Volume 10 Number 6 1982 Nucleic Acids Research

A comparison of experimental and theoretical melting maps for replicative form of φX174 DNA

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Received 19 January 1982; Accepted 23 February 1982

ABSTRACT

A previously elaborated technique for fixing a chosen partially melted state of DNA with glyoxal was used in a study of the melting process of the replicative form (RF III) of øX174DNA. Electron-microscopic maps corresponding to five points of the melting curve of RF III were obtained and compared with the theoretical melting maps obtained in (4) and (6). This comparison clearly shows that only rigorous calculations (4) and not the ones proposed by Azbel (6,7) correctly predict the course of RF III melting.

INTRODUCTION

The orthodox theory of helix-coil transition which treats DNA as a double-stranded heteropolynucleotide and allows for loop formation provides all the major characteristics of the DNA melting process on the basis of a direct computation of the partition function (see review /1/). Calculations based on this model have revealed the meaning of the narrow peaks in the differential melting curves as a fine structure effect /2/. The algorithm proposed by Fixman and Freire /3/ proved to be most adequate for computer calculations of melting curves for DNA with a given base sequence. In this way the melting curves have been calculated for a number of DNAs with known sequences /4,5/, and in /4/ the calculated curve for RFII of øX174 DNA was compared with the experimental melting curve.

Lately, alongside these rigorous calculations, Azbel has published his studies /6,7/ where he regards DNA melting as "all-or-none" cooperative denaturation of DNA regions from dozens to hundreds of base pairs long. The melting of each

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0305-1048/82/1006-1867$2.00/0

region is described by a simple analytical expression and the melting curve of the entire DNA is presented as a sum of such analytical functions. Azbel used this approach to describe the melting of RF II of ØX174 DNA. Through a computer treatment of the various ways of DNA decomposition into a number of cooperatively melting regions a variant was chosen for which the calculated differential melting curve of the entire DNA and that of its two largest restriction fragments coincided with the corresponding experimental curves. Using the relation between the melting temperature of each cooperatively melting region and its mean GC content, Azbel calculated the GC content of each region and proposed that this approach should be used for DNA sequencing.

Thus, in addition to rigorous theory, Azbel proposes an approximation to be used for the analysis of DNA melting. The problem is, however, that it is rather difficult to estimate a priori the degree of validity of the approximation used. The traditional juxtaposition of the melting curves cannot be used as a crucial test in this case for in Azbel's model the curves should coincide by definition as a result of the introduction of a large number of adjustable parameters. Another approach consists in comparing the melting maps. This is a crucial test because Azbel's / 6,7 / predicted denaturation maps for RF ØX174 are quite different from the ones obtained by rigorous calculations / 4 /. To resolve this controversy we present here the experimental melting maps for the same DNA. Such true thermal melting maps have become availiable only recently after the method was proposed for fixing melted regions of DNA at any stage of heat denaturation with low concentrations of glyoxal / 8 /. We have used this method and obtained melting maps for different temperatures in the melting range of ØX174 RF III DNA, embracing the entire melting process. These maps were compared with Azbel's data /6,7/ and the results of calculations based on rigorous theory /4/.

MATERIALS AND METHODS

ØX174 RFI DNA was obtained in the same way as in /4/;

after the superhelical DNA fraction was obtained the possible admixtures of short RNAs eliminated by gel filtration on an A-15 Biogel. RFI DNA was converted into linear RF III DNA by means of the single-site restriction endonuclease Pst I in a buffer containing 0.006 M Tris, pH 7.4, 0.006 M $MgSO_4$, 0.05 M NaCl and 0.006 M mercaptoethanol. The PstI endonuclease was kindly made available by Dr.B.A.Rebentish from the Institute of Genetics and Selection of Industrial Microorganisms. Phenol-deproteinized RF III DNA was transferred into a 0.1 x SSC solution by gel filtration on a Sephadex G-50 column. DNA melting was carried out on a Cary 219 spectrophotometer according to the procedure used in /8/. The fixation of melted regions in DNA was carried out for five points in the melting interval according to the procedure described in /8/. Immediately after the fixation procedure was finished each DNA sample was prepared for electron-microscopic visualization. The preparation of DNA for electron microscopy, the measuring of molecules and denaturational mapping were all performed according to /8/. An array of 50-90 DNA molecules was used for the construction of each melting map. The denaturation maps based on Azbel's calculations were built according to the table presented in /6,7/ using the equations given in the same paper. The denaturation maps based on rigorous theory were taken from /4/ where they had been obtained using the first data on the base sequence of ØX174 DNA /9/. These maps are not different from the ones obtained for a corrected sequence of ØX174 DNA /10/ (A.V.Vologodskii, private communication).

RESULTS

Figure 1 shows the experimental differential melting curve for ØX174 RF III DNA in 0.1 x SSC. Arrows indicate the temperatures for which denaturation maps were made. They usually corresponded to minima in the differential melting curve, where six clear-cut peaks are resolved. At five points in the melting range (indicated by arrows)in Fig.1 melted regions were fixed and the samples were prepared for electron microscopy. Figure 2 presents electron micrographs of individual DNA

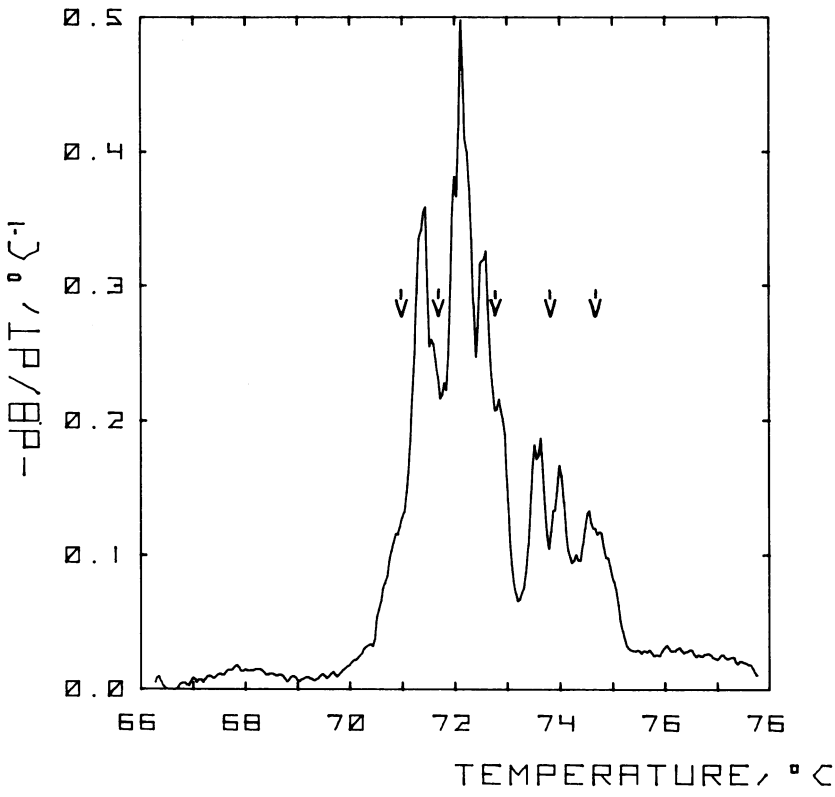

FIGURE 1. Differential melting curve for ØX174 RFII DNA in 0.1 x SSC. Arrows show the temperatures at which fixation was performed.

molecules at different stages of melting. An array of 50 DNA molecules corresponding to 25% denaturation is presented on Fig.3. This array corresponds to the second point of fixation on Fig.1. Figure 4a shows the experimental denaturation maps for an array of partially denatured DNA molecules. Beside them are theoretical denaturation maps corresponding to the same degrees of denaturation as the experimental ones: 4b are Azbel's maps and 4c are maps from /4/. The shaded part of the histograms in all maps is the remainder after the previous one is subtracted. With the data presented in this way one can clearly see the DNA regions that melt with a change of temperature within the melting range.

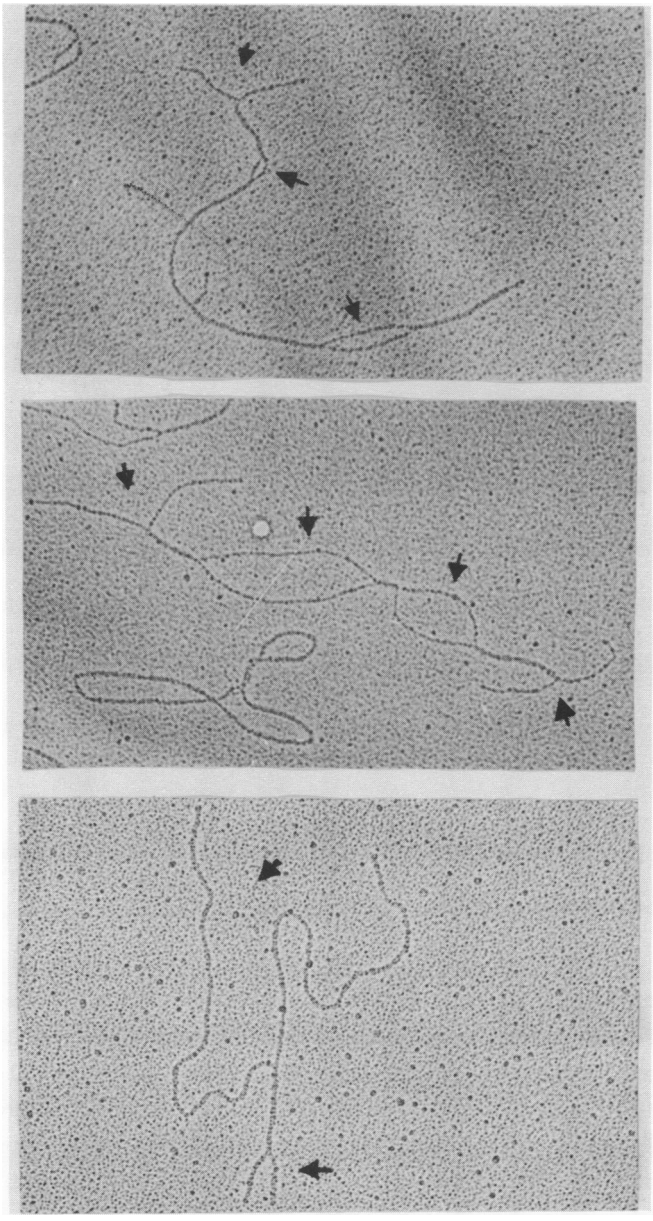

FIGURE 2. Electron micrographs of DNA molecules corresponding to different stages of melting.

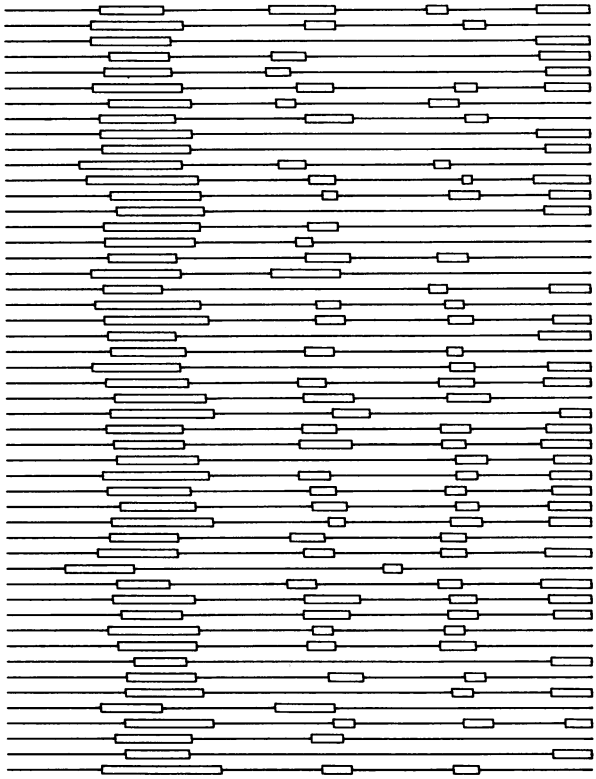

FIGURE 3. An array of DNA molecules corresponding to the second point of fixation on the RF III melting profile (see Fig.1). DNA melted regions are shown as boxes.

A comparison of the experimental maps (Figure 4a) with those based on Azbel's calculations (Figure 4b) reveals noticeable differences between the two. First of all, Azbel erroneously predicts the topography of DNA at the early stage of melting. Experiment shows that at the beginning (see maps I-II) only one end of the DNA molecule is melted (the 0 ÷ 8 region). Yet, according to Azbel (see Figure 3b) both ends of the DNA molecule should melt with practically equal probabilities and the melting region at the left-hand end is twice the size it is in experiment. Furthermore, it follows from Figure 4a (see map II) that the experimental value of 0.26 for the degree of

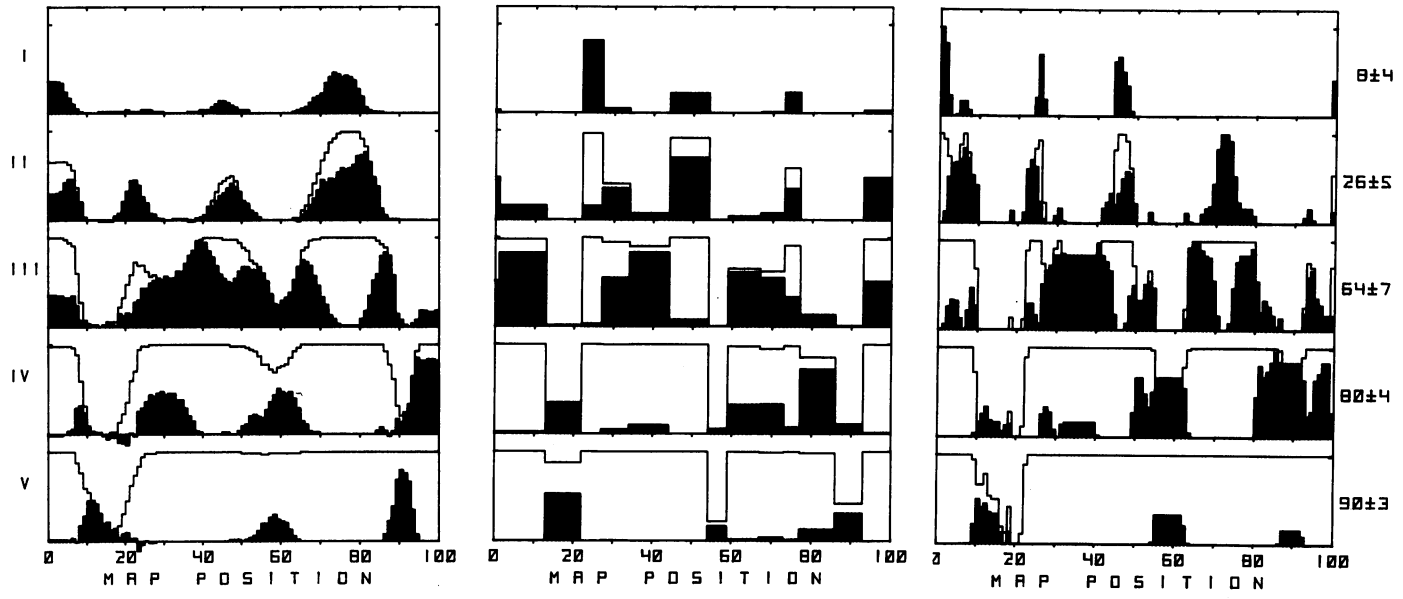

FIGURE 4. a) Experimental denaturation maps for ØX174 RF III DNA corresponding to six points within the melting range. The degree of denaturation calculated for each array of molecules is shown on the right.
b) Theoretical denaturation maps for ØX174 RF II DNA based on Azbel's data /5/.
c) Theoretical denaturation maps for ØX174 RFII DNA from /2/.
Theoretical maps are calculated for the same degree of denaturation as experimental ones. The shaded part of the histograms in all maps is the remainder after the previous one is substracted.

denaturation is largely determined by the melting of a rather long region, 65 ÷ 85, in the right-hand part of the molecule. According to Azbel (see Figure 4b), however, the melting of several DNA sites should be equally probable at this stage. The prediction of the concluding stages of melting also fails to agree with experiment: maps V and IV in Figure 4a demonstrate region 8-25 to be the most stable in ØX174 DNA: it is the only one to remain helical at the denaturation degree of 0.9. According to Azbel (corresponding maps in Figure 4b), this region melts much earlier, while at 0.9 denaturation (see map V, Figure 4b) two regions remain unmelted (one in the centre and the other at the right-hand end of the molecule.

The denaturation maps based on rigorous theory show a much better agreement with the experimental data. This theory correctly predicts the late stages of DNA melting: the last three denaturation maps almost completely coincide (cf. Figures 4a and 4c, maps III-V). Maps I and II in these Figures show that the preferential melting of one of the two ends of the DNA molecule at the beginning of the process is also correctly predicted. Only for the 0.65-0.85 region a later melting is predicted (the lag is about $1^{\circ}C$), which leads to a difference in maps II in Figures 4a and 4c.

DISCUSSION

The comparison of experimental and theoretical melting maps for ØX DNA reveals a much better agreement in the case of maps based on rigorous theory than for those based on Azbel's calculations. One important point should be emphasized. Azbel's approximation involves a decomposition of the melting process into steps in such a way that the predicted melting curve coincides with the experimental one. Furthermore, the data of /6,7/ used for our comparison are based on a decomposition which makes for the coincidence of not only the melting curves of the entire ØX DNA (5375 bp) but also of its two restriction fragments Y1 (2745 bp) and Y2(1690 bp). It seemed that the melting maps based on Azbel's approximation should coincide with the experimental ones. In reality, however, a

substantial discrepancy was revealed.

Of course Azbel's decomposition of the initial melting curve into individual peaks is not unambiguous, hence the discrepancy is not really unexpected. In /11/, a decomposition, for instance, was proposed for the same ØX174 DNA which gave a better agreement between the predicted and the experimental denaturation maps, though the authors used too large a value for the parameter $T_{GC}-T_{AT}=50^{o}C$.

As for the rigorous calculations, they do not involve any adjustable parameters and yield melting curves for RF III of ØX174 which differ from the experimental curve in the fine structure. In spite of the difference, however, on the whole such calculations correctly predict the course of DNA melting, the size of the melting regions and their localization. It was presumed in /4/ that the difference might be due to the failure to allow for the heterogeneity of the stacking interaction of DNA bases. Thus, even with these thermodynamic parameters unallowed for, rigorous theory provides a fairly correct prediction of the DNA melting process, at least it makes for much better agreement between theoretical and experimental melting maps than Azbel's calculations. It should be noted here that the comparison of experimental and theoretical denaturation maps was carried out for a low ionic strength (0.1xSSC). In the papers /1,12/ it has been shown that the DNA melting process is not completely equilibrium under such ionic conditions. Since the calculations are performed only for a completely equilibrium DNA melting process, experimental data obtained under equilibrium conditions (1xSSC /1 ,12/) should be used for the comparison with theory. However the investigation several DNAs besides ØX174 DNA (manuscript in preparation) has demonstrated that the topography of their melting in 0.1 and 1xSSC is the same, therefore our comparison of experimental and theoretical denaturation maps is valid.

Thus, the comparison of experimental and theoretical denaturation maps has demonstrated that orthodox calculations on the whole correctly describe the process of DNA melting, whereas Azbel's method leads to a picture that is hardly consistent with experiment.

ACKNOWLEDGEMENTS

It is our pleasant duty to thank Dr.B.A.Rebentish for the PstI enzyme, Dr.A.V.Vologodskii for making available the calculations of denaturation maps for the corrected sequence of ØX174 DNA, Mrs. A.M.Manko for technical assistance, Prof. Yu.S.Lazurkin and Prof. M.D.Frank-Kamenetskii for stimulating discussions.

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