STRUCTURAL AND ENERGETIC CONSIDERATIONS OF WAVE PROPAGATION IN DNA

by

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An important dynamic aspect of DNA in solution is the presence of coupled motions in its structure that involve bending, stretching, unwinding and shearing components. We have postulated these motions to arise from wave propagation in the polymer, excited through impulses generated by the random and continuous bombardment of DNA by solvent molecules along its length (Lozansky et al., 1978). This gives rise to travelling structural distortions in DNA that result in DNA breathing and in drug intercalation (Sobell et al., 1976; 1977a, b). These distortions can be thought of as premelting conformational changes in DNA, and, in this respect, could have important implications in understanding the structure of DNA active in RNA transcription, DNA replication and genetic recombination (Sobell, 1978).

In this paper, we further document the nature of these distortions and discuss their relationship to DNA breathing and to drug intercalation. A key intermediate in these processes is the multiply kinked structure, β kinked DNA. This structure has both B and A characteristics, and we postulate this structure to be a conformational intermediate in the B $\rightarrow$ A polymorphic transition. In addition, we discuss a class of intercalators known as the bis functional intercalators, molecules that have two intercalative chromophores separated by about 10.2 Å. These bifunctional intercalators appear to bind DNA in a neighbor exclusion mode, and could, therefore, be probes to detect the migratory β kinked DNA structure we have postulated.

We have already described the implications our theory has in understanding how energy is distributed along DNA to form localized premelted regions of DNA. These regions form as the result of phonon energy that originates in the structural genome DNA and could correspond to promoters. Here, we discuss this further and examine, in addition, the nature of the excitation force due to Brownian motion of solvent molecules that gives rise to wave motion in DNA. This force is temperature dependent and one would therefore expect the average energy density along the DNA molecule to reflect this. As one raises the temperature of DNA in solution,

additional localized regions of DNA premelting could arise. Events such as these could have important implications in understanding the mechanism of DNA melting.

STRUCTURAL CONSIDERATIONS OF WAVE PROPAGATION IN DNA.

Figure 1 shows Corey-Pauling-Koltun (CPK) space filling molecular models of DNA undergoing wave propagation in its structure. We envision travelling wave forms in DNA to be right-handed superhelices, containing approximately ten base pairs per turn and having a pitch of about 34 Å. These structures balance the unwinding in the helix with right-handed superhelical writhe to keep the linkage invariant (Fuller, 1971), a feature that creates minimal perturbation in DNA structure. At small amplitudes of oscillation, DNA behaves as an elastic body that accumulates strain energy in the sugar-phosphate chains through small departures of torsional angles from their equilibrium values (compare Figures 1A and B). This distortion appears to be primarily localized in the furanose rings of (alternate) deoxyribose sugar residues (i.e., normally, the puckering of the furanose ring in B DNA is C2' endo; however, the effect of introducing strain energy into the helix is to alter the magnitude of this puckering). At larger amplitudes of oscillation (see Figure 1C), the enhanced strain energy in the sugar-phosphate chains tend to flatten out the furanose ring. Finally, at some critical oscillation amplitude (this corresponds to a threshold energy that can be provided either by occasional collisions by "hot" solvent molecules at the tail of the Maxwell-Boltzmann distribution or by constructive interference between elastic travelling waves), alternate sugar residues can "snap into" a C3' endo sugar conformation with a concomitant partial unstacking of base-pairs. This conformational change results in the formation of localized regions of β kinked DNA (see Figure 1D) and corresponds to an inelastic distortion in DNA structure that leads to DNA breathing and to drug intercalation.

In thinking about the structure of DNA in solution, therefore, it is impera-

tive to keep these dynamical wave forms in mind. Attempts to estimate the average number of base-pairs per turn of the helix are misleading if one has a static picture of DNA in solution (Levitt, 1978). The presence of wave propagation in DNA distorts its structure for two reasons. First, phonon energy is initially partitioned between the solvent and the polymer when DNA enters solution and this energy remains localized in its structure in the form of permanent conformational change (Lozansky et al., 1978, and see section below). Second, travelling structural distortions due to wave propagation appear continuously as the result of the Maxwell-Boltzmann fluctuations, and their presence acts to further distort the DNA double helix. Although our model building studies have suggested these wave disturbances to take the form of right-handed superhelical structures having approximately ten base-pairs per turn, it is possible that this value is an underestimate and that, depending on the amplitude of the oscillatory wave, this is between ten and eleven base-pairs per turn. Such an estimate would be in better agreement with our current concepts that β kinked DNA is a key intermediate in the B $\rightarrow$ A polymorphic transition (see discussion below). We are therefore investigating the detailed structures of these wave forms using energy minimization methods and will report on this at a later time.

DNA BREATHING AND DRUG INTERCALATION.

We have recently proposed a structural mechanism that interrelates DNA breathing with drug intercalation (Sobell et al., 1976; Sobell et al., 1977a, b; Sobell, 1978; Lozansky et al., 1978). This mechanism involves, as a key structural intermediate, β kinked DNA. Once formed, β kinked DNA can then undergo further elastic deformations to form the neighbor exclusion intercalative struc-

ture. This structure is the maximally unwound form possible for double stranded DNA. Further unwinding results in weakening, and, eventually, in the disruption of base-pairing. We envision a combination of these activated DNA forms to exist transiently along the helix, giving rise to DNA breathing and to drug intercalation. These concepts have been summarized schematically in Figure 2, and with space filling CPK molecular models in Figure 3.

BIS INTERCALATION -- A PROBE FOR β KINKED STRUCTURAL DISTORTIONS IN DNA?

An important class of intercalative drugs and dyes that has received considerable attention in recent years are the bis intercalators -- bifunctional intercalating agents that have two chromophore groups separated by about 10.2 Å (Wakelin and Waring, 1976; Ughetto and Waring, 1977; Le Pecq et al., 1975; Butour et al., 1978; Dervan and Becker, 1978). These molecules intercalate into DNA in a neighbor exclusion mode (i.e., the intercalative chromophore groups are separated by two base-pairs when binding to DNA). One example is echinomycin, a polypeptide containing antibiotic that binds to DNA and inhibits RNA synthesis. This molecule has two quinoxaline ring systems connected to an octapeptide through amide linkages (see Figure 4), and probably possesses a pseudo-dyad axis of symmetry that plays an important role in its structural interactions with DNA (see Figure 5). The presence of these two intercalative chromophore groups held 10.2 Å apart by the rigid octapeptide chain suggests that echinomycin recognizes a naturally occurring structural intermediate related to RNA synthesis. We have postulated this intermediate to be β kinked DNA, a key intermediate in DNA unwinding (Sobell, 1978, and see below). It is of interest that echinomycin is some 4-5 times more potent than actinomycin in its ability to inhibit RNA synthesis, a feature that makes echinomycin an extremely potent antitumour agent.

Unfortunately, however, its enhanced cytotoxicity limits its clinical usefulness.

Drugs containing two intercalative chromophore groups have been synthesized in recent years (Le Pecq et al., 1975; Dervan and Becker, 1978). These include bis acridine and bis ethidium (as well as mixed acridine-ethidium) polyintercalating agents. These molecules bind DNA very tightly (i.e., binding constants approaching 10^9 liters/mole have been observed for the bis acridine intercalators). Although there are no data as yet concerning the kinetic rate constants for these associations, our model predicts the on rate constants for monofunctional and bifunctional intercalation to be very similar (since we postulate β kinked DNA to be the common structural intermediate) and this can be tested in future years.

β KINKED DNA AS A STRUCTURAL INTERMEDIATE IN THE B $\rightleftharpoons$ A TRANSITION.

Since β kinked DNA has both B and A elements in its structure, it may serve as the structural intermediate in the B $\rightleftharpoons$ A transition (see Figure 6). This follows, since sugar residues alternate between C2' endo and C3' endo conformations with concomitant changes in the glycosidic torsional angles. Each C2' endo nucleotide unit exists in the high anti conformation, a conformation that is typically observed in the B family of DNA helices (Ivanov et al., 1973, 1974). Each C3' endo nucleotide unit, on the other hand, exists in the low anti conformation, a conformation that characterizes the A family.

The B $\rightleftharpoons$ A transition has been studied under a variety of conditions. First detected in oriented fibers of Na DNA at 75% relative humidity by X-ray diffraction (Franklin and Gosling, 1953), the transition has also been observed in 70-80% ethanol-water mixtures with a variety of other methods (Brahms and Mommaerts,

1964; Ivanov et al., 1973, 1974; Girod et al., 1973; Erfurth and Peticolas, 1975; Herbeck et al., 1976). The transition occurs over a very narrow range of humidity and ethanol-water concentrations and is therefore highly cooperative. Cross linking DNA by photodimerization inhibits the $B \rightleftharpoons A$ transition (Herbeck et al., 1976), as does a variety of intercalative drugs and dyes (Venable, J., personal communication).

We envision small regions of β kinked DNA to serve as dynamic interfaces between B and A DNA. A moving structure such as this could leave behind either B or A DNA depending on the relative stabilities of these forms at given conditions. Although our model predicts that double stranded RNA can undergo a conformational change from A RNA to β kinked RNA (so as to allow for drug intercalation), the subsequent conversion of β kinked RNA to the B structure may not be possible for steric reasons. It is for this reason that double stranded RNA and the RNA-DNA hybrid helix exist solely as A structures (Langridge and Gomatos, 1963; Milman et al., 1967).

ENERGETIC CONSIDERATIONS OF WAVE PROPAGATION IN DNA.

Energy for wave propagation in DNA that results in DNA kinking and in other premelting conformational changes in its structure comes from Brownian motion of solvent molecules that continuously collide with the polymer along its length. The appropriate equation to describe wave propagation in a non-uniform elastic media with damping is

$$\rho(x) \frac{\partial^2 u(x,t)}{\partial t^2} = \frac{\partial}{\partial x} \left[E(x) \frac{\partial u(x,t)}{\partial x} \right] - h \frac{\partial u(x,t)}{\partial t} + F(x,t) \quad (1)$$

where $u(x,t)$ - is the vector displacement of the polymer

$\rho(x)$ - is the density of the polymer

$E(x)$ - is the elasticity of the polymer

h - is the viscous damping of polymer action by solvent

$F(x,t)$ - is the excitation force due to bombardment of the polymer by solvent molecules

For a simple case involving nonuniformity, let us consider a polymer consisting of two domains, one with parameters ρ, E_1 when $0 \leq x \leq a$ and the other with parameters ρ, E_2 when $a \leq x \leq b$, that experiences an impulse, P , at time $t = 0$ and $x = x_0$, due to a single collision with a solvent molecule. See Figure 7. In this case, equation (1) can be rewritten as follows

$$\frac{\partial^2 u_1(x,t)}{\partial t^2} = c_1^2 \frac{\partial^2 u_1(x,t)}{\partial x^2} - 2\gamma \frac{\partial u_1(x,t)}{\partial t} \quad (2)$$

$$\frac{\partial^2 u_2(x,t)}{\partial t^2} = c_2^2 \frac{\partial^2 u_2(x,t)}{\partial x^2} - 2\gamma \frac{\partial u_2(x,t)}{\partial t} \quad (3)$$

where

$$u(x,t) = \begin{cases} u_1(x,t), & 0 \leq x \leq a \\ u_2(x,t), & a \leq x \leq b \end{cases}$$

$$\gamma = \frac{h}{2g}, \quad c_1^2 = \frac{E_1}{g}, \quad c_2^2 = \frac{E_2}{g}$$

The boundary conditions for the system of equations (2) and (3) are

$$u_1(0, t) = 0 \quad (4a)$$

$$u_2(b, t) = 0 \quad (4b)$$

$$u_1(a, t) = u_2(a, t) \quad (4c)$$

$$E_1 \frac{\partial u_1(a, t)}{\partial x} = E_2 \frac{\partial u_2(a, t)}{\partial x} \quad (4d)$$

where we have assumed that the ends of the polymer remain fixed.

The initial conditions of this system are

$$u(x, 0) = 0$$

$$\frac{\partial u(x, 0)}{\partial t} = \frac{P}{g} \delta(x - x_0)$$

where $\delta(x - x_0)$ is the delta function.

The solution of the problem (2) - (5b) can be written in the form

(see appendix)

$$u(x, t) = \sum_{n=1}^{\infty} e^{-\gamma t} \frac{P}{g} \frac{\sin \frac{\lambda_n}{c_1} x_0}{\| \phi_n \|^2 \sqrt{\lambda_n^2 - \gamma^2} \sin \frac{\lambda_n}{c_1} a} \sin \sqrt{\lambda_n^2 - \gamma^2} t \phi_n(x) \quad (6)$$

where

$$\Phi_n(x) = \begin{cases} \sin \frac{\lambda_n}{c_1} x / \sin \frac{\lambda_n}{c_1} a, & 0 \leq x \leq a \\ \sin \frac{\lambda_n}{c_2} (b-x) / \sin \frac{\lambda_n}{c_2} (b-a), & a \leq x \leq b \end{cases}$$

$$\|\Phi_n\|^2 = \int_0^b \Phi_n^2(x) dx$$

and λ_n are the positive roots of the following equation

$$\frac{E_1}{c_1} \cot \frac{\lambda_n}{c_1} a = \frac{E_2}{c_2} \cot \frac{\lambda_n}{c_2} (a-b)$$

These relationships hold for $0 \leq x_0 \leq a$. Similar relationships can be obtained when $a \leq x_0 \leq b$. From equation (6), one can calculate the average energy densities in each area and prove that $\langle w_1 \rangle$ is greater than $\langle w_2 \rangle$ when E_2 is greater than E_1 (these formulas are complicated and will not be shown here; however, we have already proven this qualitatively in a previous communication (Lozansky et al., 1978)). An important additional insight that follows from equation (6) is that the displacement, $U(x,t)$, and, therefore, the average energy densities along the polymer depends on temperature. This follows since the momentum is a parameter governed by the Maxwell-Boltzmann distribution.

In principle, one can carry out an analysis that involves the continuous and random bombardment of the polymer by a large number of solvent molecules. Although at first glance one might expect the average excitation force due to

Brownian motion to be zero, this is not the case in the microscopic domain, where DNA is continuously undergoing unbalanced excitation forces due to collisions with solvent molecules. This is because the collision cross sectional area of DNA corresponding to the structural genome is small, and therefore relatively few solvent molecules impinge on its surface in short time intervals. A simple analogy exists in the case of Brownian motion of a particle suspended in some liquid media. If the dimensions of the particle are large, the number of collisions per unit time with solvent molecules is high and therefore the average momenta transferred to the particle are balanced. If, on the other hand, the dimensions of the particle are 10^{-4} cm or less, an appreciable Brownian force exists and this gives rise to continuous motion of the particle (Einstein, 1910). It is interesting to note that the dimensions of the DNA structural genome are in this same order of magnitude.

Wave motion is associated with kinetic and potential energy. As we have mentioned earlier, this energy tends to be highest in those domains of the polymer that have greatest flexibility. In the case of DNA, the molecular weights of A-T and G-C base-pairs differ by only 0.4% and their molecular volumes are almost identical. This means that the density along DNA is roughly constant. However, the stacking energies between different base-pairs vary considerably (Borer et al., 1974) and this gives rise to nonuniform elasticity along the polymer.

Let us now consider a hypothetical experiment in which DNA is placed into solution at some initial time. Before equilibrium is attained (see Figure 8A), DNA absorbs energy from the solvent in the form of phonon energy and this tends to enhance the average energy density in those regions of the polymer that are most flexible. As the average energy densities in these regions rise, the amplitudes of oscillation in DNA structure also rise. This gives rise to energy dissipation back into solution in the form of heat due to viscous damping, and the rate

at which this occurs depends on the amplitudes of oscillatory motion. If the rate at which energy arrives into a given region is greater than its rate of dissipation, then the average energy density continues to rise. Eventually, a critical threshold is reached and DNA undergoes a conformational change in its structure to give rise to β kinked DNA. At equilibrium, we envision the process as follows (see Figure 8B): at a given time, an appropriate solvent molecule collides with DNA to give rise to wave propagation. As the wave propagates, part of its energy dissipates back into solution in the form of heat due to viscous damping. The remaining part reaches the β kinked DNA region and causes further base unstacking and disruption of hydrogen bonding (i.e., this corresponds to the formation of higher energy structural intermediates involved in drug intercalation and in DNA breathing). This process is reversible; at some later time, bases partially restack and hydrogen bonds reform. Part of the energy is released immediately into solution in the form of heat due to viscous damping. The remaining part travels back through the surrounding DNA regions as wave propagation, and finally dissipates as heat due to viscous damping. At equilibrium, therefore, the rates of energy entering and leaving the polymer are uniform and equal throughout its length.

We have already suggested these permanently premelted regions to correspond to promoters (Sobell et al., 1976, 1977; Lozansky et al., 1978). RNA polymerase could bind to these regions by intercalating aromatic side chains into DNA, a process that can catalyze further melting. Our model makes several important predictions. First, it predicts that intercalative drugs and dyes should bind tightly to promoter regions, a prediction readily tested with radioactive psoralin or ethidium azide. Second, these regions should be particularly prone to chemical modification by carbodiimide or formaldehyde, reagents that react with imino-

nitrogens on unpaired bases, as would exist in a region of DNA undergoing extensive DNA breathing (Lebowitz et al., 1977). Evidence for this has already appeared for superhelical PM2 DNA and SV40 DNA (Flashner et al., 1977; Hale and Lebowitz, 1978). Finally, our theory would predict that promoter regions correspond to early melting regions as detected by a differential melting curve analysis. This appears to be the case in bacteriophage λ , where there is a close correlation between known promoter regions and early melting behavior (Szybalski, W., personal communication).

HOW DNA UNWINDS.

Wave propagation in DNA gives rise to coupled motions in the polymer that involve bending, stretching, shearing and unwinding. At low oscillation amplitudes, this results in elastically deforming B DNA into right-handed superhelices. Higher energy oscillations, however, result in a conformational change in the polymer, denoted β kinked DNA. This structure can be thought of as one in a series of premelting conformational changes in DNA that eventually lead to DNA denaturation. Higher energy normal mode oscillations result in the neighbor exclusion intercalative structure, while still higher energies result in the disruption of hydrogen bonds to give rise to single stranded premelted regions. We postulate this pathway of conformational change (summarized schematically in Figure 2) to define the energy surface for unwinding the DNA double-helix.

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FIGURE CAPTIONS:

Figure 1. Corey-Pauling-Koltun (CPK) space filling molecular models of DNA to show wave propagation in its structure. A. B DNA, undistorted. B. B DNA, gently unwound to possess a right-handed superhelical writhe. C. same as B, except with larger oscillation amplitude. D. β kinked DNA. These structures correspond to normal mode oscillations in DNA that are excited through impulses generated by the random and continuous bombardment of DNA by solvent molecules along its length. See text for discussion.

Figure 2. Schematic illustration of concepts relating drug intercalation and DNA breathing. A. B DNA, gently unwound to possess a small right-handed superhelical writhe. B. β kinked DNA structure. C. β intercalative DNA structure. D. premelted form of single stranded DNA. E. A combination of these activated DNA forms that migrates isoenergetically with the propagating wave to give rise to DNA breathing and to drug intercalation. F. Adenine-thymine base-pair in closed form. G. Adenine-thymine base-pair in open, partially denatured, form. These structural intermediates correspond to premelting conformational changes in DNA and define a structural pathway to understand how DNA unwinds. See text for additional discussion.

Figure 3. Corey-Pauling-Koltun (CPK) space filling molecular models of DNA to show relationship between DNA breathing and drug intercalation. A. the combination of activated DNA forms shown schematically in Figure 2E. B. the echinomycin-DNA complex. See text for discussion.

Figure 4. Chemical structure of echinomycin.

Figure 5. Corey-Pauling-Koltun (CPK) space filling molecular model of echinomycin, proposed from energy calculations (Ughetto and Waring, 1977). Echino-

FIGURE CAPTIONS: (continued)

mycin has two quinoxaline ring systems connected through amide linkages to a rigid octapeptide chain and probably possesses a pseudo- dyad axis of symmetry that plays an important role in its structural interactions with DNA. This molecule is one in a class of molecules known as bifunctional (or bis) intercalators. See text for discussion.

Figure 6. Schematic illustration to show β kinked DNA (or RNA) as a dynamic interface connecting: A. B DNA with B DNA. B. A DNA with A DNA. C. A RNA with A RNA. D. A DNA with B DNA. See text for discussion.

Figure 7. An illustration to show a polymer consisting of two domains, one with parameters ρ, E_1 when $0 \leq x \leq a$ and the other with parameters ρ, E_2 when $a \leq x \leq b$, that experiences an impulse, P , at time $t = 0$ and $x = x_0$, due to a single collision with a solvent molecule. See text for discussion.

Figure 8. Cartoon showing a hypothetical experiment in which DNA is placed into solution at some initial time. A. Before equilibrium. B. At equilibrium. See text for discussion.

APPENDIX:

Using the method of separation of variables to solve equations (2) and (3), we write

$$u(x, t) = \psi(t) \phi(x) \quad (A1)$$

Substituting (A1) into (2) and (3), we have

$$\psi_1'' \phi_1 = c_1^2 \psi_1 \phi_1'' - 2\gamma \psi_1' \phi_1 \quad (A2)$$

$$\psi_2'' \phi_2 = c_2^2 \psi_2 \phi_2'' - 2\gamma \psi_2' \phi_2 \quad (A3)$$

Dividing (A2) and (A3) by $\psi_1 \phi_1$ and $\psi_2 \phi_2$, respectively, we obtain

$$\frac{\psi_1''}{\psi_1} + 2\gamma \frac{\psi_1'}{\psi_1} = c_1^2 \frac{\phi_1''}{\phi_1} = -\lambda_1^2 \quad (A4)$$

$$\frac{\psi_2''}{\psi_2} + 2\gamma \frac{\psi_2'}{\psi_2} = c_2^2 \frac{\phi_2''}{\phi_2} = -\lambda_2^2 \quad (A5)$$

where λ_1 and λ_2 are constants. From the boundary conditions, (4a) - (4d), it follows that $\lambda_1 = \lambda_2$ and one can write

$$\phi_1(x) = A \sin \frac{\lambda}{c_1} x ; \phi_2(x) = B \sin \frac{\lambda}{c_2} (a-x) \quad (A6)$$

$$A \sin \frac{\lambda}{c_1} b = B \sin \frac{\lambda}{c_2} (a-b) \quad (A7)$$

$$E_1 \frac{\lambda}{c_1} A \cos \frac{\lambda}{c_1} b = -E_2 \frac{\lambda}{c_2} B \cos \frac{\lambda}{c_2} (a-b) \quad (A8)$$

Dividing (A7) by (A8), we get (9). Now, from (A4) and (A5) we also have an equation

APPENDIX: (continued)

$$\psi'' + 2\gamma\psi' + \lambda^2\psi = 0 \quad (\text{A9})$$

where $\psi = \psi_1 = \psi_2$

The solution of this equation for the case $\lambda^2 > \gamma^2$ is

$$\psi(t) = e^{-\gamma t} (C_1 \sin \sqrt{\lambda^2 - \gamma^2} t + C_2 \cos \sqrt{\lambda^2 - \gamma^2} t) \quad (\text{A10})$$

If $\lambda^2 < \gamma^2$, there will be no waves. Now, we can write

$$u(x,t) = \sum_{n=1}^{\infty} e^{-\gamma t} (C_{1n} \sin \sqrt{\lambda_n^2 - \gamma^2} t + C_{2n} \cos \sqrt{\lambda_n^2 - \gamma^2} t) \phi_n(x) \quad (\text{A11})$$

where $\phi_n(x)$ is given by (7). From the initial conditions (5a) - (5b), it follows that $C_{2n} = 0$ and

$$\sum_{n=1}^{\infty} C_{1n} \sqrt{\lambda_n^2 - \gamma^2} \phi_n(x) = \frac{P}{S} \delta(x - x_0) \quad (\text{A12})$$

Functions $\phi_n(x)$ are orthogonal in the interval $[0, l]$. Multiplying both sides of (A12) by $\phi_k(x)$ and integrating from 0 to l , we get

$$C_{1n} = \frac{P \sin \frac{\lambda_n}{C_1} x_0}{S \sqrt{\lambda_n^2 - \gamma^2} \sin \frac{\lambda_n}{C_1} a \|\phi_n\|^2} \quad (\text{A13})$$

where $\|\phi_n\|^2$ is given by (8). From this, one obtains (6).

Before Equilibrium

A

At Equilibrium

B