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EFFECT OF A MECHANICAL TENSION ON THE HYDRATION OF DNA IN FIBRES

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Summary. Fiber X-ray diffraction and measurement of fibre dimensions yield information about the effects of a mechanical tension on hydration of DNA in fibres. At a given relative humidity, the mechanical tension changes the DNA conformation but does not modify the number of water molecules associated to a nucleotide. The number of water molecules per nucleotide necessary to maintain B form decreases for increasing tensions applied to the DNA fibre. Form transitions can be opposed by mechanical tensions;an energy of 1 Kcal per mole of nucleotide pairs is sufficient to prevent the B to A transition.

Recent research has shown that the superhelical torsion in DNA depends strongly on its environment (1).Theoretical calculations (2,3) and experiments (4) on the bending and twisting of closed circular DNAs as well as detailed models based on elasticity (5,6,7) are also relevant in order to understand processes like DNA packing in chromosomes for example. In the present work, some quantified results on mechanical properties of linear DNAs stretched into fibres are given. In that case it has been shown that the DNA torsion, flexibility and rigidity depend on physico-chemical parameters (humidity, temperature, base composition) (8-11). It is also known that an applied tension on DNA fibres can induce or modify changes of DNA conformation (12,13). It therefore

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appears that the tension can play the same role as hydration on conformational changes. But a quantitative relation between these two factors is lacking, even if the number of water molecules, G, associated to a nucleotide during conformational transitions of DNA can be determined (14-16). By coupling fibre X-ray data with measurement of fibre dimensions, we can follow, for a given applied tension, the change of DNA conformation during B-A transition as a function of G. The variation of the nucleotide fraction in the B form ,with G ,at different values of the applied tension is presented. It appears that the applied tension can change the DNA conformation with no modification of the average number of water molecules per nucleotide.

MATERIAL AND METHODS

In the present work, only DNA associated to sodium salt is used, namely Calf thymus DNA, containing approximately 42 % of G-C and purchased from Pharmacia. The lyophilized DNA is dissolved in NH_3 buffer, pH 7.0. The Na^+ ions are fixed at 0.5 per nucleotide by dialysis against a salt solution. That Na^+ content already allowed us to study the B-A transition (14-17). The experimental system permits to follow the fibre behaviour by X-ray diffraction and microscope observations (17). The X-ray camera is adapted in order to allow the application of a precise mechanical tension to the fibre without disturbing the r.h.(relative humidity) in its vicinity (12). The number of water molecules per nucleotide G is given by the following relation (14) : $G = K\ (V_f - V_o)/(n\ n_s)$,

The constant $K = 1.67\ 10^{19}\ mm^{-3}$, V_f and V_o (given in mm^3) are the fibre volumes at respectively a given value of r.h. and at 0 %. n and n_s are respectively the number of base pairs along the fibre axis and in the fibre section. n is determined by the ratio of the fibre length to the rise per base in the DNA helix, (L/p) (17). The number n_s is obtained at low r.h. by the ratio of the fibre section to the section of a DNA double helix (14). One should note that the number G thus determined does not take into account water molecules which remain in the fibre at 0 % r.h. (15). In this experimental work, a quantified mechanical tension is applied to fibres at 95 % r.h.. From this initial condition in which the fibre adopts the B conformation, the r.h. is decreased and the fibre behaviour is studied by X-ray diffraction and microscope observations. Note that we suppose that all nucleotide pairs of the DNA fibre are submitted to the same mechanical tension $f = F/n_s$, where F is the force applied to the DNA fibre.

RESULTS

From X-ray diffraction alone, we noticed that a tension f of $3.6\ 10^{-11}$ N is the minimal applied tension which prevents the transition from B to A at any value of r.h. (relative humidity). In that case we obtained the B-B^*transition, with B^* a deformed B conformation (12). For tensions smaller than $3.6\ 10^{-11}$ N, we obtained the transition from B to a (B^*-A) mixture when the r.h. decreases (in Table 1 are given results for this transition with $f = 2.4\ 10^{-11}$ N).

By associating X-ray data to microscope observations, it appears that the ratio L/p which must remain constant with the r.h. at $f = 3.6\ 10^{-11}$ N, actually diminishes by a few percent when the r.h. decreases below 86 % (Table 1). So, it seems that below 86 % r.h. a certain fraction of A form is present even if it cannot be identified by X-ray diffraction. By using the fibre length variation with r.h. (Fig.1) and the value of the helical parameters of DNA (Table 1), the fraction X_{B^*} of nucleotides in the B^* form is calculated. When the A form is

Table 1 : Variation of the rise per nucleotide as a function of r.h. and the applied tension

	B-A Transition			
r.h. %	f = 0	$f = 2.4\ 10^{-11}$	$f = 3.6\ 10^{-11}$	
	p	p	p	10^{-7} L/p
95	3.37 (B)	3.40 (B)	3.40 (B)	1.24
92	3.36 (B)	3.40 (B)	3.40 (B)	1.24
90	3.36 (B)- 2.58 (A)	3.38 (B)	3.40 (B)	1.24
86	2.57 (A)	3.37 (B^*)-2.57 (A)	3.38 (B)	1.24
80	2.57 (A)	3.35 (B^*)-2.57 (A)	3.35 (B^*)	1.22
75	2.56 (A)	3.33 (B^*)-2.56 (A)	3.34 (B^*)	1.20
66	2.56 (A)	3.30 (B^*)-2.56 (A)	3.31 (B^*)	1.20

p rise per nucleotide (DNA form) given in Å
f applied tension (in Newton) on a nucleotide pair ($\Delta f/f \simeq 10$ %)
L/p ratio of the fibre length to the rise per nucleotide

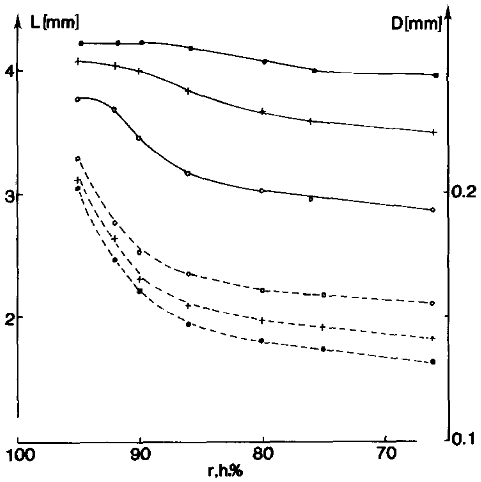

Figure 1 : B-A transition : variations of the length L (——) and diameter D (---) with the r.h.
f : applied tension on nucleotide pairs (in Newton)
(o) f = 0 (L = 2.40 mm, D = 0.149 mm at 0 % r.h.)
(x) f = $2.4\ 10^{-11}$ (L = 3.15 mm, D = 0.130 mm at 0 % r.h.)
(●) f = $3.6\ 10^{-11}$ (L = 3.65 mm, D = 0.121 mm at 0 % r.h.)

not obtained alone but present in (A-B*) mixture, the fraction $X_B{}^*$ is calculated as follows :

$$X_B{}^* = (L/n - p_A)/(p_B{}^* - p_A)$$

where p_A and $p_B{}^*$ are respectively the rise per nucleotide in A and B* forms and L the fibre length. The above expression is established from the relation : $L = n_A\ p_A + n_B{}^*\ p_B{}^*$
where n_A and $n_B{}^*$ are respectively the number of base pairs along the fibre axis in A and B* forms ($X_B{}^* = n_B{}^*/n$, $X_A = n_A/n$, $n = n_A + n_B{}^*$).

As the very slight variations of p_A with r.h. (Table 1) have no effect on values of $X_B{}^*$, we neglected the deformation of the A form and only took into account deformations of the B form with the tension (p_A = 2.56 A for the calculation of $X_B{}^*$ at any value of the tension).

One should note that with or without a tension the same relation between r.h. and G is observed. The effect of the tension is to shift the conformational transition toward lower values of G (Fig.2). When no tension is applied, X_B varies

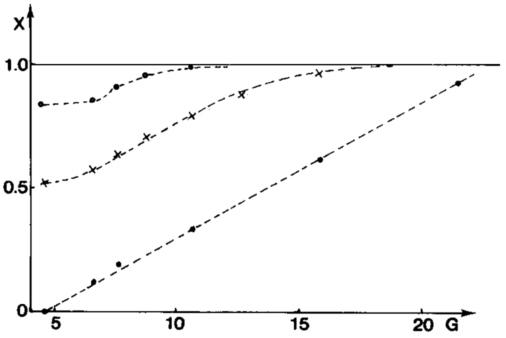

Figure 2 : B-A transition : Fraction (X) of nucleotides in the B form (or in the B* form) as a function of the number (G) of water molecules per nucleotide.
f : applied tension on nucleotide pairs (in Newton)
(●) f = 0; (x) f = 2.4 10^{-11}; (o) f = 3.6 10^{-11}.

linearly with G (Fig.2) according to the cooperativity of the B-A transition (14). But with a tension applied to the fibre, $X_B{}^*$ does not vary linearly with G, this behaviour is probably due to the simultaneous effects of a continuous B-B* deformation and of the B-A cooperative transition.

Figure 2 shows also that the value of X_B increases with the tension for a given value of G. So with 10 water molecules per nucleotide, for example, the DNA fibre is completely in the B form when a tension f = 3.6 10^{-11} N is applied, while without tension, B represents only 30 % of the A-B mixture. We also noticed (Fig. 2) that the DNA adopts the β form with at least 10, 15 or 22 water molecules per nucleotide with values of the tension respectively equal to 3.6 10^{-11}, 2.4 10^{-11} and 0 N. With $H = G_{B0} - G_{Bf}$, where G_{B0} and G_{Bf} are respectively the number of water molecules per nucleotide necessary for the DNA to adopt the B form without or with a tension f, we observed that the ratio H/f is a constant (equal to 3.0 10^{11} N^{-1} in the present case). Accordingly one obtains, by extrapolation, that G_{Bf} = 0 for f = fm = 7.4 10^{-11} N , this means that the DNA adopts the B form at any value of G when a tension of 7.4 10^{-11} N is applied to a nucleotide pair (actually such a tension cannot be used at 95 % r.h. because it breaks the fibre). Consequently, we can calculate the minimum value of the energy necessary to prevent the transition of all the nucleotides in the fibre from the B to A conformation (at any value of G). This energy (per mole of nucleotide pairs) is determined according to the expression : $W_m = N_A f_m q$ where $q = p_B - p_A = 3.4 - 2.56 = 0.84$ A and N_A is Avogadro's number. We obtained $W_m = 0.9$ kcal/mole (precision of 15 %).

DISCUSSION

Measurement of DNA fibre dimensions associated to X-ray diffraction have given precise information on the change of the number of water molecules associated to a nucleotide during conformational transitions of natural DNA or polynucleotides (14,18). In the present work ,it is shown that a mechanical tension applied to fibres of DNA in the B form can modify the DNA conformation and have the same effect as hydration.

At a given r.h., the number of water molecules associated to a nucleotide does not depend on the applied tension, but this number increases with the quantity of Na^+ ions associated to nucleotides. During the B-A transition, the number of water molecules stabilizing the B conformation is near to 20 per nucleotide for samples mounted free of tension (14-16). That number (G_B) decreases as a linear function of the applied tension and reaches the value of 0 when the applied tension is about $7.4\ 10^{-11}$ N per nucleotide pair. By using this last value one can note that an energy of about 1 Kcal per mole of nucleotide pairs is enough to prevent to the B to A transition whatever the value of G. We noted that this energy value depends on others physico-chemical parameters. It increases, for example, with the amount of Na+ ions and the percentage of

A-T base pairs in natural DNA (the value of 1 kcal/mole corresponds to 58 % of (A-T) and 0.5 Na^+ per nucleotide in solution).

When a nucleotide is submitted to a tension corresponding to an energy lower than 1 Kcal/mole and is associated to an average number of water molecules lower than G_B, a fraction of nucleotides adopts the B conformation and the others the A form (Fig. 2). So,it appears that all the nucleotides in the fibre are not associated to the same amount of water molecules and that the effects of a mechanical tension on DNA conformational changes can be opposed to those of other physico-chemical parameters and mainly to dehydration. Such effects could be of some importance for the interactions of DNA with proteins which intervene in the genetic expression within the cell nucleus.

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