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DOES DNA HAVE TWO STRUCTURES IN SOLUTION THAT COEXIST AT EQUILIBRIUM?

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ABSTRACT:

In this paper, we postulate the existence of two discrete structures for different regions of DNA at equilibrium in solution. These structures correspond to B DNA and to an inelastically deformed DNA structure, denoted β kinked DNA. β kinked DNA corresponds to a second order phase transition in the polymer, different regions of DNA undergoing this transition at different temperatures, and arises from a specific normal mode oscillation in DNA structure that is excited by Brownian motion of solvent molecules. It is possible that these multiply-kinked premelted regions play an important role in RNA polymerase-promoter recognition.

What is the structure of DNA in solution at equilibrium?

Is it only B DNA?

In this paper, we propose that different regions of DNA could have two discrete structures that coexist at equilibrium at a given temperature: B DNA and β kinked DNA. β kinked DNA corresponds to a second order phase transition in the polymer (different regions of DNA undergoing this transition at different temperatures) and arises from a specific normal mode oscillation in DNA structure that is excited by Brownian motion of solvent molecules.

Thus, DNA in solution at lower temperatures could exist only in the B form. At higher temperatures, however, permanently premelted β kinked DNA regions could appear. Due to their enhanced flexibility, these regions could be particularly prone to undergo DNA breathing motions (i.e., further base unstacking and hydrogen-bond breakage) due to thermal fluctuations. These breathing motions arise from both local fluctuations and, in addition, from fluctuations originating in neighboring regions that propagate energy in the form of mechanical waves (i.e., acoustic phonons) along DNA.

Our theory allows us to understand a variety of action at a distance phenomena observed for DNA (1-3). It provides a model for RNA polymerase-promoter recognition and, possibly, for other protein-DNA interactions as well (4-7). It allows us to understand how transient fluctuations elsewhere on the polymer can give rise to DNA breathing and to (mono- and bis- functional) drug intercalation (8-12). Finally, it provides an insight into the transition state intermediate that connects B with A DNA in the $B \rightleftharpoons A$ transition (13).

A preliminary description of our theory has already appeared (14). This paper further describes the theory and its biological implications.

NATURE OF NORMAL MODE OSCILLATION IN DNA STRUCTURE THAT GIVES RISE TO THE
FORMATION OF β KINKED DNA.

We envision DNA in solution to be continuously bombarded by solvent molecules along its length. Although the vast majority of these solvent collisions transfer energy into the polymer to give rise to anharmonic motions in its structure that eventually dissipate energy back into solution due to viscous damping, occasionally a solvent molecule with the appropriate momentum (i.e., having its direction oriented along the dyad axes in DNA) collides with DNA and this gives rise to harmonic motion in the polymer.

By harmonic motion, we mean a specific normal mode oscillation in DNA structure that either remains localized to a site or travels along the helix in the form of a normal mode wave. At low amplitudes, these harmonic motions cause DNA to oscillate between B DNA and a right-handed superhelical variant of B DNA that contains approximately 10 base-pairs per turn and has a pitch of about 34 Å (compare Fig. 1B and C). Such structures balance the unwinding in the helix with right-handed superhelical writhe to keep the linkage invariant (15), a feature that creates minimal perturbation in DNA structure. Higher amplitude harmonic motions give rise to similar structures except that these contain somewhat more than 10 base-pairs per turn and have base-pairs inclined more acutely to the helix axis (see Fig. 1D). Their presence could contribute to the apparant net unwinding of DNA and the altered tilt of base-pairs relative to the helix axis, features observed for DNA in solution (see discussion).

At small amplitudes of oscillation, DNA behaves as an elastic body that accumulates strain energy in its structure through small changes in torsional angles that define the geometry of the sugar-phosphate backbone. These changes are localized primarily in the furanose

rings of alternate deoxyribose sugar residues (normally, the puckering of the furanose ring in B DNA is C3' exo; however, the effect of introducing strain energy into the helix is to alter the magnitude and direction of this puckering). At larger amplitudes of oscillation, the enhanced strain energy in the sugar-phosphate chains begins to flatten out the furanose ring. Finally, at some critical oscillation amplitude, alternate sugars "snap into" a C3' endo sugar conformation with a concomitant partial unstacking of base-pairs (see Fig. 1E). This structure (denoted β kinked DNA) corresponds to an inelastic distortion in DNA structure and arises from a transient high energy normal mode oscillation in the helix localized at a specific site.

We are attempting to compute the detailed energy surface that defines this pathway of conformational change more precisely, and will report on this at a later time.

STRUCTURE OF DNA IN SOLUTION AT EQUILIBRIUM.

The structural considerations described above lead us to postulate the existence of two discrete structures for DNA at equilibrium. That is, at any given temperature, DNA may consist of Watson-Crick B DNA regions and other regions that are permanently β kinked. We emphasize that these β kinked regions are not fluctuational conformational changes that occur transiently along the polymer -- rather, they are permanent conformational changes associated with the absorption of heat from solution as the system approaches equilibrium.

We identify these multiply-kinked premelted regions as promoters. This makes the strong prediction that promoter regions (when activated) should be permanently accessible for attachment by the RNA polymerase enzyme. An important component in RNA polymerase-promoter recognition, therefore, is the interaction between RNA polymerase and these freely breathing DNA regions (by partial

intercalation of aromatic side chains into DNA) and through recognition of other structural elements (possibly a specific class of nucleotide sequences) to form the tight binding complex (5-8). Our model describes the process of RNA polymerase-promoter recognition in a simple and attractive way in comparison to alternative models that require large (and therefore less likely) fluctuations leading to a similar recognition process.

In addition to these permanently premelted β kinked promoter regions, short regions of β kinked DNA can form transiently elsewhere along the helix due to thermal fluctuations that exist at equilibrium (see below). These structures can undergo breathing motions during their lifetime to allow drug intercalation -- structural aspects of this process have already been described elsewhere (14, 16-17).

WAVE PROPAGATION (PHONONS) IN DNA.

We now ask -- can acoustic waves exist in DNA, continuously propagating back and forth along the helix?

By an acoustic wave we mean a normal mode wave in the DNA helix that can travel at the speed of sound along the polymer. Such waves could arise due to thermal fluctuations at equilibrium (i.e., as discussed previously, when an appropriate "hot" solvent molecule strikes DNA this gives rise to a normal mode oscillation in DNA structure that can either remain at that site or travel along the molecule in the form of a normal mode wave). Of course, any travelling wave in DNA will experience damping due to viscous drag by the solvent; however, we think it possible that waves of the type we describe could propagate significant distances along the helix before being completely damped.

If this is true, then an important consequence of phonon propagation in DNA is the altered nature of fluctuations that exist along the helix at equili-

brium. This is because -- when calculating the magnitude of fluctuations at specific places on DNA -- one has to take into account not only fluctuations that arise locally at this site but, in addition, fluctuations that occur at a distance and travel to this site in the form of phonons. Since DNA is a heterogeneous polymer (having variable flexibility due to different stacking energies between base-pairs in different sequences), it follows (see appendix) that the probability a specific region experiences a fluctuation with a given energy depends on the elasticity of this region and of neighboring regions. Generally, more flexible DNA regions will experience larger fluctuations. The magnitude of these enhanced fluctuations increase the probability that transient DNA conformational change can occur (i.e., B DNA $\rightarrow$ β kinked DNA). Subsequent additional fluctuations in this region could then give rise to breathing motions to permit intercalation by a drug molecule into DNA.

The same considerations apply when one discusses quasi-equilibrium processes. For example, if one raises the temperature of a DNA solution very slowly one can expect fluctuational changes in conformation to arise along the polymer. As energy continues to enter the polymer, the probability that a fluctuation exceeds the threshold energies needed for inelastic deformation increases at specific places. Eventually, at a given temperature, a new equilibrium structure for DNA will be formed. It is for this reason that we have postulated two discrete structures for DNA to coexist at equilibrium.

DISCUSSION.

How does our model for DNA structure and energetics relate to the data? First of all, it clarifies structural aspects of DNA breathing and drug intercalation -- two phenomena that reflect the dynamic structure of DNA in solution.

Second, it allows understanding of premelting phenomena in DNA and relates this to the process of protein-DNA recognition -- in particular, to RNA polymerase-promoter recognition. Third, it provides a reasonable explanation for action at a distance phenomena in DNA.

One of the clearest examples of action at a distance along DNA is the arabinose operon (3). Here, it has been demonstrated that the araC promoter interacts with the araBAD promoter, even though these DNA regions are separated by about 150 base-pairs. Under conditions where one promoter is active -- the other is not. This limitation in promoter function is relieved by cleavage between the promoters at a site close to the araBAD promoter by the BamI restriction nuclease. This now allows the araC promoter to operate in the presence of araC protein and L-arabinose, conditions under which the araC promoter is normally inactive.

Additional physico-chemical evidence for action at a distance along DNA has been presented by Wells and his colleagues (1, 2). The properties of the duplex block polymer $d(C_{15}A_{15})d(T_{15}G_{15})$ were examined by thermal denaturation and nuclease sensitivity in the absence and presence of drugs (actinomycin and netropsin) which bind specifically to only one end of the block polymer. These studies indicate that the properties of one region of a DNA molecule can be influenced by another remote region.

Another question frequently raised is: How many base-pairs per turn does DNA have in solution? Wang (18) has recently provided evidence that DNA has 10.4 base-pairs per turn at room temperature. Levitt (19) using semi-empirical energy calculations proposes DNA to have an equilibrium structure centered at about 10.5 base-pairs per turn. An interesting feature of Levitt's model is the predicted base tilt of 78° relative to the helix axis and a pro-

pellor-like twist between base-pairs of about 28° . Crothers and his colleagues (20), using transient electric dichroism to study the structure of monodisperse rod-like DNA molecules in solution estimate that base-pairs are inclined 73° or less to the helix axis, a value consistent with Levitt's proposal.

Our model, however, provides an alternative interpretation to these data. The presence of phonons in DNA distorts the helix for two reasons. First, there may be regions of DNA that remain permanently β kinked and therefore partially unwound. Second, travelling wave structures of the type we have described appear continuously due to fluctuations and their presence further acts to distort the DNA double helix. For these reasons, DNA in solution may have an average structure possessing slightly more than 10 base-pairs per turn and having a base tilt somewhat less than 84° relative to the helix axis.

Of course, one can argue that significant wave propagation in DNA is not possible due to viscous damping by the solvent. Clearly, this question must be resolved by experiment. Such an experiment has been done recently by Maret et al. (21), and to our knowledge represents the first experiment of its kind. They have used inelastic laser light (Brillouin) scattering on partially crystalline fibers of calf thymus DNA to detect acoustic phonons travelling at about 1800 m/sec along DNA. These data appear to confirm the existence of wave propagation along the DNA molecule.

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APPENDIX:

The importance of phonon propagation in DNA can be easily demonstrated if one considers a wave arising from a single fluctuation and propagating along the polymer. Since different regions of DNA have different elasticities, transmitted and reflected waves will arise at the boundaries. From Fresnel's formulae, the coefficients of transmission and reflection are

$$T = \frac{2\sqrt{E_1}}{\sqrt{E_1} + \sqrt{E_2}}, \quad R = \frac{\sqrt{E_1} - \sqrt{E_2}}{\sqrt{E_1} + \sqrt{E_2}}$$

where E_1 and E_2 correspond to different values of Young's modulus in two neighboring regions of DNA (we have assumed the density of DNA to be constant along its length).

From this one can easily obtain a relationship between the transmitted and incident energy density. This is

$$\frac{W_t}{W_i} = |T|^2 = \frac{4E_1}{(\sqrt{E_1} + \sqrt{E_2})^2}$$

If $E_1 \ll E_2$ and the wave originates in area 1 then only a small part of the energy will be transmitted into area 2. On the other hand, if $E_1 \gg E_2$ the transmitted energy density is: $W_t \approx 4W_i$. Notice that it makes no difference where the fluctuation originates -- the energy of this fluctuation will be distributed with higher density in the region having lower elasticity (i.e., higher flexibility).

Of course, DNA has many regions with different elasticities and therefore multiple reflections and transmissions from a single fluctuation have to be taken into account. In addition, one must consider not only the effect of one fluctua-

tion but more generally the influence of multiple fluctuations having different energies and arising at different times. This problem becomes very complicated and we will describe it elsewhere.

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FIGURE CAPTIONS:

Figure 1. Illustration of show the normal mode oscillation in DNA structure that gives rise to the formation of β kinked DNA. A. B DNA, drawn by computer graphics. B. B DNA, as visualized with the Corey-Pauling-Koltun (CPK) space filling molecular models. C. Low amplitude normal mode oscillation. D. Higher amplitude normal mode oscillation. E. β kinked DNA structure. F. β kinked DNA, drawn by computer graphics. See text for detailed discussion.