The B – A transition in superhelical DNA

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ABSTRACT

Relaxation of a DNA superhelical stress due to the B to A transition induced by trifluoroethanol has been studied by assessing the change of DNA orientation in a flow gradient. Using DNAs of different superhelical densities, a decrease in the winding angle during the B→A shift of DNA was found to be 1.5° per base pair in solution. Accepting the winding angle for B-DNA in solution to be 34.1°, that for A-DNA must have a value of 32.6° which agrees with the X-ray data for A-DNA in the condensed state. The date obtained within the B – A transition interval make it possible to conclude that there is an increase in winding at each B/A junction, which is about 5° per one junction.

INTRODUCTION

The B to A transition of DNA in solution is known to be induced by addition of a non-electrolyte such as ethanol or trifluoroethanol (1–3), which reduces water activity, i.e. relative humidity (4,5). Earlier we have shown that the transition is cooperative with a length of cooperativity $\nu \sim 20$ base pairs (2,3). This means, that in the middle of the transition, a long DNA molecule is subdivided into alternating A and B stretches with the mean length ν, the energy of a B/A junction thus formed being $F_j = RT \ln\nu$ (2).

Circular dichroism (CD) is a convenient method for the tracing the shift into the A conformation, which allows one to obtain a so-called transition curve, i.e. a fraction of the A form, θ, as a function of non-electrolyte content (6).

The formation of a B/A junction affects the hydrodynamic properties of a DNA molecule. Specifically, the drop of DNA orientation in flow, observed in the B-A transition interval, follows the quantity of B/A junctions (7,8).

Let us now consider superhelical DNAs. As was shown both theoretically (9,10) and experimentally (11), though a negative superhelicity must facilitate a shift into the A form with a winding angle lesser than that for the B form, its effect upon the transition curve is next to nothing. This reflects a small change of the winding angle per base pair, $t_A - t_B$. Nevertheless, if an essential part of the molecule adopts the A form, then the number of titrated superhelical turns, τ, would be altered to a marked degree. Thus, a possibility arises for estimating the winding angle of the A form in solution providing that the winding angle for the B form is known. (According to many studies (12–14), the mean value of t_B in solution is $34.1 \pm 0.1°$ per base pair.) Indeed, a change in the equilibrium winding angle by $\Delta t = t_A - t_B$ will change the number of superhelical turns by $\Delta\tau = -\Delta t \cdot N$ or

$$\Delta\sigma = -\Delta t / t_B \qquad [1]$$

Here N is the number of base pairs in a DNA molecule and σ stands for superhelical density.

The change of σ can easily be measured by the aforementioned method of orientation in flow since a covalently closed molecule with a zero superhelical density possesses the maximum orientability in flow with respect to negatively and positively supercoiled molecules. What is only needed now is to select DNA molecules of such superhelical densities which would relax completely in consequence of the B-A transition.

MATERIALS AND METHODS

DNA samples with different superhelical densities were obtained by treating the pUC19 plasmid with topoisomerase I in the presence of ethidium bromide as described in ref. (15).

Open circular DNA (ocDNA, with one single-stranded cut) was obtained by treating the pUC19 plasmid with DNAase I in the presence of an excess of ethidium bromide (16).

Determination of superhelical density was fulfilled by measuring DNA relaxation with an intercalator, ethidium bromide. The relaxation was traced by following a linear dichroism (LD) change of flow-oriented DNA. Fig. 1 illustrates that the total superhelical stress relaxation corresponds to a 2–3-fold increase in relative LD. Accepting the unwinding angle at one intercalation site to be 26°, the superhelical density can be estimated from the amount of the bound dye needed for complete relaxation. We have used samples with σ values of 0, −0.006, −0.012, −0.020, −0.034, −0.045.

Linear dichroism (LD) was registered with a Jobin Yvon Mark III dichrograph supplied with an achromatic quarter-wavelength plate. To orient DNA, we pumped the solution through a cell which consisted of two cyclindric cavities connected by a 1-mm slit between quartz plates. The back and forth movement of the DNA solution within the cell was realized using a periodic change of air pressure in the cavities. The mean velocity gradient of the liquid in the slit due to friction of the liquid with the walls was about 500 s^{-1}.

The B-A transition was accomplished in the direction from the A to B form by adding a water component into the initial 80 percent trifluoroethanol/water solution (A form). The two-component solution contained 0.25 mM NaCl and 0.1 mM EDTA.

Continuous registration. A distinctive technical feature of this study is a continuous change in the fraction of trifluoroethanol

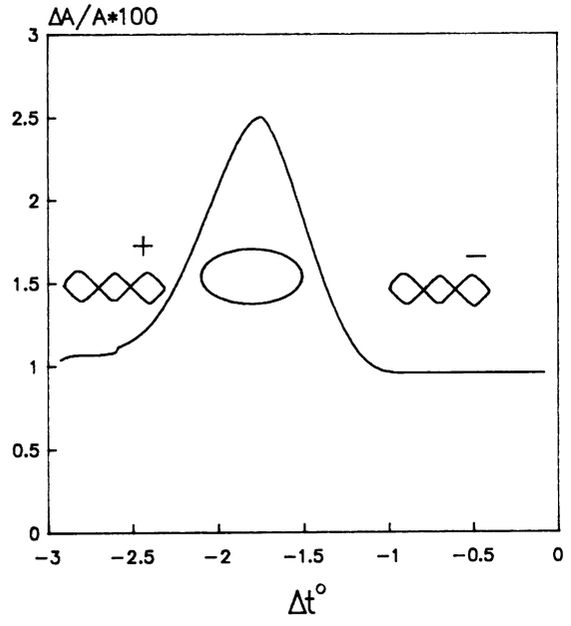

Figure 1. Relaxation of pUC19 plasmid DNA upon ethidium bromide binding. $\Delta A/A$ stands for the relative linear dichroism of flow-oriented DNA molecules. Δt stands for the decrease in the average winding angle of DNA due to ethidium intercalation, recalculated from a 26° unwinding value by one dye molecule. (For the conditions and other details see text.)

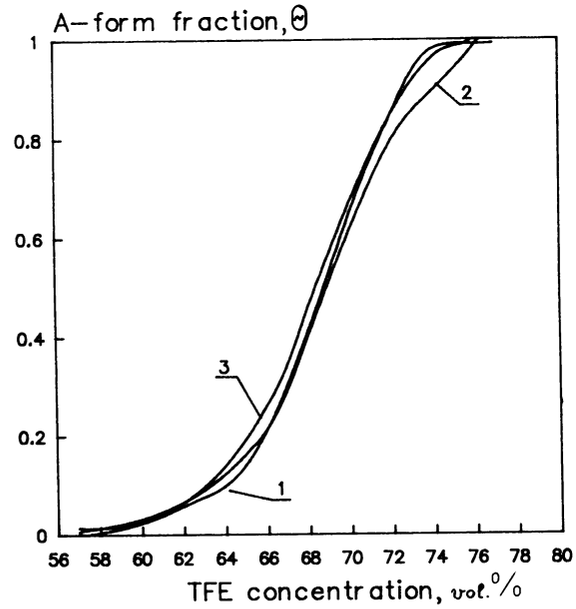

Figure 2. The B to A transition curves for the pUC19 DNA molecules of a different superhelicity. (1) open circular DNA; (2) covalently closed DNA with $\sigma = 0$; (3) $\sigma = -0.04$. θ is the fraction of the A form obtained from circular dichroism change at 270 nm. (For the conditions see MATERIALS AND METHODS.)

(TFE) due to continuous administration of the water component into the cell for measuring the linear dichroism. This allows one to obtain smooth (rather than punctuated) curves, which is important in order to determine accurately the event of complete relaxation of superhelical density.

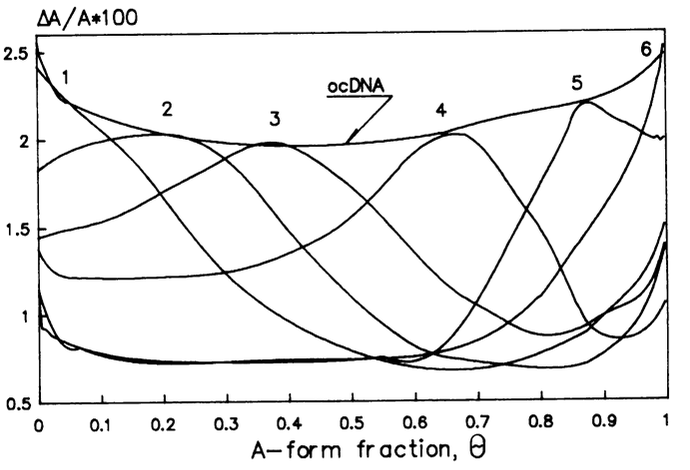

Figure 3. Relaxation of circular pUC19 DNAs of different superhelical densities at the B−A transitions. $\Delta A/A$ stands for the reduced linear dichroism of the flow oriented DNA and θ for the A-form fraction. The superhelical densities, σ, are as follows: 0 (curve 1), −0.006 (2), −0.012 (3), −0.20 (4), −0.034 (5), −0.045 (6). OC is a curve for open circular DNA with one single-stranded nick.

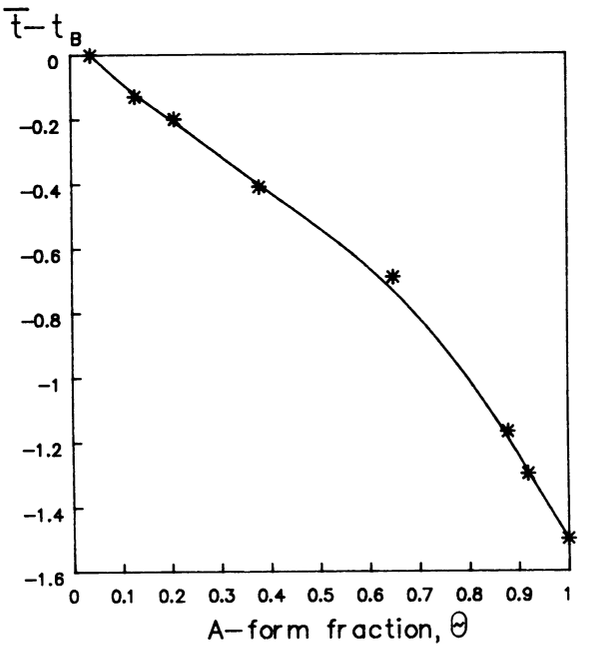

Figure 4. Change of the average DNA winding angle, $t(\theta) - t(B)$, within the B-A transition interval. (Determined from the data of Fig. 3 using Eq. [2].)

RESULTS AND DISCUSSION

Figure 2 shows B-A transition curves for three samples of a different topology. A very small (if any) effect of superhelicity upon the B-A transition is seen in accord with the above mentioned theoretical and experimental data (9−11).

At the same time, the transition into the A form affects the hydrodynamic properties of circular DNA as revealed in LD measurements. When a number of DNA samples with different superhelical densities ($-0.045 \leq \sigma \leq 0$) were transformed into the A form in the presence of TFE, then their linear dichroism $\Delta A/A$

in the B-A transition interval appeared to alter depending on their initial superhelical density (Fig.3).

First of all, let us consider a curve labeled with ocDNA which corresponds to our earlier data with the linear calf thymus DNA (7). As was shown there, the observed concave curve results from the B/A junctions: the pattern closely replicates an ellipitic dependence for the number of B/A boundaries, the feature which is predicted by the statistic-mechanical description of a cooperative transition within the framework of the one-dimensional Ising model (7,8).

The other curves, numbered from 1 to 6, are for covalently closed circular DNAs of the pUC19 plasmid with different superhelical densities. One can see that each of the DNA samples goes through a state with a zero superhelical density: it occurs at points where linear dichroism increases up to that for ocDNA. According to Eq. [1], the mean value of double helix unwinding in these kissing points can be estimated:

$$\Delta t = t(\theta) - t_B = \sigma_i \cdot t_B \qquad [2]$$

where σ_i stands for σ of the very sample which relaxes at a given point.

The dependence $\Delta t = f(\theta)$ thus obtained is presented in Fig. 4.

If all the twisting of the double helix within the B-A transition range were composed of partial twistings of the B and A-segments only, one should have $\bar{t}(\theta) = t_A \cdot \theta + t_B \cdot (1-\theta)$ or

$$\Delta t(\theta) = (t_A - t_B) \cdot \theta \qquad [3]$$

So, the dependence $\Delta(t) = f(\theta)$ would be a linear one.

The observed convex pattern in Fig.4 may be explained by a local increase in the twist at B/A junctions. Indeed, due to a rather small cooperativity length of the B-A transition ($\nu \sim 20$ base pairs), the number of junctions is great enough (2,3,7). Their linear density $n(\theta)/N$ attains a maximum at $\theta = 1/2$, $n(1/2)/N = 1/\nu$. Here, n is the number of B- (or A-) segments and N is the total number of base pairs in the DNA. If the change of helical winding at one B/A junction equals t_j, then an addend, which is proportional to the number of junctions, must be included into Eq. 3:

$$\Delta t(\theta) = (t_A - t_B)\ \theta + t_j\ n(\theta)/N \qquad [4]$$

The maximum deviation of this function from that determined by Eq. 3 must be observed at [theta] = 1/2 and has the value t_j/ν. It follows from Fig. 4 that the deviation at $\theta = 1/2$ equals 0.25° per base pair. Accounting for $\nu \sim 20$ b.p., one gets the value for $t_j \sim 5°$ per/junction. Such a value for the extra winding at a B/A junction is in accord with the results obtained in conformational calculation of the structure for a B/A junction (17).

However, one cannot be sure yet that the convex pattern in Fig.4 is definitely due to a rewinding at the junctions. Other explanations such as a heterogeneity of the A form winding angles for different sequences, are also possible. In any event, these alternatives do not affect the main result of this study; the estimation of a difference between the winding angles for the B and A forms in solution. As follows from Fig. 4, this difference is equal to $1.5 \pm 0.2°$ per base pair.

The mean value for the B-DNA winding angle in solution is known to be $t_B = 34.1 \pm 0.1°$ per base pair (12–14). Hence, $t_A = 32.6 \pm 0.3°$ per base pair, which corresponds to a helical repeat of the A form about 11 base pairs. Thus, the helical repeat of the A form is similar in solution and in the condensed state, in contrast to the B form for which it is ~10.5 in solution and 10.0 in the condensed state.

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