

Duplex-single strand denaturing transition in DNA oligomers

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We have measured the temperature driven denaturing, or melting transition in poly d(A)-poly d(T) DNA oligomers of various lengths in different buffer conditions. Our findings are in clear disagreement with two state, reaction kinetics model, and we find that the so-called zipper model, where denaturing proceeds through opening of the duplex at the ends describes well the temperature dependence of the average number of open base pairs. Analysis of the length dependence of the transition parameters however suggest that bubble formation is important and that the transition, in the thermodynamic limit, is continuous, albeit close to first order.

Replication and translation, the fundamental processes in biology, involve the separation of double stranded DNA [1]. Duplex-single strand transition occurs also when DNA is heated[2, 3], upon changing the buffer surrounding the DNA, or under the influence of an external force or torque[4-6]. This so-called denaturing transition has been extensively studied both in natural and synthetic duplexes. Aside from its inherent interest, the process is also of primary importance for applications in the biotech arena. For example, thermal denaturation is an essential step in the Polymerase Chain Reaction amplification procedure.

For the thermally driven transition of native DNA segments of several thousand base pairs the transition occurs in discrete steps, these steps being determined by the particular base pair sequences [7, 8]. In long native DNA the transition is smooth, presumably reflecting a large number of discrete steps occurring at different temperatures. The denaturing transition was also investigated in short oligomers, in the vast majority of cases with random distribution of CG and TA base pairs.

Several models have been proposed to describe this duplex-single strand transition. The standard view of the thermal denaturation of DNA is that it represents the classic competition between energy and entropy. At low temperatures, the thermodynamics is dominated by the binding energy of the base pairs. As the temperature is raised, sections of the DNA separate to take advantage of the greater entropy available to two separated single DNA strands, as opposed to smaller entropy to be found in the smaller configuration space accessible to tightly inter-wound double-stranded DNA. In the thermodynamic limit (or for circular DNA) melting transition occurs because of the growth and accretion of denaturation bubbles. For finite size oligomers "fraying" at the ends of a section of linear DNA is likely to be important as we will discuss below.

Here we address the simplest possible scenario: the denaturing of short oligomers, where each strand is a homopolymer (composed of identical base pairs). Under such circumstances variations of base pair interaction energies (different for CG and AT pairs) do not occur, and

the oligomer can be regarded as a duplex held together by identical base pair interaction energy at the different sites. We are not aware of experiments which address the situation where differences between binding energies associated with different base pairs and other complications do not arise and which thus would allow the experimental test of simple, but important descriptions of the melting transition.

For finite oligomers, the following argument can be made: the binding energy between two bases located at the end of the molecule is smaller than the binding energy for pairs away from the ends, consequently the unbinding occurs most likely by a "zipper" like sequential opening of the base pairs, starting at the ends where the binding is weakest. Such model for DNA melting has been proposed by C. Kittel [9]. In this so-called zipper model, the melting of a linear DNA oligomer occurs entirely as a result of strand separation at the ends. One assigns an energy $-\epsilon_0$ to each bound base pair, and an entropy equal to S_0 to each unbound pair. Then, the partition function of an N-base-pair oligomer is given by

$$Q_N = \sum_{N_1 + N_2 \leq N} e^{(N_1 + N_2)(S_0 - \epsilon_0)/k_B T} \quad (1)$$

where N_1 and N_2 are the number of separated base pairs at the two ends of the linear strand. The zipper model, and this partition function, ought to be reasonably accurate as long as one can ignore the effects of excluded volume, which should be the case for oligomers that are not too long, and if the oligomer is uniform. Here, we use the zipper model as a fitting form for the experimental data. A much simpler model, assuming that only two configurations occur, completely closed and completely separated strands, has also been used to describe denaturing. We call this model the "two-state" model; for this description the appropriate partition function has the form:

$$Q_N = 1 + \exp \left[-N \left(\frac{\epsilon_0}{k_B T} - S_0 \right) \right] \quad (2)$$

The two-state model predicts a first order melting transition in the thermodynamic limit ($N \rightarrow \infty$). As it turns

out, the zipper model also leads to the same conclusion [9]. Using the partition functions as given in Eqs. (1) and (2), physical quantities, such as the average number of paired bases, and the distribution of oligomers with different numbers of open base pairs, can be calculated.

The zipper model is a theoretical scenario in which denaturation takes place via unraveling at the ends of a DNA duplex. However, another contributing factor in the thermal denaturation of long DNA molecules is the denatured "bubble," a portion of denatured DNA bounded by duplexed segments. Entropic considerations militate in favor of an accumulation of such bubbles in a sufficiently long DNA molecule. In fact, the most physically reasonable picture of the denaturing transition is in terms of the proliferation and merging of denatured bubbles. Poland and Scheraga [10, 11] have proposed a model of the transition based on this notion. This model admits of elaboration and is amenable to analysis in the context of field-theoretical approaches to the statistical mechanics of critical phenomena. It is consistent with either a continuous or a first order transition, depending on the influence of base pair inhomogeneity [12, 13] and excluded volume [14]. Furthermore, the Poland-Scheraga model, along with the closely-related model of Peyrard and Bishop [15], produces results that are consistent with scaling and hyperscaling analysis of both continuous and first order transitions [16].

In this paper we focus on the average number of open base pairs as function of temperature, using the intensity of the UV absorption at the wavelength of 260 nm. This parameter predominantly measures base stacking which is directly related to the number of open base pairs [17]. Other spectroscopic methods are also available for monitoring the melting transition. In a separate study [18] we have demonstrated that for the oligomer dA15/dT15 three different spectroscopic methods (UV absorption, CD spectroscopy, and a fluorescence based method) give rise to identical (within experimental error) melting curves. We believe therefore that the assumption we make, namely that the measured UV absorption correctly represents the average number of open base pairs is justified.

We have used synthetic poly(A) and poly(T) oligomers of three different lengths - 15, 30 and 60 bases, PAGE purified, purchased from Operon Technologies. Single strands were dissolved in 1.5 M Phosphate Buffered Saline (PBS). For recombination, solutions of complementary strands were mixed in equimolar ratio, warmed up to 90°C in a water bath, followed by a slow cool down to room temperature. This resulted in complete recombination of the complementary strands as confirmed with hypochromicity measurements. A quantity of few pL was isolated from the stock solutions and it was dissolved in 500 µL of 50 mM PBS buffer adjusting the final DNA concentration to 1 OD. For measurement in buffers of higher molarities the appropriate volumes of

1.5 M PBS were added to the samples in order to obtain 100 mM and 200 mM buffer concentrations. This led to a slight dilution of DNA solutions. Absorption measurements were done in a standard quartz cuvette with a Beckman-Coulter 640 UV/Vis spectrophotometer with an integrated Peltier heating block and a temperature controller that enable temperature control between 10°C and 90°C. Temperature dependent absorption measurements were done in steps of 1 K. Before the absorption measurement the samples were thermalized at every temperature for 5 min - the time needed for the cuvette and solution inside to reach the temperature of the heating block. The absorption is smaller for a DNA duplex than for the same DNA in single strand form [17]; this is referred to as hyperchromicity. The main component of this effect is the screening of the intra-base excitations by dipole-dipole interactions between stacked bases, with significantly smaller screening for a single strand DNA on which bases are unstacked. For poly d(A)-poly d(T) this difference, the ratio of the intensity for single strand and duplex DNA, is 1.4 (see below). In Fig. 1, the temperature dependence of the UV absorption intensity is displayed for three different oligomer lengths. For all oligomers we observe a smooth transition from the duplex to the single strand state, with the transition temperature (defined as the half-point of the transition-see below) and width, increasing with increasing length. The linear slope visible in the melting curves after the sigmoidal transition region is a well-known phenomenon attributed to residual base unstacking in the single strands. The linear slope before the transition is indicative of temperature driven conformational changes in the double helix; this phenomenon, known as "premelting," is not well understood [19]. These phenomena are not accounted for in the models above: the first one is not related to strand separation, while the degrees of freedom relevant for the second are not taken into account by the zipper model.

We start with a comparison between the experimental results and the two state and zipper models discussed above. Such comparison is shown in Fig. 1. In the zipper model, the fitting parameter ϵ_0 , (in Kelvin) was allowed to vary between 6405 and 7090, and the parameter S_0 was fixed at 20.8. The points utilized for the fit were those closest to the transition. The dominant temperature dependence of the UV absorption in the vicinity of the melting transition is due to the separation of base pairs. At temperatures significantly higher and lower than the nominal melting temperature, the absorbance exhibits a temperature dependence as a result of effects that are unrelated to the denaturation of the DNA, as discussed above.

The fitting results for zipper model are summarized in the table below (Table 1). The binding energy increases with salt concentration, because of ionic screening (the temperature at the midpoint is T_m). The fact that the two-state model fits the data for the 15mer but not the 60mer

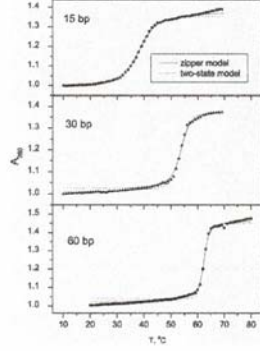


FIG. 1: Temperature dependence of the UV absorption measured at 260 nm for poly d(A)-poly d(T) oligomers of three different lengths -15, 30 and 60 base pairs (bp). All curves are at a molarity of 100mM.

| bp | molarity/mM | $-\epsilon_0/K$ | S_0 |
|----|-------------|-----------------|-------|
| | 50 | 6405 | |
| 15 | 100 | 6524 | 20.8 |
| | 200 | 6611 | |
| | 50 | 6742 | |
| 30 | 100 | 6822 | 20.8 |
| | 200 | 6916 | |
| | 50 | 6848 | |
| 60 | 100 | 6991 | 20.8 |
| | 200 | 7090 | |

TABLE 1: Parameters used to fit (see text) the measured absorption curves to the zipper model.

indicates that gradual opening of the duplex plays an important role in the melting in the case of larger oligomers.

Figure 2 displays the temperature derivative of the UV absorption. In this case the absorption has been normalized so that the integrated weight under each peak is equal to one in all cases. Two curves are shown for each data set. One represents the results of taking the derivative of the best fit of tchezipper model to the data. The other was obtained by taking the temperature derivative of a three-point Lagrange interpolation through the data. While there are systematic differences between the two derivative curves, the tendencies of both are the same, as can be seen in Fig. 3, representing a log-log plot of the maximum of the derivative curve against the number of base pairs in the oligomers. This last figure is relevant to the analysis discussed below.

In light of the likely relevance of standard scaling notions to DNA melting, we have applied finite size scaling to our data. According to finite size scaling analysis, the specific heat of a d-dimensional system with (linear) size L that undergoes a continuous phase transition will take

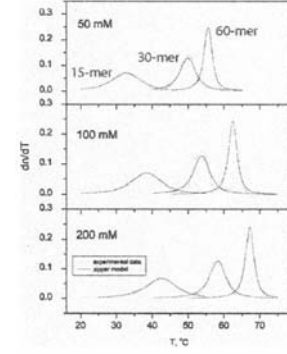


FIG. 2: Temperature dependence of the derivative of the UV absorption of poly d(A)-poly d(T) at three different molarities. Here, the absorption has been normalized so that the area under each peak is one. The full line is obtained fitting the absorption curves with the zipper model and then taking the derivative ("zipper interpolation"), the dotted line is obtained using a three-point interpolation of the experimental data. The parameters used for the zipper fits are given in the table. In all cases, the height of the maximum, and the temperature at which this maximum occurs, increase mono-tonically with the size of the oligomer.

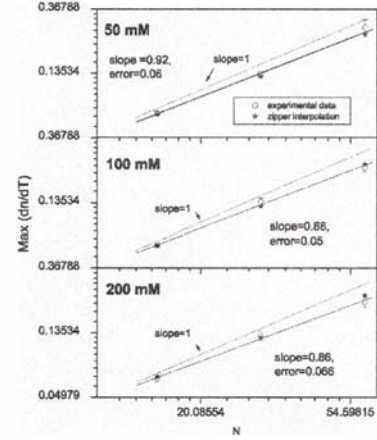


FIG. 3: Log-log plots of the maximum of the derivative of the melting curves against the size of the oligomer at the three molarities. The two sets of points refer to two different interpolations of the data, see caption to Fig.2. The best-fit linear regression fit is shown. Also displayed is a line with unit slope, representing a first order transition in the large N limit.

the form

$$c \propto |t|^{d\nu-2} f(L|t|^\nu) \quad (3)$$

in the immediate vicinity of the transition. In the above expression, t is the reduced temperature and ν is the correlation function exponent. As no finite system exhibits thermodynamic singularities, the behavior of the function f is such that the singularity in t in the prefactor

is cancelled as $t \tilde{\Delta}' > 0$. This implies a specific heat at the bulk transition temperature that goes as $L^{(2-d)/\nu}$. In the case at hand, $d = 1$, so the specific heat at the bulk transition temperature scales as $L^{(2-\nu)/\nu}$. This sort of dependence on L also characterizes the maximum in the specific heat. The temperature derivative of the UV absorption should behave in essentially the same way as does the specific heat at the denaturing transition, against L , and we have evaluated the maximum values of dn/dT using two procedures. The first involves the Lagrange interpolation through the data. The second, which we call the "Zipper interpolation" refers to a theoretical fit to the observed temperature dependence, using Eq. (I), with \tilde{a} , as a free parameter for each oligomer and identifying the maximum of the derivative of the fit. The length dependence of the maxima are displayed in Fig. 3, and we find that the optimal fit is consistent with a specific heat that scales as $L^{0.86}$ which implies a $\nu = 1.075$ and a specific heat diverging as $|t|^{-0.925}$ in an infinite system. Consequently, the transition is continuous, but close to first order. Our findings are, therefore, consistent with the picture that bubble formation, in addition to opening at the ends, is important for the denaturing process. This is so even in the case of relatively short oligomers.

The experiments and analysis given above lead to several conclusions. First, it is clear from Fig 2 that the zipper model is a better fit to the experiments than the two state model for longer oligomers. This is in agreement with observations [17] that the dependence of the melting temperature on oligomer concentration is not significant above a length of about 14, while for shorter oligomers the transition is a chemical equilibrium between single strand and duplex species, which depends on concentration [20]. Contrasting our results obtained on different lengths suggest that bubble formation, and a scaling scenario of the denaturing transition, is likely to be important. Experiments on longer oligomers, where the bubble formation process is expected to be more important would be desirable in order to distinguish between the zipper model and phase transition scenario. Finally we note that we have analyzed only the average, or mean number of open base pairs. The measurements described here do not offer insight into effects associated with fluctuations.

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