCl_2$. As follows from the presented curves, $(1-\theta)$ shows a weak temperature dependence up to 55 °C. In the temperature range from ≈ 55 to 80 °C, a distinct increase in $(1-\theta)$ is observed, and for temperatures higher than 80 °C the temperature dependence of $(1-\theta)$ becomes insignificant (as for temperatures lower than 55 °C). In the temperature range ≈ 65–75 °C, an anomaly appears in the $(1-\theta)$ curves and they depart from the typical S-shape. A local maximum in the $(1-\theta)$ value appears, and increases with increasing concentration of NaDNA in solution.

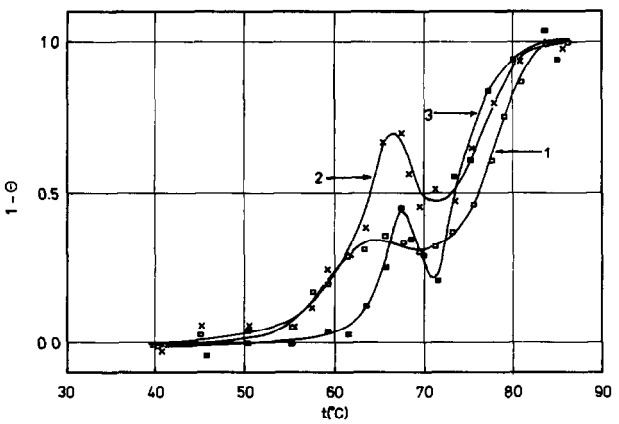

Fig. 6. Temperature dependences of $(1-\theta)$ for different concentrations c of NaDNA in 0.005 M $MgCl_2$; 1: $c = 0.5$ g l^{-1}; 2: $c = 1$ g l^{-1}; 3: $c = 2$ g l^{-1}.

The melting temperatures determined for NaDNA solution in 0.01 M NaCl on the basis of the $(1-\theta)=f(t)$ curves fall in the range 72–76 °C. The melting temperatures T_M obtained for solutions of NaDNA in 0.005 M $MgCl_2$ take values from 73 to 76 °C. We did not find an explicit relation between the melting temperature and the concentration of NaDNA in solution. The accuracy of melting temperature determination was estimated to be ± 2 °C.

The melting temperatures T_M of NaDNA determined by the thermal conductivity method in 0.01 M NaCl solution are in agreement with the values obtained by Pankowski [32], who applied the electrical conductivity method to material from the same producer and in an identical solution (0.01 M NaCl).

The width of the melting interval was determined as the difference between the temperatures T_1 and T_2 at which the tangent to the $(1-\theta)=f(t)$ curve at the point $(1-\theta)=0.5$ takes the ordinate values of 0 and 1, respectively. For the NaDNA solutions in 0.01 M NaCl, a significant scattering of ΔT_M results from 5 to 19 °C was obtained. For the NaDNA solutions in 0.005 M $MgCl_2$ the values of ΔT_M vary from 9 to 12 °C.

A comparison of the results obtained with the literature data is difficult because of the many factors (for instance, buffers or pH) which modify DNA molecular structure, affecting its thermodynamic stability and thus the value of the melting temperature T_M [15,19,33–35].

As has been mentioned earlier, the $(1-\theta)=f(t)$ curves exhibit an anomaly with respect to the typical S-shape. This anomaly appears in the temperature range preceding the melting temperature range. Anomalies in the premelting temperature range have been observed with many measurement techniques [36–43]. They are manifested by an increase in reactivity with formaldehyde [40,41] or by atypical changes in the circular dichroism spectra [37,38,42,43]. An investigation by the laser Raman spectroscopy technique also demonstrated premelting changes at temperatures above 50 °C [39].

The degree of hydration has been found to affect strongly the helix conformation and the stability of DNA [36,44]. A DNA chain is assumed to be surrounded by a hydration layer which stabilizes the double-strand DNA structure. Considerable changes in this hydration layer occur in the premelting temperature range. These changes are the abstraction of dipolar water molecules from the DNA chain, partial release of Na^+ ions together with their hydration sphere from the chain, and an increase in the mobility of the water dipoles bound in the hydration centers [36]. These processes are already taking place in the premelting temperature interval, and they can probably produce significant changes in the double-strand structure prior to the actual melting.

The analysis of the energy transport phenomena in liquids containing structurally complicated DNA macromolecules does not at present permit

the quantitative evaluation of the parameters which characterize the macromolecular structure. However, in the liquid media considered, the results of energy transport measurements indicate the possibility of qualitative analysis of the observed phenomena on the basis of the obtained temperature dependences of $(1-\theta)$. In the temperature range up to $\approx 60\,^{\circ}\mathrm{C}$ the changes occur mainly within the second order structure. If the resulting changes do not significantly affect the spatial conformation of the whole DNA chain, they should not affect the macroscopic process of energy transport. This means that the process of thermal phonon scattering in the liquid under study is the same. On the other hand, essential changes in the solution structure should alter the process of thermal phonon scattering, thus affecting the thermal energy transport. Such significant changes take place during the melting process. Their effect is revealed in the character of the dependences $(1-\theta)=f(t)$ determined by the thermal conductivity method in the DNA melting temperature range.

The premelting anomalies increase with increasing concentration of NaDNA in solution. An increase in this concentration, however, should not affect the Watson–Crick bond energy or the value of the melting temperature T_{M}. In the present work we observed no systematic or significant changes of the melting temperature associated with the DNA concentration. Nevertheless, increasing concentration of molecules in solution may favor molecule association into aggregates, thus changing the conditions of phonon scattering in the premelting temperature range prior to the DNA melting temperature range. It seems that anomalies of the observed course of $(1-\theta)=f(t)$ may be due to the aggregation of DNA molecules and changes of the hydration layer structure of single molecules.

CONCLUSIONS

On the basis of the results obtained in our investigations, it can be concluded that the applied method of measurement on an apparatus constructed to provide high precision permits observations of the molecular conformational changes of DNA in solutions. The temperature dependences of $(1-\theta)=f(t)$ can be used to determine the melting temperature of DNA molecules.

The $(1-\theta)=f(t)$ curves exhibit anomalies in the so called premelting temperature interval, in this way enabling the premelting phenomena to be recorded by means of thermal conductivity measurements.

The only disadvantages of this method are the large volume of solution required to carry out the experiment (60 ml) and the impossibility of analyzing quantitatively the thermal denaturation process in terms of the theory of thermal conductivity in liquids.

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