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Determination of DNA cooperativity factor

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ABSTRACT

The paper presents measurements of the difference in the melting temperature of a ColE1 DNA region when it is located inside the DNA helix and at its end. A direct comparison of calculations based on the rigorous theory of helix-coil transition with experimental data for 0.2 M Na^+ (the conditions for fully reversible melting) yielded the value of $2.5\text{-}5\times10^{-5}$ for the cooperatively factor σ. We discuss the reversibility of DNA melting and the possibility of applying the "all-or-nothing" concept to the melting of DNA regions.

INTRODUCTION

The helix-coil transition in DNA has been studied for more than 25 years, and practically all the parameters of the basic model for DNA melting have been determined. For instance, there are accurate data concerning the melting temperatures of AT and GC pairs /1/ and their melting enthalpy /2,3/. Nevertheless the cooperatively factor $\sigma = \exp(-F_s/RT)$ (where F_s is the free energy of the boundary) has been determined with a rather low accuracy: $\sigma = 10^{-4} \div 10^{-5}$. The most reliable value of this parameter was found by comparing theory to the results of the melting of double-stranded homopolynucleotides /4/.

Although the theoretical differential curves of DNA melting are highly sensitive to this parameter, it cannot very well be determined from a direct comparison of complete theoretical and experimental melting curves. The reason for this is that a detailed profile of theoretical melting curves cannot be fitted to the experimental ones by variation of the cooperatively factor. To determine the value of σ from an analysis of differential melting curves one needs an experi-

mental system where the melting of one and the same region can be observed with different boundary conditions. The present paper is about getting such data and their analysis.

A description of the experimental system required was found in /5/. That paper dealt with the melting of ColE1 DNA. The denaturation of ColE1 DNA was mapped at different stages of the process, allowing every peak of the differential melting curve to be matched with a cooperatively melting region of DNA. For instance, the very first isolated peak of the differential melting curve (Figure 1) was found to correspond to the melting out of a ~350bp-long region. This region is at the end of the molecule when DNA is cleft by the EcoR1 restriction endonuclease but it is in the middle of the helix and still the first to melt when circular ColE1 DNA has been treated with Sma1 (single-site cleavage). Since we deal with the same region in both cases, the shift of the melting temperature with changing position relative to the helix ends is entirely due to boundary effects. Therefore by measuring the temperature shift we get the necessary information for determining the cooperativity factor. Knowledge of the nucleotide sequence in this DNA region was essential for a quantitative analysis of the experiments. After 1/4 of the ColE1 DNA was sequenced /6/ the measurements of the difference in the melting temperature of the region concerned were analysed and a reliable estimation of the cooperativity factor σ was made. Analysis of the experimental results has shown that a quantitative interpretation of the differential melting curves should involve a rigorous solution of the basic model. A representation of the melting process as a sum of a few cooperative transitions /7 - 9/ ignores the entropic effects which considerably affect the profile of the differential melting curves.

MATERIALS AND METHODS

1. ColE1 plasmid DNA was extracted as described in /5/.
2. The enzymes EcoR1 and Sma1 were kindly donated by Dr. B.S.Naroditskii from the Ivanovskii Institute of Virology,

USSR Academy of Medicine.

3. DNA cleavage by Sma1 (DNA-Sma) was carried out at 30°C in a solution containing 15 mM Tris-HCl, 6 mM $MgCl_2$, 15 mM KCl (pH 9.0). EcoR1 was applied under the following conditions: 25°C, 100 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$ (pH 7.8). Such DNA preparation is denoted as DNA-EcoR.The cleavage reaction was controlled by electrophoresis in agarose gel. The reaction was stopped by adding EDTA up to 20 mM, then DNA was transferred into a 0.1xSSC solution through gel filtration on a G-50 Sephadex column (SSC is a salt solution containing 0.15 M NaCl and 0.015 M sodium citrate, pH 7).

4. DNA solutions with different Na^+ contents were prepared by adding the necessary amount of 10xSSC to the initial DNA solution in 0.1xSSC.

5. DNA melting was performed on a Cary 219 double-beam recording spectrophotometer (Varian, Switzerland) equipped with a specially designed thermostatically controlled cell, with the temperature changing continuously at a constant rate of 0.1°C per minute. The melting curves were differentiated as in /5/ by means of an HP9825A calculator (Hewlett-Packard, USA) interfaced with an HP9864A digitizer.

6. Determination of the distance (δT_1) between the first peaks in the differential melting curves for DNA-EcoR and DNA-Sma. When the distance between the first peaks, δT_1, exceeded 0.5°C it was determined from the differential melting curve of a composite preparation of DNA-EcoR and DNA-Sma. Smaller values of δT_1 make the peaks in composite melting curves overlap, so δT_1 was determined by superposing the differential melting curves for DNA-EcoR and DNA-Sma. This method was based on the fact that the cleavage of ColE1 DNA by EcoR1 or Sma1 did not appreciably change the profile of the differential melting curve beyond the first peak. We found two relative positions of the differential melting curves ($d\vartheta/dT$) corresponding to the minimum of the expression

$$\int \left| \frac{d\vartheta^{(Sma)}}{dT} - \frac{d\vartheta^{(EcoR)}}{dT} \right| dT \qquad (1)$$

The first position of the curves corresponded to integration in (1) over the entire melting range outside the first peak while the second position corresponded to integration over the range of the first peak only. The value of δT_1 was assumed to equal the relative displacement of the curves when switched from the first to the second position.

This procedure eliminated the possible shift between the melting curves for DNA-EcoR and DNA-Sma due to errors in Na^+ concentration. In this way δT_1 was determined with an error of $\pm 0.02^{\circ}C$.

7. Theoretical calculation of the melting curves. For theoretical calculations we used the algorithm /10/. The value of the parameters corresponding to the 1xSSC buffer were: melting enthalpy for AT pairs 8550 cal/mol /2,3/, melting temperature for AT and GC pairs $343.0^{\circ}C$ and $384.0^{\circ}C$ respectively /11/ and loop-weighting factor $\alpha = 3/2$ /4/. The parameter σ varied. We used the nucleotide sequence of the pA03 plasmid /6/ including the complete ColE1 DNA region under study. Linear pA03 DNA molecules obtained by treatment with EcoR1 and Bpa1 endonucleases corresponded in the calculations to DNA-EcoR and DNA-Sma.

RESULTS

Figure 1 shows the differential curves for the melting in 0.1xSSC of linear ColE1 DNA molecules obtained by cleavage with EcoR1 (DNA-EcoR, curve a) and Sma1 (DNA-Sma, curve b). The main difference lies in the position of the first peak: it occurs at a lower temperature in 1a. The difference is clearly demonstrated by Figure 2a which shows a section of the differential melting curve for a composite DNA preparation containing approximately equal amounts of DNA-EcoR and DNA-Sma. A considerably smaller shift in the first peak is observed between the differential melting curves of DNA-EcoR and DNA-Sma (δT_1) in a 1xSSC buffer (Figure 3). In this case the peaks are not resolved in the differential melting curve of a composite preparation (Figure 4) and the shift had to be determined through an optimum superposition of the curves outside the first peak range (see item 6, MATERIALS AND METHODS).

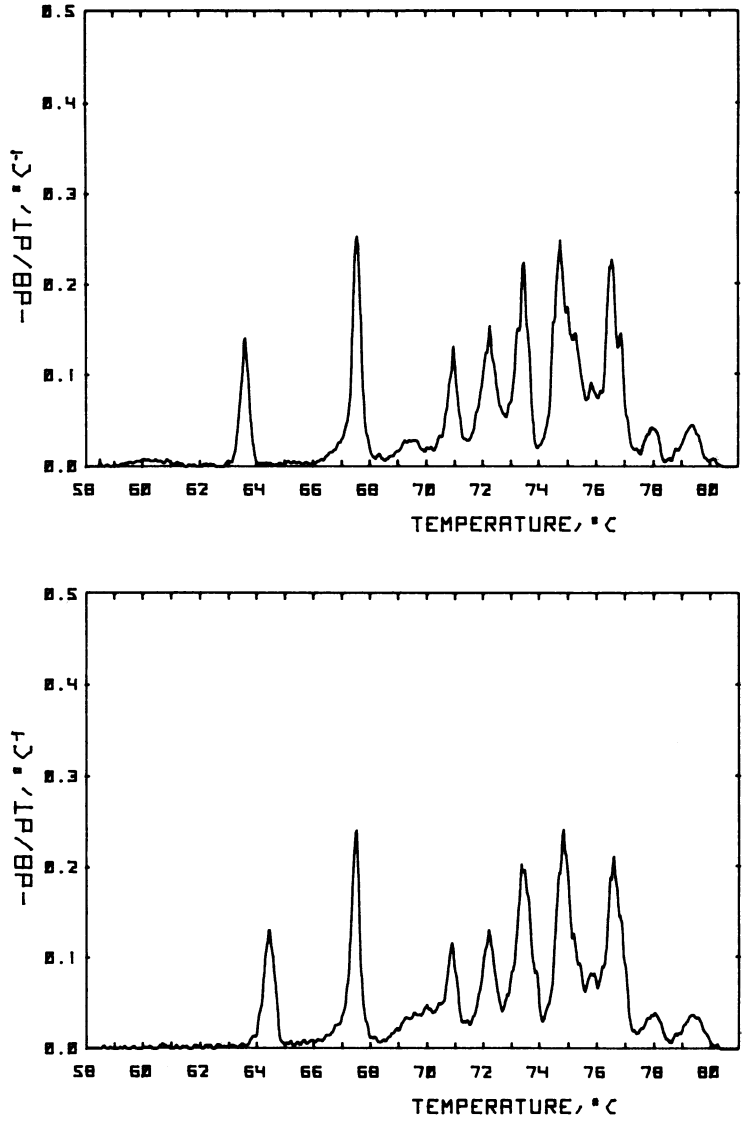

Figure 1. a) Differential melting curve for ColE1 DNA in 0.1xSSC linearized with EcoR1 endonuclease (DNA-EcoR).
b) Differential melting curve for ColE1 DNA in 0.1xSSC linearized with Sma1 endonuclease (DNA-Sma).

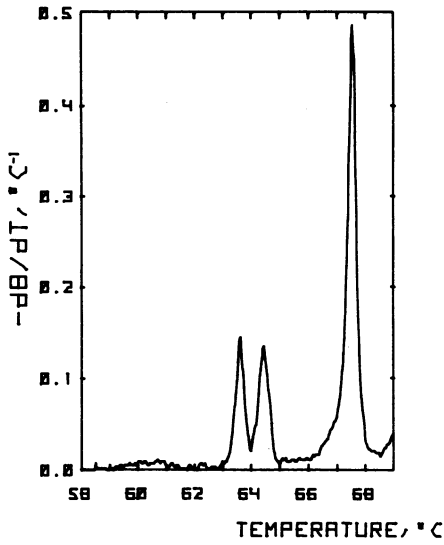

Figure 2. Part of the differential melting curve for a composite preparation of DNA in 0.1xSSC containing equal amounts of DNA-EcoR and DNA-Sma.

The melting curves of DNA-EcoR and DNA-Sma were recorded for different ionic strengths in the range from 0.01 to 0.4 M Na^+. For each ionic strength the value of δT_1 was determined. Figure 5 shows the dependence of δT_1 on the concentration of Na^+. The value of δT_1 grows from ≈ 0.3 to $\approx 1.7^{\circ}C$ with the Na^+ concentration dropping from 0.1 to 0.01 M, but δT_1 changes negligibly in the range from 0.1 to 0.4 M Na^+.

As shown in /12/, at $[Na^+] \lesssim 0.1$ M the melting process is usually not equilibrium throughout the transition range. Yet, equilibrium melting is a necessary condition for the applicability of theoretical analysis based on the equilibrium model of the helix-coil transition. Therefore we checked the reversibility of melting in the first peak for DNA-EcoR and DNA-Sma in 0.1xSSC.

In these experiments DNA was subjected to partial denaturation corresponding to the first-peak melting, then cooled at the same rate while the differential curve of renaturation was recorded. The results presented in Figure 6 shows that the

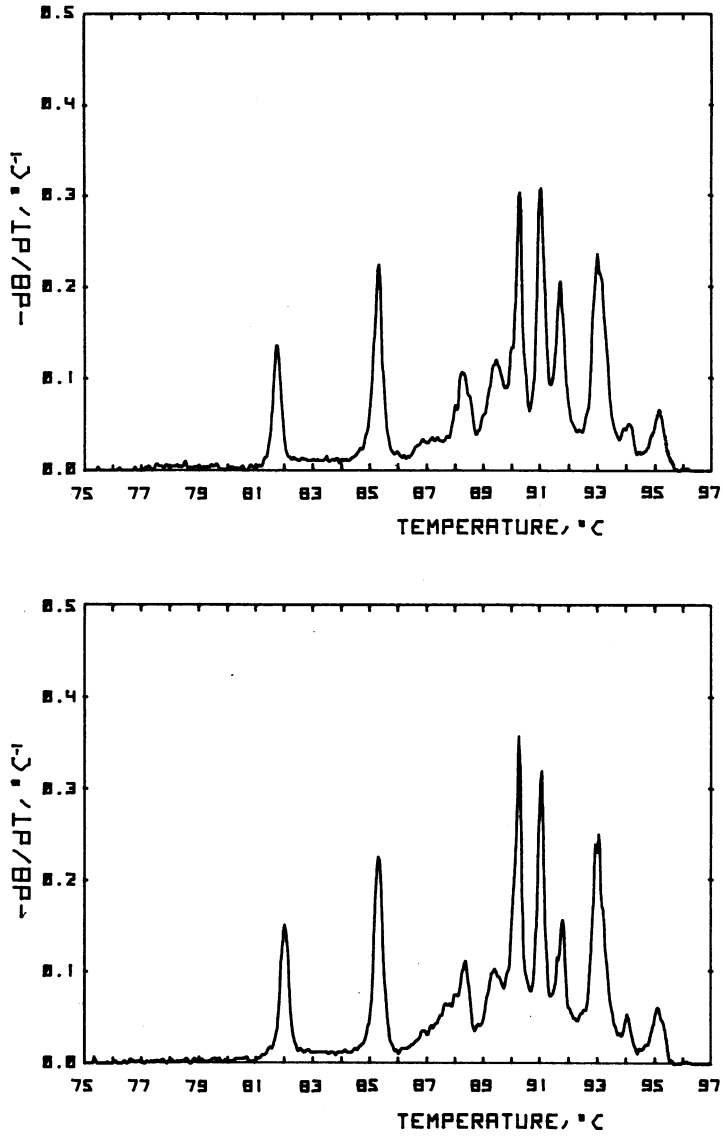

Figure 3. a) Differential melting curve for ColE1 DNA in 1.0xSSC linearized with EcoR1.
b) Differential melting curve for ColE1 DNA in 1.0xSSC linearized with Sma1.

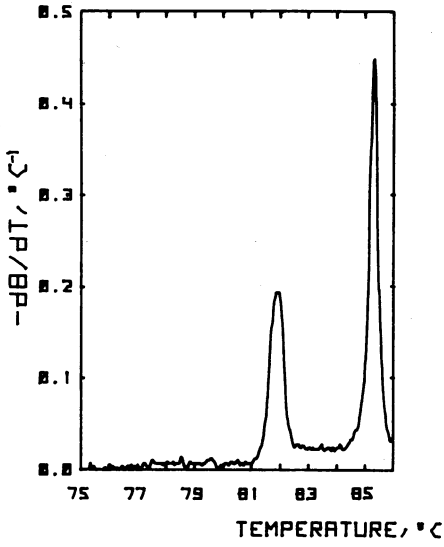

Figure 4. Part of the differential melting curve for a composite preparation of DNA in 1.0xSSC containing equal amounts of DNA-EcoR and DNA-Sma.

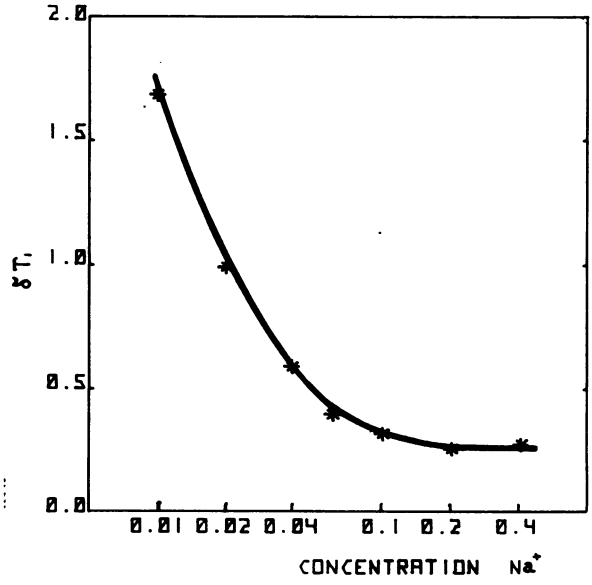

Figure 5. Dependence of the temperature difference for the first peak in the differential melting curves of DNA-EcoR and DNA-Sma (δT_1) on $[Na^+]$.

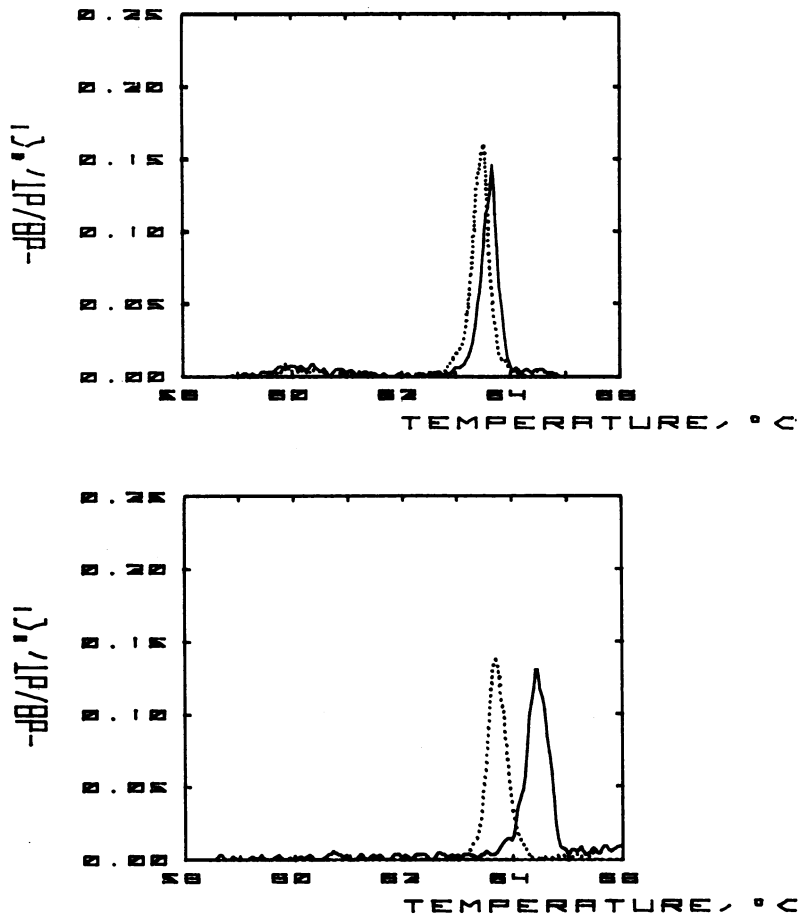

Figure 6. Differential curves of partial denaturation (_____) and renaturation (.....) of linear ColE1 DNA forms
a) DNA-EcoR in 0.1xSSC
b) DNA-Sma in 0.1xSSC

melting of DNA-Sma in 0.1xSSC is not reversible even in the first peak of the differential melting curve. In contrast, the melting of DNA-EcoR is reversible in the range of the first peak. The peaks in the differential melting curve corresponding to a non-equilibrium process should be shifted towards higher temperatures /12/, hence we should get a larger value of δT_1. Similar experiments carried out in 1xSSC showed the

melting to the equilibrium for both DNA preparations. Thus the observed growth of δT_1 with decreasing ionic strength can at least partly be explained by the non-equilibrium melting of DNA-Sma at low ionic strength. On the other hand these results show that the experimental values of δT_1 can be compared with theoretical calculations only at a high ionic strength.

We used for our theoretical analysis the data obtained in 1xSSC. The value of δT_1 under these conditions is $0.26 \pm 0.02^{\circ}C$.

Differential melting curves were calculated for pA03 plasmid DNA. Its base sequence is known, it constitutes 1/4 of ColE1 DNA /6/ and includes the complete ColE1 DNA region under study /5/ (see item 7, MATERIALS AND METHODS). The calculations corresponded to breaks in circular pA03 DNA at positions 1611 and 140 according to /6/. The first variant corresponds to DNA-EcoR and the second one provides the same melting conditions for the region as DNA-Sma. All curves were renormalized for the entire length of ColE1 DNA. Figure 7 presents partial theoretical differential melting curves (first peak) computed for different values of σ from 1.25×10^{-5} to 1×10^{-4}. Clearly a change of σ changes the amplitude of the peaks as well as the distance between them. Table 1 shows the calculated values of δT_1 and the amplitudes (A_1) of the peaks for EcoR1 and Sma1 - treated DNA corresponding to different values of σ. The experimental values of $A_1 = 0.15$ and $\delta T_1 = 0.26 \pm 0.02$ are best met by those theoretical values obtained for σ from 2.5×10^{-5} to 5×10^{-5}.

DISCUSSION

The value of the cooperativity factor $\sigma = (2.5 \div 5.0) \times 10^{-5}$ was obtained here by a direct comparison of experimental and theoretical melting curves for natural DNA. This value is quite consistent with the one yielded by theoretical analysis of homopolymer melting curves /4/. Ours, however, is a more direct approach since instead of analysing the shape of the whole curve we measure an effect which is entirely due to changing boundary conditions for the melting of one and the same region. It should be noted that although the basic helix--coil transition model cannot accurately predict the absolute

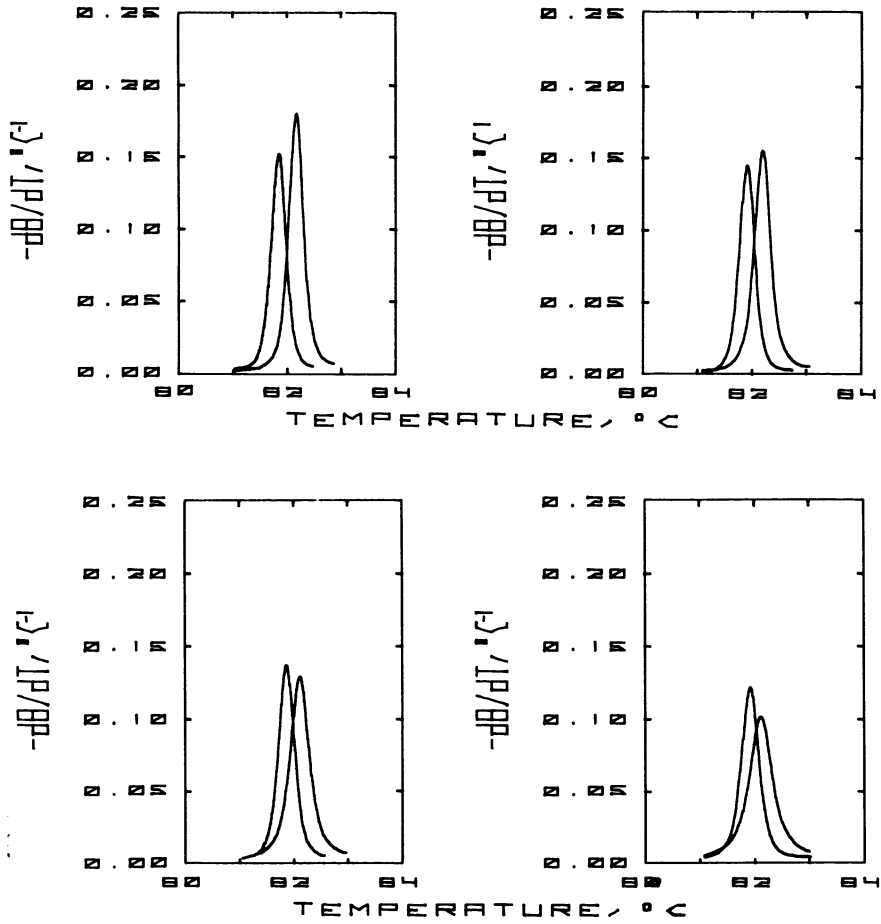

Figure 7. Theoretical partial differential melting curves calculated for different values of σ
a) $\sigma = 1.25 \cdot 10^{-5}$ b) $\sigma = 2.50 \cdot 10^{-5}$
c) $\sigma = 5.00 \cdot 10^{-5}$ d) $\sigma = 1.00 \cdot 10^{-4}$

melting temperature of a DNA region because in the present form it fails to allow for the heterogeneity of stacking interaction, it must correctly predict the temperature shift due to changed boundary conditions. Yet, a comparison of the experimental results with calculations only makes it possible to find the product $\sigma \cdot n^{-\alpha}$, where n is the size (bp) of the melting region and α is the loop-weighting factor /1/. The

TABLE 1. Theoretical values of the distance between peaks (δT_1) and their amplitudes (A_1) for two different cases of single-site cleavage corresponding to different values of σ .

σ	T_1	A_1^{EcoR}	A_1^{Sma}
$3,13x10^{-6}$	0,40	0,156	0,214
$1,25x10^{-5}$	0,32	0,151	0,179
$2,5x10^{-5}$	0,28	0,145	0,155
$5,0x10^{-5}$	0,24	0,136	0,128
$1,0x10^{-4}$	0,20	0,121	0,101
$2,0x10^{-4}$	0,16	0,100	0,077

reason for this is that in the theoretical model σ always enters the combination $\sigma \cdot n^{-\alpha}$ while n remains constant in a real experiment. Our value of the cooperativity factor σ is based on the use of the generally accepted value of the loop factor $\alpha = 3/2$ /1,4/.

Analysis of our experimental results shows that their quantitative interpretation requires a rigorous solution of the basic model. Azbel's /7-9/ representation of DNA melting as a sum of a few "all-or-nothing" transitions leads to erroneous results. Indeed, analysis of this simplified model would yield the following simple expression for the difference in the melting temperatures of the same region located inside or on the end of the helical area (7-9):

$$\delta T_m = \frac{T_m}{n} \cdot \frac{F_s + f(n)}{\Delta H} \qquad (2)$$

Here T_m is the melting temperature of the region when it is located at the end of the molecule, F_s is the free energy of the boundary, ΔH is the melting enthalpy per base pair, $f(n) = \alpha RT \ln(n)$ is a term allowing for the non-additive nature of loop entropy.

Let us introduce into (2) the values F_s = 7000 cal/mol, which corresponds to $\sigma = 5x10^{-5}$, f(n) = 6200 cal/mol for n=350 bp and ΔH = 8840 cal/mol. In the case of a rigorous solution of the basic model these values would yield $\delta T_1 = 0.24^{\circ}C$.

Yet, the expression (2) gives $\delta T_m \simeq 1.5^{\circ}C$. The discrepancy is most probably caused by two factors.

First, the simplified model of the process does not allow for the degeneration of the final or initial DNA states. Indeed, when the melting region is inside the helical area its initial state (helix) is not degenerate while its final state involves multiple localizations of the boundaries of the denatured area. As a result δT_m decreases by the value

$$\frac{T_m}{n \cdot \Delta H} \cdot RT \cdot \ln W$$

Analysis of the theoretical denaturation maps for the region concerned shows each boundary of the melted area to the localized with an accuracy to within $\sim 30 \div 60$ bp. This corresponds to $W \sim 1000 \div 5000$ and further decreases δT_m by $\sim 0.7 \div 0.8^{\circ}C$. These effects caused by different degrees of degeneration cannot be allowed for by Azbel's model. Therefore that model cannot claim to provide a rigorous quantitative description of DNA melting.

Secondly, it seems that the melting of the first region in DNA-Sma is somewhat shifted towards lower temperature by the denaturation of a still low-melting short section. This section is located at the very end of the DNA-EcoR helix and here melts independently of the first large region. In DNA-Sma, however, its melting temperature coincides with the starting point for the melting of the first large region (see Figure 2). This is confirmed by detailed theoretical calculations which of course allow for the effect of this factor on the value of δT_1.

We found the melting of the first DNA-Sma region in 0.1xSSC inside the helical area to be a non-equilibrium process (see Figure 6). At first glance this results seems to contradict the model for the emergence of irreversibility /1, 12/ suggesting that the process becomes nonequilibrium only after the number of helical regions decreases in the course of denaturation. However our data prove quite consistent with this scheme if it is somewhat generalized. Indeed the first-peak melting region was found to have a sequence

/6/ wherein approximately 80bp-long stretches at both ends have a lower GC content (~20%) than the rest (32%). Therefore the renaturation of the entire melted region at a low ionic strength occurs at a lower temperature which allows the renaturation of the AT-rich terminal stretches. Now if the region is at the end of the DNA molecule its melting temperature gets lower and never rises above the renaturation temperature of the inner AT-rich terminal stretch. Thus the model for the emergence of irreversibility in DNA melting may be complemented with a situation when the melting is non-equilibrium at a low ionic strength because of the lower thermal stability of long enough terminal stretches of a cooperativeely melting region. As a result irreversibility may manifest itself quite early in the melting process, as with ColE1 DNA.

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