ELSEVIER Thermochimica Acta 284 (1996) 109–114

thermochimica acta

The calculation of the energy of transition associated with inversions in double-stranded DNA using a rising temperature method[1]

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Abstract

Inspection of the literature has shown that in carefully buffered aqueous solutions, DNA will undergo a reversible inversion between ambient and 90°C. The process of the inversions is followed by UV absorbance of the DNA strand when it is heated and can also be followed using micro-DSC. The data are recorded as temperature against extent of the process and the midpoint is often termed 'inversion or melting'. If the extent of the process is designated by θ, and the temperature by T, then the general process of rising temperature kinetic evaluation can be applied. This is the basis for a kinetic analysis of a number of transitions. This approach is investigated in the present study.

Keywords: Denaturation; DNA; Kinetics; Melting

1. Introduction

The melting curves for DNA oligomers are generally shown as a plot of θ, the fraction melted, against temperature T, and the melting point T_m is quoted [1] as the temperature of the melting curve at $\theta = 0.5$. However, in a normal melting process, one would expect that the phenomenon would occur at one temperature. The presence of a distribution in molecular weight which occurs in macromolecules, and the occurrence of localized melting which is the explanation of the melting curves in DNA would imply that a kinetic aspect to the melting process in such instances should be considered.

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[1] Presented at the 24th North American Thermal Analysis Society Conference, San Francisco, CA, U.S.A., 10–13 September 1995

PII S0040-6031(96)02889-4

A kinetic approach to the published melting curves for DNA can be attempted and is illustrated in the present study. In practice the experimental data would be determined by the species subjected in solution to a programmed temperature rise so that the study comes under the true definition of a thermal analysis procedure. When a solution of duplex DNA is heated above a characteristic temperature, its native structure collapses and its two complementary strands separate and assume the random coil conformation. This process is accompanied by a qualitative change in the DNA physical properties. The native state of the DNA can be monitored by its ultra-violet (UV) absorbance at a defined wave number, which increases by approximately 40% when the DNA denatures. This is described as a melting curve and the temperature at its midpoint is known as its melting temperature or T_m. T_m depends on the nature of the solvent used, and the pH of the solution [2].

The UV/vis spectra of the oligomer duplex can be recorded with a UV/vis spectrophotometer equipped with a thermoset cuvette holder. The thermally induced denaturation can be investigated by heating the oligomer in a 10 mM phosphate buffer solution from 2 to 95°C at a heating rate of 0.3°C min^{-1}. The absorbance is read every 0.1°C at 268 nm. A plot of the fraction denatured versus temperature is made and the T_m is usually determined from the midpoint of the transition [3] (at $\theta = 0.5$). The data used in this present communication are taken from the literature, but derived as indicated above.

A kinetic interpolation would consider possible variations of θ with time to be governed by first-order, second-order and the Avrami–Erofeev expressions. These are the type of kinetic expressions generally put forward to cover physical transitions such as recrystallization and were used here by analogy to study this phenomenon. The expression for first order can be written in either the differential form or the integral form. In interpreting kinetic data from rising temperature experiments, the use of an integral form of the kinetic expression needs an approximation to be made and so in this present application a differential approach is used. Generally the differential equation takes the form

$$d\theta/dt = kf(\theta) \tag{1}$$

where θ is the extent of reaction and t the time at constant temperature.

When applied to an isothermal expression, the form $f(\theta)$ is

$$f(\theta) = (1-\theta)^n \tag{2}$$

with $n = 1$ for a first-order expression, $n = 2$ for a second-order expression and for the Avrami–Erofeev equation

$$f(\theta) = 2(1-\theta)(\ln(1-\theta)^{1/2} \tag{3}$$

The power to which the log expression in the Avrami–Erofeev equation was found to fit in the present analysis was as indicated in Eq. (3), although other values, i.e. 1/3, 1/4, are found in the literature.

With rising temperature experiments, the heating rate β is linear and k (the specific reaction rate) at any specified temperature can be written as

$$(\mathrm{d}\theta/\mathrm{d}T)\beta = kf(\theta)$$

and

$$k = (\mathrm{d}\theta/\mathrm{d}T)\beta/f(\theta) \tag{4}$$

The experimental plot is θ versus T, so $\mathrm{d}\theta/\mathrm{d}T$ can be read from such plots and the specific rate constant k can be calculated, provided a correct choice of $f(\theta)$ is assigned. In the present instance, the correct assignment of $f(\theta)$ is judged from the correlation coefficient obtained in the Arrhenius plot from the relation

$$\ln k = \ln A - E/RT \tag{5}$$

where A is the pre-exponential term, E is the energy of activation for the inversion and R is the gas constant.

2. Results and disscusion

Typical plots of θ against temperature taken from the literature are shown in Fig. 1 [2]. Other data of this kind are available but the information shown in this figure serves as an example where a kinetic evaluation process can be demonstrated. In this present study, we have taken data from the literature which were originally analyzed to give a 'melting point' and subjected it to a kinetic analysis using Eq. (4) to determine the value for the specific reaction rate at different temperatures. The decision on the correct

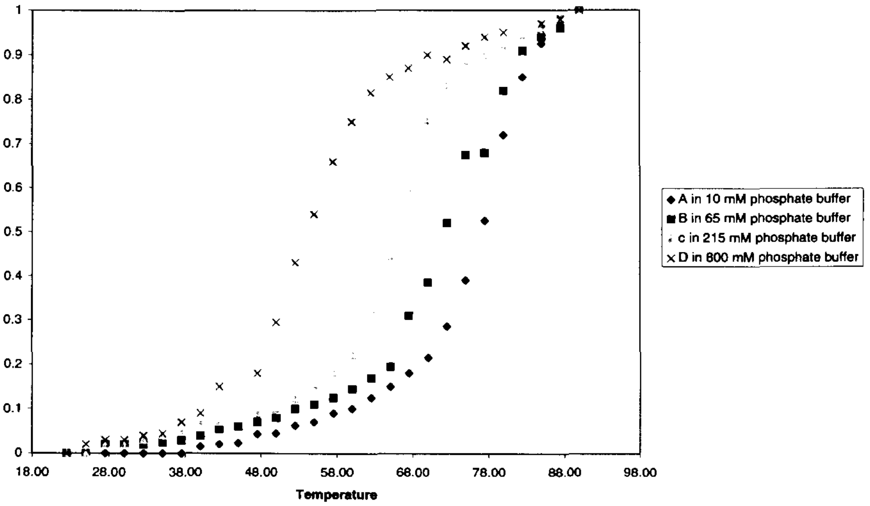

Fig. 1. Typical plots of DNA in different concentrations of phosphate buffer, where θ is the fraction decomposed.

choice of $f(\theta)$ was made partly using selection rules [4, 5] and partly, as already noted, by assessing the resultant Arrhenius plot of $\ln k$ versus $1/T$ using values of R^2, the coefficient of determination. An Arrhenius plot selected from one of these reactions is illustrated in Fig. 2. A typical tabulation of data needed for calculation of the Arrhenius data is provided in Table 1, and Table 2 summarizes the data collected in the runs shown in Figs. 1 and 2. The data given in the table only cover curves A, B, and C. Curve D proved more intractable. This could be because the choice of $f(\theta)$ was too restricted or the inversion under these conditions might be kinetically controlled by more than

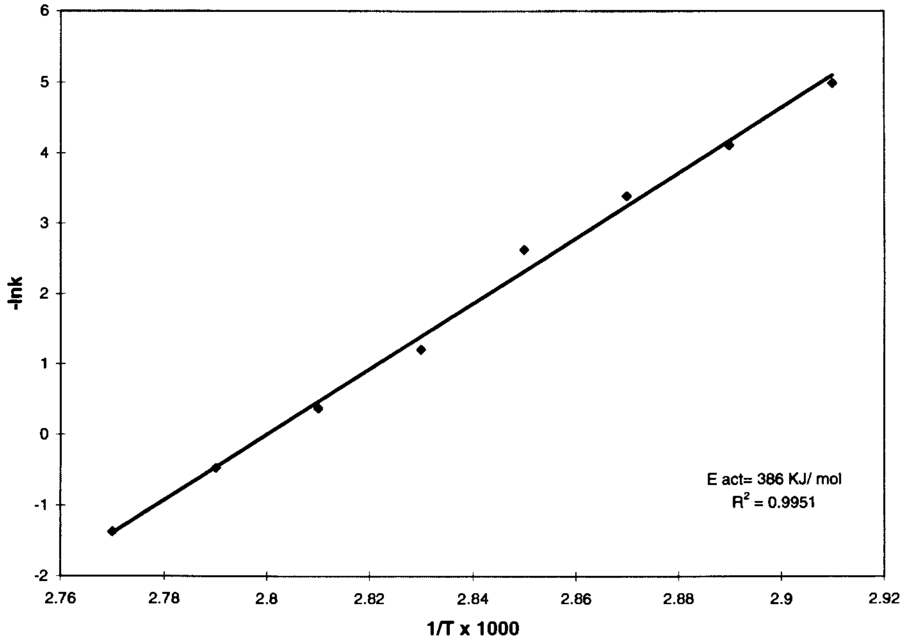

Fig. 2. Arrhenius plot for curve A of Fig. 1 showing Avrami–Erofeev rate kinetics.

Table 1
Data needed for calculation of the Arrhenius parameters

Temp./°C	$1/T \times 1000$	θ	$d\theta/dT$	$f(\theta)$	$-\ln k$
343.15	2.91	0.215	0.014	0.70	5.21
345.65	2.89	0.285	0.0428	0.80	4.59
348.15	2.87	0.390	0.042	0.80	4.22
350.65	2.85	0.525	0.054	0.80	3.92
353.15	2.83	0.720	0.078	0.60	3.30
355.65	2.81	0.850	0.052	0.40	3.28
358.15	2.79	0.925	0.030	0.20	3.29
360.65	2.77	0.965	0.016	0.10	3.28

Table 2
Data from the 3 curves shown in Fig. 1

Curve number	$f(\theta)$	E_{act}/kJ mol^{-1}
A	$2(1-\theta)(-\ln(1-\theta))^{1/2}$	386
B	$(1-\theta)^2$	221
C	$(1-\theta)^2$	220

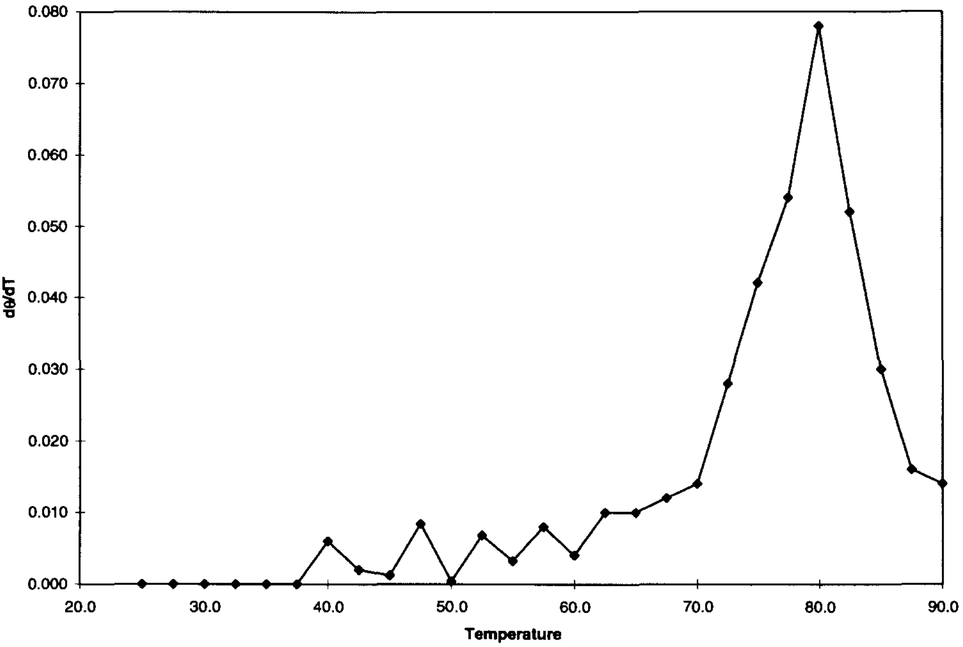

Fig. 3. A typical plot of $d\theta/dT$ versus temperature.

a single process [6]. In the use of this method, the evaluation of $d\theta/dT$ was achieved by using a spread sheet method involving reading temperature taken at 5°C intervals and approximating $d\theta/dT = \Delta T/5$ for these 5-degree intervals. A typical plot is shown in Fig. 3 and a more precise evaluation of $d\theta/dT$ could be achieved by narrowing the temperature interval, for example to 2°C.

Finally, a comment on the fact that different kinetic expressions describe the transition. If melting is involved, then a process of nucleation followed by the growth of a reaction interface would be a possible process describing the kinetics. This could lead to a variety of possible kinetic expressions as found in the present study. The rate of alteration of the reaction boundary in transitions is set out in model form in various books [7, 8].

3. Conclusion

It can be seen that the data can easily be represented by a set of kinetic parameters properly obtained by using all the normal approaches associated with rising temperature kinetic evaluations, as compared with the pseudo-thermodynamic approach which has to cope with the so-called melting being spread over a large temperature range. The use of associating the inversion with melting is somewhat dangerous, as it encourages the further calcuation of other thermodynamic functions associated with this phenomenon on a noncritical basis.

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