# On the flexibility of the boundaries between the $\bar{A}$-form and $\bar{B}$-form sections in DNA molecule

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ABSTRACT

The degree of orientation of DNA in a flow has been studied within the interval of the $\bar{B}$ - $\bar{A}$ transition induced by ethanol. The orientation of the $\bar{B}$ DNA (60-65% ethanol, v/v) and that of the $\bar{A}$ DNA (80-82% ethanol) are nearly identical. This means that both conformations have similar persistence lengths and that there is no aggregation in the course of formation of the $\bar{A}$ form. Within the transition range (65-78% ethanol) the orientation attains a sharp minimum which coincides with the half-transition point (73% ethanol). The cooperative character of the $\bar{B}$ - $\bar{A}$ transition presupposes the existence of boundaries between the alternating sections of the $\bar{A}$ and $\bar{B}$ conformations that may entail an increased flexibility of the DNA molecule and a corresponding drop of orientation. Theory predicts an elliptical dependence of the number of boundaries on the proportion of the $\bar{A}$ form. The experimental degree of orientation follows the same pattern. Quantitative evaluation shows that the flexibility of a boundary is small, so that several dozen of boundaries are required to simulate free rotation.

## INTRODUCTION

Two co-operative conformational transitions are known in DNA at present: the helix-coil[1] and the $\bar{B}$ - $\bar{A}$ [2,3] ones, the latter having no base pair disruption. While the first one is well studied and explained by statistical physics[4-6], the $\bar{B}$ to $\bar{A}$ transition is as yet imperfectly understood. Moreover, there are even doubts as to the possibility of achieving this transition intramolecularly, i.e. with no aggregation[7,8].

We have already had, while working on the ethanol-induced $\bar{B}$ - $\bar{A}$ transition in solution, evidence of the co-operative character of the $\bar{B}$ - $\bar{A}$ change[9,10]. This implies that each DNA molecule is subdivided into alternating sections of the $\bar{A}$ and $\bar{B}$ conformations. The average length of these sections, $\gamma_0$ , at the half-transition point characterizes the degree of co-operativity. Having applied the Ising model to the description of the $\bar{B}$ - $\bar{A}$ transition, we obtained a value in the order of ten base pairs for the co-operativity length[10,11]. Recently the value of such an order has been obtained by an independent way[12]. Thus, the $\bar{B}$ - $\bar{A}$ transition involves less co-

-operativity then the helix-coil transition with its $\gamma_o \sim 10^3 - 10^4$ base pairs[4-6].

The purpose of the present study is to detect the boundaries between the $\bar{A}$-form and $\bar{B}$-form sections. The boundary must look as a distortion of the regular helix and have an increased energy. Hence DNA may be expected to have an increased flexibility at these sites. Experimentally this can be studied by observing changes in specific viscosity or in the degree of DNA orientation in flow within the $\bar{B}$ - $\bar{A}$ transition interval. So, these may be expected to have a minimum value at the half-transition point. In the study of Frisman et al.[13] the specific viscosity of DNA in alcohol-water solvents was measured but not in the $\bar{B}$ - $\bar{A}$ transition range. The present work deals with the orientation method.

## METHODS

To orient DNA we used a cell similar to that described by Chung and Holzwarth[14]. Glass capillaries 33mm long with the internal diameter 0.5mm are placed along the optical path; the total length of the optical path was 35mm. The solution is pumped through the cell by a 100 $cm^3$ syringe. The piston of the syringe is moved to and fro for 20 sec in each direction by an electric motor with an automatic switch. The cell is set in a Cary-118 spectrophotometer, and the degree of DNA orientation, b, is measured by the increase in the absorption at 260 nm, $\delta A_f$ [14]:

$$b = \frac{2\,\delta A_f}{A(1 - 3\sin^2 TL)} \qquad (1)$$

Here A is the absorbancy value of the solution when the pump is off. $\delta A_f$ is the increase in the absorbancy due to orientation of DNA. TL is the inclination angle of base pairs to the helix axis. If $TL < 15°$ then $3\sin^2 TL$ is small and the orientation is equal to:

$$b = \frac{2\,\delta A_f}{A} \qquad (2)$$

It was checked that the time, required to achieve an equilibrium ($\sim$3 sec), was much less than the time of one passage of the piston ($\sim$20 sec).

Calf thymus DNA ("Sigma") with mol.weight of about $10^7$ was used. The DNA concentration in our experiments was 5-7 $\mu g/ml$. The initial solution contained 82% ethanol (v/v) and a small NaCl concentration (indicated in Figures).

The $\bar{A}$ to $\bar{B}$ transition was carried out by addition of the calculated

quantities of water as described in our previous paper[10]. After each water addition a circular dichroism (CD) spectrum was recorded by a Jobin-Ivon Mark III dichrograph in an ordinary quartz cell, and the flow orientation value, b, was measured in the multicapillary cell. The $\bar{B}$ - $\bar{A}$ transition profile was obtained by plotting the CD magnitude at 270 nm as a function of ethanol concentration.

## EXPERIMENTAL

In Fig.1 two curves are shown: the curve of the $\bar{B}$ - $\bar{A}$ transition (left ordinate) and the V-shaped curve which represents the dependence of the orientation value of the same sample within the transition interval (right ordinate). Clearly the orientation minimum coincides with the point $a_t$, where the number of the boundaries should be maximum.

Another important feature is the equality of the orientation values for the $\bar{B}$ form (left plateau) and the $\bar{A}$ form (right plateau). This shows that both conformations have similar persistence lengths, i.e. $\bar{B}$ and $\bar{A}$ forms have the same flexibility (or nearly the same, if one takes into account the fact that the base pairs in the $\bar{A}$ form can be inclined; see equation (1) ). At the same time the equality of the orientation values for the $\bar{A}$ and $\bar{B}$ forms proves the absence of aggregation in the course of the $\bar{B}$ - $\bar{A}$ transition. (In addition, we did not observe any change in the molar absorbance in our conditions when going from 66 to 82% ethanol; see also our previous paper[10].) So, the $\bar{B}$ - $\bar{A}$ transition can indeed proceed within one molecule, i.e. intramolecularly.

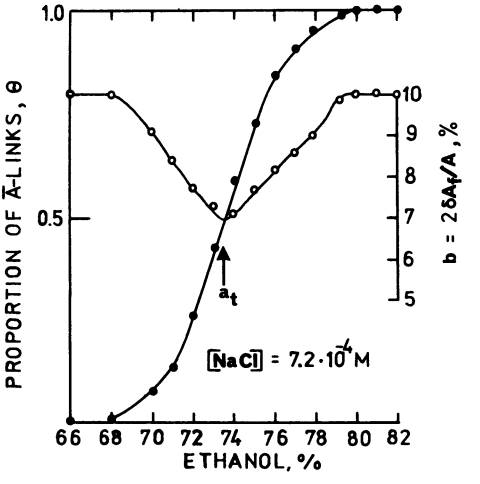


Fig.1. $\bar{B}$ - $\bar{A}$ transition of DNA in water-ethanol solution. $\theta$ is the relative CD change at 270nm. Right ordinate is for the orientation curve (open circles)

The drop of orientation in the transition interval is rather small, but significant: the experimental data presented in Fig.1 show that the "b" value changes from 10 to 7%. This indicates that the DNA molecule is only slightly more flexible at the boundaries, than in the body of the regular helix. It should be remembered that the number of the boundaries is very high since the co-operativity length for the $\bar{B}$ - $\bar{A}$ transition is in the order of ten base pairs[10,11]. One can, therefore, suppose that the "joint" between the $\bar{A}$ and $\bar{B}$ sections is rather smooth and has no free hinge.

## THEORETICAL

Our interpretation would be verified if one could compare the whole experimental curve of the change in the "b" value within the transition interval with the theoretical dependence. The latter would be rather hard to obtain in the general case. The situation is, however, much simplified in our case by the fact that the orientation values observed in the experiments and their relative changes are small enough. This ensures a simple relation between the change in the degree of orientation and the portion of DNA base pairs in the $\bar{A}$ (or $\bar{B}$) form. To begin with, in the range of low orientations (low gradients rotational diffusion coefficient of a molecule (see, e.g. ref.[15]). The latter, in its turn, is inversely proportional to the molecular volume of the DNA coil. Consequently, it must hold that

$$b/b_o = v/v_o \qquad (3)$$

Here the values for the ideal helices are labelled with the index "$_o$", and those in the transition range have no index. Since $v=(\bar{h}^2)^{3/2}$, where $\bar{h}^2$ is the mean square distance between the ends of a DNA molecule, our task is reduced to finding $\bar{h}^2$ value in the interval of the $\bar{B}$ - $\bar{A}$ transition. This problem is similar to that of the viscosity change in the case of the helix-coil transition of DNA[16,17].

Assume that the only consequence of subdividing a DNA molecule into alternating $\bar{A}$ and $\bar{B}$ sections is an increase in its flexibility, i.e. the formation of "hinges" at the sites of boundaries between $\bar{A}$ and $\bar{B}$ segments. Of course, these hinges do not have complete flexibility. The degree of flexibility of the hinge can be characterized by a parameter $\beta$, so that if n is the number segments ($\bar{A}$ or $\bar{B}$), the corresponding number of fully flexible hinges will be $\beta n$. According to the persistence model (see, e.g. refs.[16,17]) the mean square distance between the ends of DNA molecule equals to:

$$\bar{h}^2 = 2a^2 \left( \frac{\gamma L}{\beta a} - 1 + e^{-\gamma L/\beta a} \right) \frac{\beta N}{\gamma} \qquad (4)$$

Here $a$ is the persistence length of the regular helix; $L$ is the length of one link of the chain (one nucleotide); $N$ is the number of base pairs; $\gamma = N/n$. The small variation of the relative orientation value (and therefore of the $\bar{h}^2$ value as well) in the transition interval implies that $\gamma L/\beta a \gg 1$. Hence, the equation (4) boils down to:

$$\bar{h}^2 = 2aNL \left( 1 - \frac{\beta a}{\gamma L} \right) \qquad (5)$$

Note, that although the equation (4) is written for a specific case of uniform disposition of the hinges, eq. (5) is valid for a broad class of interhinge distributions including the random one[16,17]. Using eqs. (1) and (5) along with the relation $a\beta/\gamma L \ll 1$, one gets:

$$b/b_o = v/v_o = \frac{(\bar{h}^2)^{3/2}}{(\bar{h}_o^2)^{3/2}} = 1 - \frac{3}{2}\frac{\beta a}{\gamma L} \qquad (6)$$

Within the framework of the homogeneous Ising model for the $\bar{B} - \bar{A}$ transition[10] the value of $\gamma$ , which is the mean total length of the $\bar{A}$ and $\bar{B}$ section, equals to:

$$\gamma = \frac{\gamma_o}{\sqrt{\theta(1-\theta)}} \qquad (7)$$

where $\theta$ is the proportion of the $\bar{A}$ conformatuon.

Finally one has :

$$b/b_o = 1 - \frac{3}{2}\frac{a\beta}{L\gamma_o}\sqrt{\theta(1-\theta)} \qquad (8)$$

This is the curve of an ellipse. Fig.2 shows a very good correspondence between the experimental dependence at the indicated ionic strengthes (points) and the theoretic ellipse (line), provided that the only adjustible parameter, $\frac{3}{2}\frac{a\beta}{L\gamma_o} = 0.6$.

Since $a/L$ is equal to approx.200 base pairs (the persistence length[17,18]) and $\gamma_o \sim 10^1$ base pairs (the co-operativity length of the $\bar{B} - \bar{A}$ transition[10]), the flexibility of the boundaries $\beta \sim 1/50$. The $\beta$ is equal to 1 when free

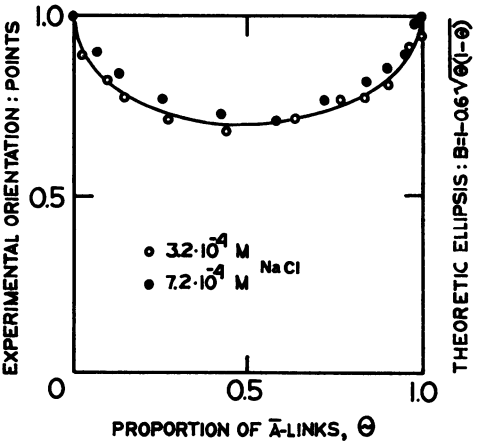


Fig.2. Dependence of relative orientation, $B = b/b_o$, on the proportion of the $\bar{A}$ form, $\Theta$ (see text).

rotation at a boundary site is possible. Thus, the boundary between the $\bar{A}$ and $\bar{B}$ sections is rather rigid, so that several dozen boundaries are necessary to create free rotation.

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