ELSEVIER

Biophysical Chemistry 75 (1998) 177–186

Biophysical Chemistry

Denaturation of supercoiled DNA: a Monte Carlo study

Sudip Kundu[a], Ansuman Lahiri[1,b], Ashoke Ranjan Thakur[a,*]

[a] *Department of Biophysics, Molecular Biology and Genetics, University of Calcutta, 92 Acharya Prafulla Chandra Road, Calcutta 700 009, India*

[b] *Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta 700 037, India*

Received 3 March 1998; received in revised form 26 August 1998; accepted 10 September 1998

Abstract

A theoretical investigation of the denaturation characteristics of a supercoiled DNA has been presented employing a Metropolis Monte Carlo algorithm to examine the overall melting profiles of a supercoiled plasmid as the temperature is varied. We show that in contrast to a previously presented algorithm, this much simpler method is sufficient to explain almost all the overall denaturation characteristics and it also correctly calculates the detailed denaturation probabilities of each base pair at various degrees of supercoiling. We also present for the first time a theoretical investigation of the alkaline denaturation of a supercoiled plasmid. Although one can qualitatively reproduce the denaturation profiles using the present Monte Carlo algorithm, the agreement with experiment is not as good as in the case of thermal denaturation. The possible sources of discrepancy between theory and experiment have been discussed.

Keywords: Supercoiled DNA; Denaturation; Monte Carlo

1. Introduction

Although more than 30 years have passed since the discovery of DNA supercoiling [1], interest in it is still very much alive [2–5]. This is due to the realisation that DNA in living organisms almost always exists in supercoiled form and the excess free energy of supercoiled DNA facilitates several biochemical processes, like transcription, replication, recombination and the binding of unwinding proteins [6–9].

A supercoiled DNA can be imagined as a closed circular ring made up of two intertwined DNA strands. The strands are composed of complementary base pairs which under proper conditions form hydrogen bonds with each other and give rise to a double-stranded helical pattern designated as the B-DNA structure. The B-DNA structure is additionally stabilised through stacking interactions between successive bases of each strands. Under elevated temperature or change in

* Corresponding author. Tel.: +91 350 8386/6387; fax: +91 33 3510359; e-mail: arthakur@cubmb.ernet.in

[1] Present address: Centre for Structural Biochemistry, Karolinska Institute, NOVUM 14157, Huddinge, Sweden.

0301-4622/98/$ - see front matter
PII S0301-4622(98)00204-X

the pH and ionic strength of the solution, the hydrogen bonds may be broken and the base stacking disrupted. Study of such denaturation transitions, both experimentally and theoretically, has helped a lot in understanding the role of supercoiling in influencing DNA structure and energetics.

Theoretical calculation of partition function of a supercoiled DNA undergoing conformational transition has received much attention over the years. A population of supercoiled DNA will follow a distribution probability of its conformational state at thermodynamic equilibrium given by $Z = \sum_i e^{-G_i/RT}$; where G_i is the free energy of conformation of the ith state, R is the universal gas constant, T is absolute temperature and Z is the partition function. It was perceived early on that the methods usually employed for rapid evaluation of partition function for linear DNA are ill adapted for this case. The problem resided in the fact that whereas in the case of a linear DNA molecule one can consider the transition to be influenced by the states of a small number of neighbouring bases, in the case of a supercoiled DNA such a view is essentially incorrect. The topological constraint is defined by $L_k = T_w + W_r$ for closed circular DNA, where L_k, the linking number, is the number of times the two strands of a closed DNA duplex cross over each other; T_w, the twisting number of a DNA, is the number of base pairs per turn of the double helix; and W_r, the writhing number, is the number of times a duplex axis crosses over itself in space. Under these constraints the states of all the bases in the molecules are correlated, i.e. dependent on each other, at least in principle.

The first rigorous approach to this problem was formulated for calculating the denaturation probabilities of a supercoiled DNA [10]. In this work, two algorithms were suggested. One was a modification of an earlier algorithm by the same group for calculating the denaturation probabilities of a linear DNA [11] such that the proper contribution of the supercoiling energy was taken care of. The second algorithm was much simpler but involved a very substantial amount of approximation. It was based on the idea of self consistent calculation of the required quantities. The first algorithm, although quite accurate, had the disadvantage that it could not be extended to include more than two states for the base pairs. An approximate approach to take care of multiple states under transition was suggested much later [12].

However, a fresh approach was suggested to solve the denaturation problem [13]. This method involves counting of low energy states and also the states available through thermal fluctuations. A number of biologically important sequences were examined proceeding from this approach [14]. At the same time, another statistical mechanical approach was made by Kastura et al. to predict the non-B-form transition probability in a supercoiled DNA [15]. This method has introduced a kind of renormalisation of statistical weight for each of the secondary structures (B-form, interior loop, Z-form and cruciform) considered under transition.

For the present study, we utilise the importance sampling scheme in Monte Carlo methods. This avoids the necessity to calculate the partition function while yielding all the equilibrium averages of interest. We have simulated the thermal and alkaline pH induced denaturation transitions in supercoiled DNA. Sun et al. [16] have developed a Metropolis Monte Carlo algorithm to analyze the strand separation transitions in superhelical DNA. They have used a complicated set of moves, called shuffling operation, that randomize the location of unpaired regions to minimize the correlation among the sampled states. In this calculation a very large value of nucleation energy was used to locate the instability in the β-lactamase gene region of plasmid pBR322. The probability of change of secondary structure of a base pair was much less since nucleation energy had been taken to be large. Hence successively sampled states remained highly correlated and equilibrium distribution could be reached only very slowly. For this the authors had to use the shuffling operation so as to decorrelate successive sampled states. Furthermore, they did not apply their algorithm for determination of denaturation profile of supercoiled DNA. We have used a low nucleation energy ($\epsilon_0 = 2.5$ kcal/mol) [17,18] in our calculation which has helped us to do away

with the necessity of introducing shuffling procedure to reach an uncorrelated state. It had also been shown [19] that at nucleation energy (ϵ_0) values higher than $\epsilon_0 = 2.5$ kcal/mol the melting transition becomes sharper and loses the true transition characteristics of a supercoiled DNA.

2. Method

The bases in a supercoiled DNA can be imagined to have a multiple number of states available to them, depending on the type of transition being analyzed. In thermal denaturation, according to the usual practice, we designate the base pairs to be in the helix state (h) and those unpaired, in the coiled state (c). For alkaline denaturation, since the disruption in base pairing may be due to ionization of the nitrogeneous bases, we introduce a third type of state, which we designate as ionised coil (ic).

In the Metropolis Monte Carlo scheme [20] used here, one considers a fundamental move consisting of the following two steps:

1. generate a candidate state, j, from the current state, i, according to a specified probabilistic rule; and
2. use the energy difference between the two states to determine which step is to be selected as the new current state.

In step 2, the Metropolis criterion [21] is to be used: accept the state j with probability = $\exp[(G_i - G_j)/K_B T]$ if $G_j > G_i$ and with probability = 1 if $G_j < G_i$. Here G_i and G_j are the free energies of the ith and jth states, respectively.

For the particular model presented here, the above scheme is realised in the following manner.

Each configuration of the DNA is represented by a sequence of base states: h, c or ic. The energy of the system changes when one base pair undergoes a transition. Contributions to this energy change come from different sources.

The factor that creates the major difference between the linear and supercoiled DNA is designated as the supercoiling free energy. This energy originates from the elastic deformation of the DNA duplex under the condition of supercoiling and has contributions from both twist- and bent-deformed potential. The writhing energy arises from bending deformation and is assumed to vary with the square of the bending. Radius of curvature at each point of the bent molecule is equal to the radius of a circle whose circumference is equal to the contour length of each writhing turn. Under these considerations the supercoiling free energy of DNA which is N base pairs long, having n base pairs in the coil state is given by [19,22]

$$G_s = \frac{2\pi^2 C(\Delta Lk + n/A_\beta)^2}{N + (\alpha - 1)n} \tag{1}$$

where ΔLk is the linking difference, A_β is the number of base pairs per turn of canonical B-DNA, and C and α are the functions of persistence length and torsional rigidity of the DNA. It should be noted that the functional form of supercoiling energy expression does not depend on the exact partitioning of the effective linking difference into twisting and writhing because T_w and W_r are related to the total linking number L_k through the linear relationship $L_k = T_w + W_r$. It will only change the value of C and α (for details of expression see Sen and Majumdar [19] and Sen and Lahiri [22]). Fluorescence depolarization, time resolved fluorescence and dynamic light scattering measurements based on gel shifts and several theoretical analyses showed that the torsional rigidity of the double helical DNA varies in wide range from 1×10^{-19} to 4×10^{-19} erg-cm [23–28]. The persistence length of a duplex DNA also varies in a wide range [29]. The value of C used by us in the calculation is within the range derived from the reported values of bendability and torsional rigidity. The change in the supercoiling free energy ΔG_s when one base pair goes from helix to coil state or vice versa is calculated from Eq. (1) accordingly.

The other factors come from the disruption of the hydrogen bonded structures and stacking between the bases. Taking the helical state as the reference, the free energy change during helix–coil transition is given by

$$\Delta G_{hc} = \epsilon - T\Delta S + \epsilon_0 + \Delta G_s \tag{2}$$

where $(\epsilon - T\Delta S)$ represents the free energy of a broken base pair and ϵ_0 is the nucleation energy for denaturation with ΔS determining the change in entropy per base pair due to melting and ϵ represents the enthalpy change. In general, these parameters are dependent on the base sequence.

When we consider the helix to ionised coil transition, formation of ionised coils introduces two more terms in addition to helix–coil transition; one is the ionisation energy and the other is the screened coulomb interaction. A simple derivation from the Henderson–Hasselbach equation under conditions of reversibility leads to the equation:

$$\Delta G_{\text{ion}} = 2.303(\text{p}K_0 - \text{pH})K_B T \tag{3}$$

The free energy change involved in the screened coulomb interaction [30] between the ionised bases is given by

$$\Delta G_{\text{elec}} = \sum_{ij} \frac{q_i q_j \text{e}^{-\kappa r_{ij}}}{\epsilon_r r_{ij}} \tag{4}$$

where κ is the Debye parameter, r_{ij} is the distance between the ith and jth base pairs, ϵ_r is the dielectric constant of the medium, q_i and q_j are the normal electronic charges of the ith and jth base pairs, respectively, and $\text{p}K_0$, pH and K_B have the usual meaning. So, the energy needed for the transition from helix to ionised coil is given by

$$\Delta G_{hic} = \Delta G_{hc} + \Delta G_{\text{ion}} + \Delta G_{\text{elec}} \tag{5}$$

For coil to ionised coil transition the energy parameters needed are only the ionisation energy and screened coulomb interaction energy, i.e.

$$\Delta G_{cic} = \Delta G_{\text{elec}} + \Delta G_{\text{ion}} \tag{6}$$

The energy parameters described above are more or less sequence dependent, e.g. the energy needed for breaking the AT base pair is different from that of the GC base pair. The energy needed for transition depends also on the nearest neighbour base pair, whether it is in helical state, coiled state or ionised coil state.

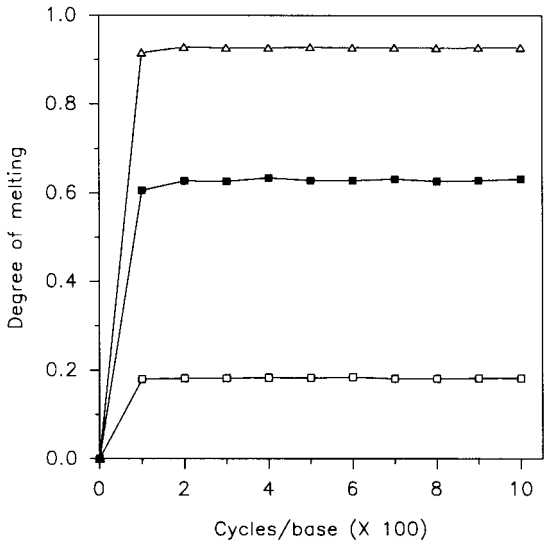

Fig. 1. The attainment of equilibrium is shown. The hollow square corresponds to 55°C, the solid square to 75°C and the hollow triangle to 95°C.

For two-state transition in thermal denaturation, starting from an initial configuration where all the base pairs are in helical state, we consider the first base pair and apply the usual Metropolis Monte Carlo technique. The next base pairs are then sequentially chosen and this process is continued to the end of the sequence; one may call it one Monte Carlo step per base pair (MCS/base). The first few cycles are taken for the equilibration of the system (Fig. 1). For calculation of any parameter, discussed in the result, the ensemble is considered after attaining the equilibrium.

In the analysis of transitions involving three states, the choice of a state to make transition from the original state to any of the other two is made by the following rule: a random number is generated and compared with 0.5 — if it is less than 0.5, the choice for transition to one state is made, if not, then it is tested for transition to the other state. The method used for acceptance or rejection of the new state is the same as described above. The equilibrium values are calculated from runs of 60 000 MCS/base, rejecting the result of the first 10 000 MCS/base. We have ensured that these are the true equilibrium states and they do not show any correlation. We have calculated the

degree of melting [which is $c/(c+h)$ for thermal denaturation and for alkaline denaturation $(c+ic)/(c+h+ic)$] taking the averages of the values generated at the intervals 5 MCS/base, 7 MCS/base and 9 MCS/base and the results from these three calculations show no appreciable difference. The values used to generate the denaturation profile, both thermal and alkaline, are determined by taking the average of the values generated at intervals of 5 MCS/base. For calculation of denaturation probability profiles at a fixed temperature, 50 blocks of 100 MCS/base are employed for averaging. The programs are implemented in FORTRAN 77 and run on a Pentium-133 processor. A standard run involving 1000 MCS/base for ϕX174 plasmid (5386 base pairs) is completed in approximately 22 cpu-sec.

3. Results and discussions

The method, outlined above, has been utilised to calculate the melting characteristics of a supercoiled DNA ϕX174 ($\Delta Lk = -31$) in TEA buffer, where the effect of base pair heterogeneity is minimised. We have plotted the overall melting profile of the DNA as a function of temperature in Fig. 2 and compared it with the experimental data [31]. The parameters used in the energy expressions for our Monte Carlo calculations are $\epsilon = 7.9$ kcal/mol, $\epsilon_0 = 2.5$ kcal/mol, $\Delta S = 12$ e.u. and $\alpha = 23.4$, as reported by Sen and Majumdar [19]. The value of C is taken as 1.5 kcal/mol which lies within the reasonable range as discussed previously. The Monte Carlo denaturation profile is in good agreement with the experimental data. The discrepancy at lower temperature may be due to the residual denaturation [32]. Lyubchenko et al. [32] have demonstrated that denaturing gradient gel electrophoresis shows an early melting of covalently closed circular supercoiled DNA compared to the linear DNA. This may explain the discrepancy observed when theoretical results are compared to experimental data obtained by their group earlier [31]. The nucleation energy parameter ϵ_0 used by Sun et al. [16] fails to explain even qualitatively the experimental denaturation profile, as provided in [31] (Fig. 3). This is in sharp contrast to the close match

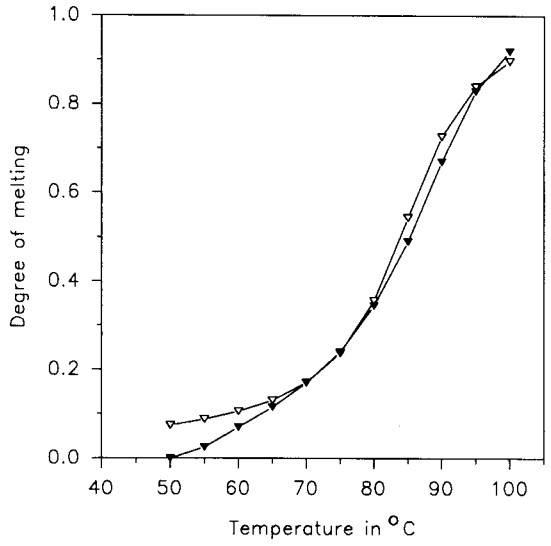

Fig. 2. Comparison of theoretically derived (hollow triangle) and experimentally observed (solid triangle) melting profiles of ϕXI74 DNA in TEA buffer (see text).

obtained in our theoretical analysis using the nucleation energy parameter value ($\epsilon_0 = 2.5$ kcal/mol) given in [17,18] (Fig. 3). When ϕX174 DNA is taken in 7.2 M $NaClO_4$ solution, the strong chaotropic activity cause the AT and GC

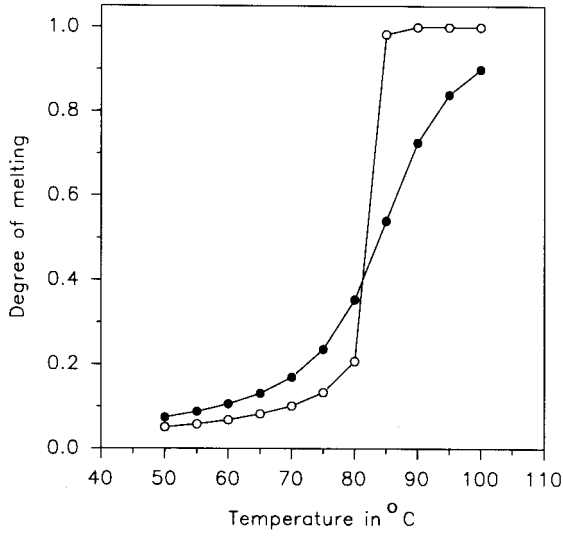

Fig. 3. The curve represents the melting profile with nucleation energy (ϵ_0) = 10.0 kcal/mol (hollow circle) and $\epsilon_0 = 2.5$ kcal/mol (solid circle).

base pairs to show different denaturation energies. Under these conditions also the melting profile simulation study through Monte Carlo method using identical supercoil density yielded good agreement with the experimental data (Fig. 4).

In Fig. 5 we have plotted the melting probabilities of the individual base pairs of a naturally occurring supercoiled pBR322 plasmid DNA which was considered as a heteropolymer with $\epsilon_{AT} = 7.2$ kcal/mol and $\epsilon_{GC} = 8.2$ kcal/mol [12]. We have included in the same figure the melting probabilities of a hypothetical homopolymer of the same length and supercoiling density (Fig. 5a). The difference in their profile is striking. The pBR322 plasmid has very pronounced peaks between 3200 and 3300 and also at the region between 4150 and 4350 for native levels of supercoiling, which is in complete agreement with other theoretical calculations [13]. These are the terminator and the promoter regions of the β-lactamase gene. Similar analysis with ϕX174 showed multiple regions of high peaks (Fig. 6). These overlaps all the gene encoded in the DNA sequence. It is interesting to note that our method is sensitive enough to identify the transcribing regions from any segment of DNA through a melting probability calculation. In Fig. 5b,c we see that there is no significant change in the peak regions with change of supercoil density, except for an increment of peak height with supercoil density.

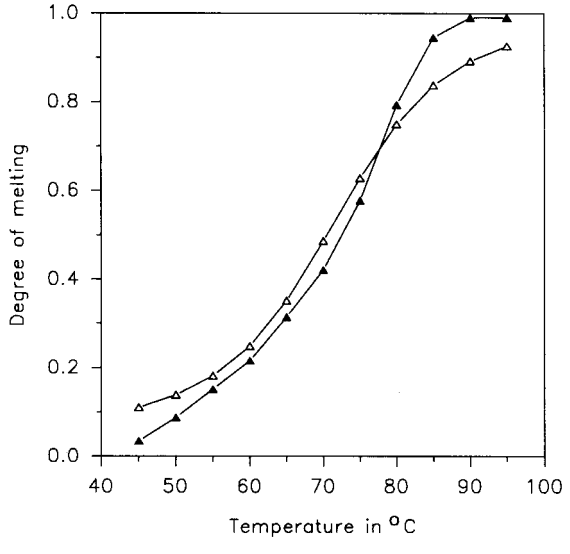

Fig. 4. The melting profile of ϕX174 DNA in 7.2 M $NaClO_4$ solution is represented by a solid triangle (hollow triangle for theoretical profile).

As an extension to the three-state transition problem of this Monte Carlo simulation technique we next studied the alkali denaturation melting profile of ϕX174 DNA. We have taken $pk_{0AT} = 10.47$ and $pk_{0GC} = 10.0$ [33]; $\epsilon_{AT} = 7.2$ kcal/mol and $\epsilon_{GC} = 8.2$ kcal/mol [12]; dielectric constant $\epsilon_r = 80.0$; $\kappa^{-1} = 4.4$ Å for ionic strength corresponding to 0.5 M NaCl solution and temperature 40°C. The distance between successive base pairs in normal double helical B-DNA is 3.4 Å. In the case of denaturation, the flexibility arising along the DNA chain may change the distance between successive base pairs; furthermore, since the supercoiled DNA actually is not linear but bent (in many places), the distance between ith and $(i+j)$th base pair can vary. For the sake of simplicity, we have taken different $r_{i,i+1}$ values to calculate the effective distance between i and $(i+j)$th segments, neglecting the angle between successive segments. We have examined how the melting profiles change with change in $r_{i,i+1}$ under the above assumptions. We have also compared the denaturation profiles with change of number of interacting bases. Increasing the number of interacting bases from $n = 2$, the denaturation profiles (Fig. 7) do not cause drastic change for the value of κ chosen. The nature of saturation with increment of n value is the same for all r (the distance between successive base pairs) values considered (data not shown). The value of α is a function of bendability and torsional rigidity of the DNA at different states. Sen and Lahiri [22] showed that for non-ionised polymers the value of α is 23.4 under thermal denaturation condition. For elevated pH, the DNA may be considered as a heteropolymer polyelectrolyte which has three states: helix, coiled and ionised coil. The stiffness of the polymer is expected to be higher for predominantly charged coil regions and hence the value of α would change. Fig. 8 presents the alkali denaturation profile of ϕX174 with the value $\alpha = 23.4$ (taken

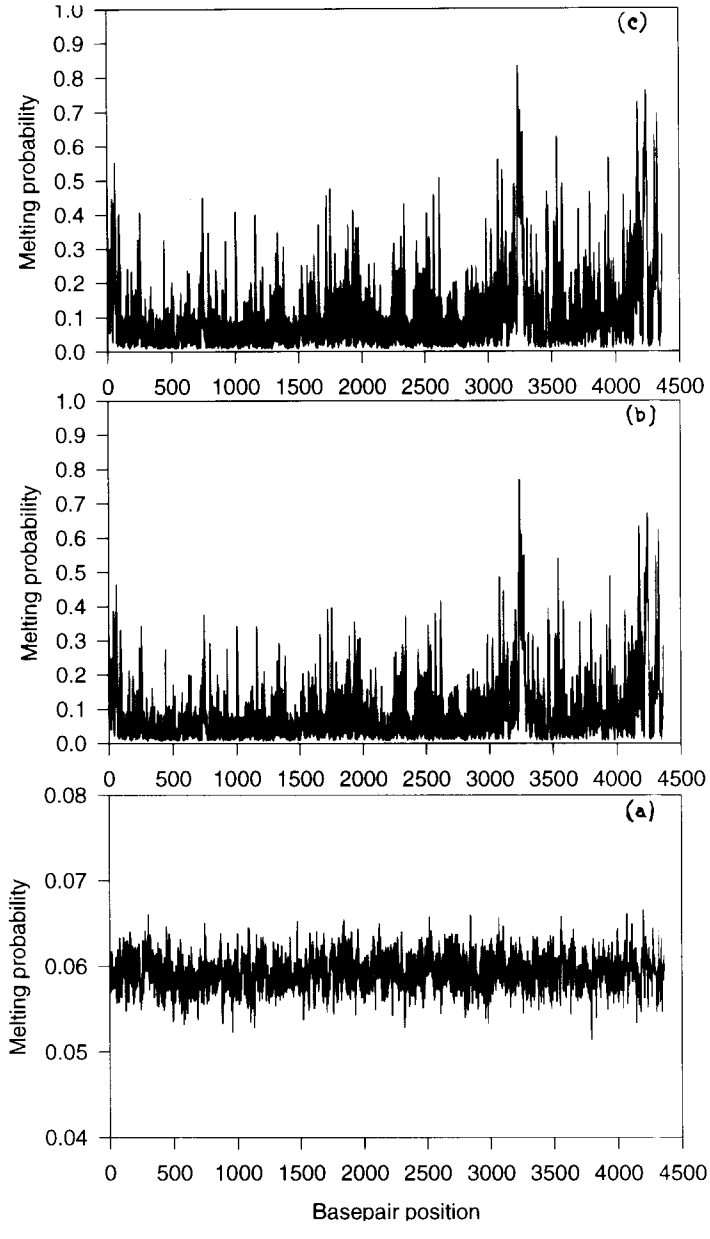

Fig. 5. (a) The melting probability of a hypothetical homopolymer of pBR322 DNA with $\Delta L_k = -30$. (b,c) The melting probabilities of the same DNA at the base pair level considering that as a heteropolymer with $\Delta L_k = -30$ and $\Delta L_k = -41$, respectively.

from thermal denaturation) and $\alpha = 10$. In both cases, the distance between successive base pair is taken as 3.4 Å. If the value of α were taken to be greater than 23.4, then the transition would have been sharper and hence not representative for a supercoiled DNA (data not shown). When compared with the experimental data [34] the Monte Carlo profile with $\alpha = 23.4$ shows slightly higher cooperativity whereas the profile with $\alpha = 10$ shows a slightly less cooperativity. The nature of

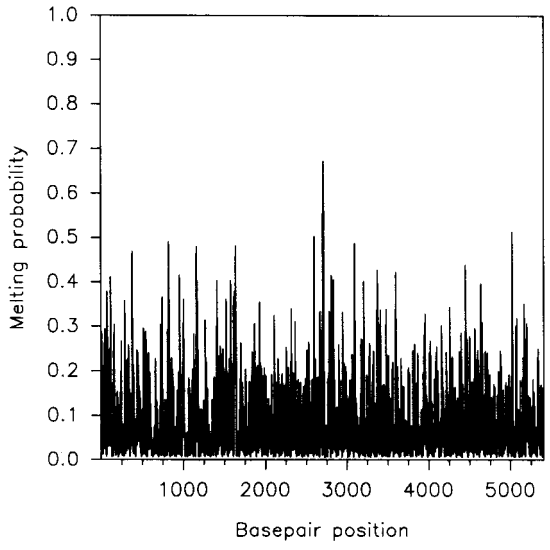

Fig. 6. The melting probability of the ϕX174 (supercoiled density, $\sigma = -0.06$) at the base pair level.

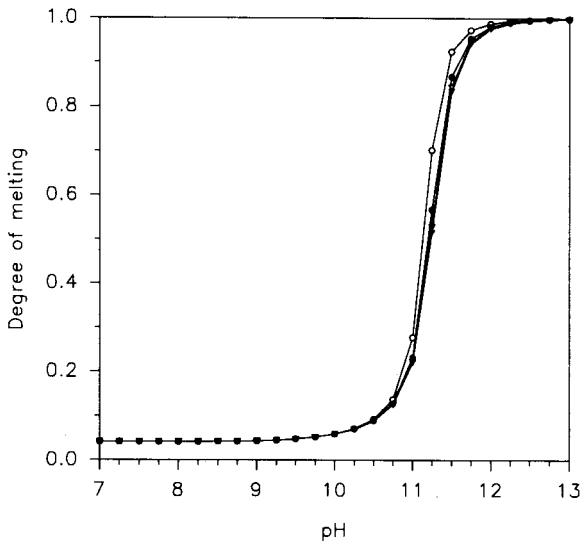

Fig. 7. Alkaline denaturation profiles of ϕX174 DNA for different electrostatic energy. The energy has been truncated after n base pairs; where $n = 1$ (hollow circle), $n = 2$ (solid circle), $n = 3$ (hollow triangle) and $n = 4$ (solid triangle).

the profile with the value $\alpha = 10$ is similar to the experimental value having a rightward shift. The persistence length of a polymer varies to a large extent at different ionic conditions [29] and consequently the bendability will also change. So, with change of pH, the bendability and hence the value of α would change. But in the case of an ionized polymer, the value of α and its exact nature as a function of pH remains unknown. Currently a number of groups have suggested that the effective persistence length and torsional rigidity at different pH should be calculated considering the DNA to be an elastic plus electrostatic rod [3]. By incorporating the dependence of α on pH we might be able to reduce the discrepancy. In Fig. 8, we have also shown the change of denaturation profiles with changes of $r_{i,i+1}$ value. Once we can determine the exact nature of the variability of these values from results of more rigorous experiments, we would be able to reproduce the alkali denaturation profile more accurately by this theoretical approach. Finally, it should be noted that throughout our calculation we have considered twisting and bending to be independent, although there are recent works [35,5] which suggest the existence of such dependence.

The present method is of general applicability in that it can in principle be extended to take into account any amount of complexity. At high supercoiling limit, these considerations are expected to throw light on the structural features of non-B-form DNA like V-form DNA. It has been shown that when a plasmid DNA is taken to a highly denatured state through alkali treatment it sometimes attains a physical state, defined as denatured supercoiled DNA. This structure has been shown to harbour large regions of single stranded DNA and behaves as a better biochemical substrate than supercoiled DNA [36]. Similar structure has been shown to exist as a replication intermediate by Baker et al. [37]. The present analysis is a step towards the development of an algorithm for generating such a structure through Monte Carlo simulation.

4. Conclusions

We have proposed a Metropolis Monte Carlo algorithm for calculating both the overall melting profile of a supercoiled DNA and the detailed melting probabilities of specific base sequences

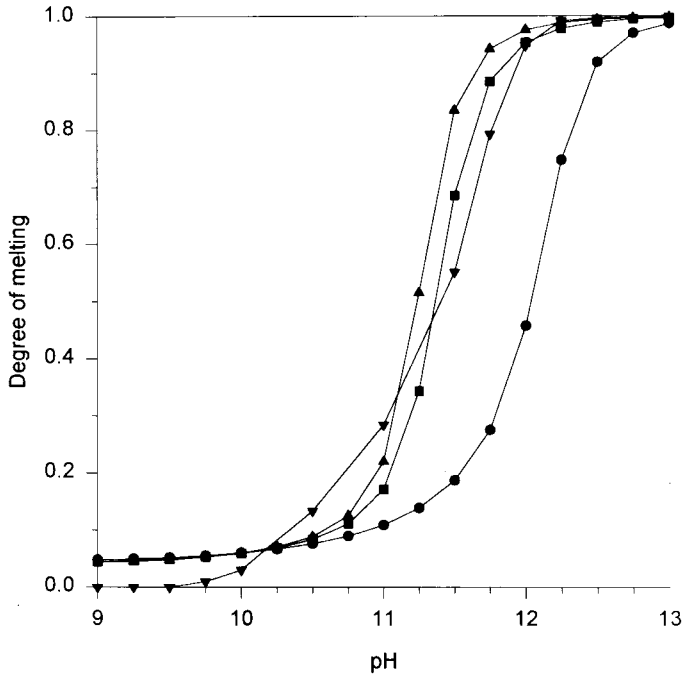

Fig. 8. Alkaline denaturation profiles of ϕX174 DNA with $\alpha = 23.4$ and $\alpha = 10$ (with $r_{ij} = 3.4$ Å) are represented by the triangle (upward) and circle, respectively. The square reprents the denaturation profile with $r_{ij} = 3.1$ Å and $\alpha = 23.4$. Here, the value of $C = 1.66$ kcal/mol has been used. The corresponding experimental melting profile is represented by triangle (downward, see text).

within the supercoiled molecule. The analysis has the novelty that it is sensitive enough to identify transcribing regions and may have good predictive values for unknown sequences. We have also proposed a multistate transition algorithm which can be used for both temperature and alkali transition. We found that the thermal denaturation profile agreed closely with the experimental data and previous theoretical analysis. The base denaturation probabilities also show good agreement with experiment and theoretical results. The method, conceptually very simple, is of general applicability and of comparable accuracy with other more specified methods.

Acknowledgements

We thank Department of Biotechnology, India for financial support vide grant no. BT/TF/15/14/91 and for use of computational facilities at the Distributed Information Centre at Calcutta University.

References

[1] J. Vinograd, J. Lebowitz, R. Radloff, R. Watson, P. Laipis, Proc. Natl. Acad. Sci. USA 53 (1965) 1104.
[2] T. Schlick, Curr. Opin. Struct. Biol. 5 (1995) 245.
[3] G. Ramachandran, T. Schlick, Biopolymers 41 (1997) 5.
[4] V.V. Rybenkov, A.V. Vologodskii, N.R. Cozzarelli, Nucl. Acids Res. 25 (1997) 1412.
[5] J.J. Delrow, P.J. Heath, J.M. Schurr, Biophys. J. 73 (1997) 2688.
[6] M. Gellert, Annu. Rev. Biochem. 50 (1981) 879.
[7] J.C. Wang, Annu. Rev. Biochem. 54 (1985) 665.
[8] K. Rippe, P.H. von Hippel, J. Langowski, Trends Biochem. Sci 20 (1995) 500.
[9] J.B. Clendenning, J.M. Schurr, Biophys. Chem. 52 (1994) 227.
[10] V.V. Anshelevich, A.V. Vologodskii, A.V. Lukashin, M.D. Frank-Kamenetskii, Biopolymers 18 (1979) 2733.
[11] A.A. Vedenov, A.M. Dykhne, M.D. Frank-Kamenetskii, Sov. Phys. Usp. 14 (1972) 715.
[12] V.V. Anshelevich, A.V. Vologodskii, M.D. Frank-Kamenetskii, J. Biomol. Struct. Dyn. 6 (1988) 247.
[13] C.J. Benham, J. Chem. Phys. 92 (1990) 6294.
[14] C.J. Benham, Proc. Natl. Acad. Sci. USA 90 (1993) 2999.
[15] S. Katsura, F. Makishima, H. Nishimura, J. Biomol. Struct. Dyn. 10 (1993) 639.

[16] H. Sun, M. Mezei, R. Fye, C.J. Benham, J. Chem. Phys. 103 (1995) 8653.
[17] R. Rein, M. Pollack, J. Chem. Phys. 47 (1967) 2039.
[18] C.R. Cantor, P.R. Schimmel, Biophysical Chemistry, part 111, Freeman, New York, 1980, pp. 1208–1209
[19] S. Sen, R. Majumdar, Biopolymers 27 (1988) 1479.
[20] D. Stauffer, F.W. Hehl, V. Winkelmann, J.G. Zabolitzky, Computer Simulation and Computer Algebra, Springer-Verlag, Berlin, 1988.
[21] N. Metropolis, A.W. Rosenbluth, A.H. Teller, E. Teller, J. Chem. Phys. 21 (1953) 1087.
[22] S. Sen, A. Lahiri, Biophys. Chem. 40 (1991) 33.
[23] M.D. Barkley, B.H. Zimm, J. Chem. Phys. 70 (1979) 2991.
[24] A.V. Vologodskii, V.V. Anshelevich, A.V. Lukashin, M.D. Frank-Kamenetskii, Nature 280 (1979) 294.
[25] D.S. Horowitz, J.C. Wang, J. Mol. Biol. 173 (1984) 75.
[26] J.B. Clendenning, A.N. Naimushin, B.S. Fujimoto, D.W. Stewart, J.M. Schurr, Biophys. Chem. 52 (1994) 191.
[27] J.A. Gebe, S.A. Allison, J.B. Clendenning, J.M. Schurr, Biophys. J. 68 (1995) 619.
[28] A.A. Gorin, V.B. Zhurkin, W.K. Olson, J. Mol. Biol. 247 (1995) 34.
[29] P.J. Hagerman, Ann. Rev. Biophys. Chem 17 (1988) 265.
[30] C. Tanford, Physical Chemistry of Macromolecules, John Wiley and Sons, Inc., 1961, pp. 482–489
[31] A.V. Gagua, B.N. Belintsev, Yu.L. Lyubchenko, Nature 294 (1981) 662.
[32] Yu.L. Lyubchenko, L.S. Shlyakhtenko, Nucl. Acids Res. 16 (1988) 3269.
[33] D.Y. Lando, S.G. Haroutiunian, A.M. Kulba, E.B. Dalian, P. Orioli, S. Mangani, A.A. Akhrem, J. Biomol. Struct. Dyn. 12 (1994) 355.
[34] W. Strider, M.N. Camien, R.C. Warner, J. Biol. Chem. 256 (1981) 7820.
[35] M. Duget, Nucl. Acids Res. 21 (1993) 463.
[36] C.R. Santra, S.K. Mukherjee, A.R. Thakur, Ind. J. Biochem. Biophys. 30 (1993) 123.
[37] T.A. Baker, K. Senkimizu, B.E. Funnel, A. Kornberg, Cell 45 (1986) 53.