CBS-QCI model chemistry gives a value for $\Delta H^\circ_{f,0}$ (-8.83 ± 0.5 kcal/mol) in good agreement with experiment (-9.34 ± 0.01 kcal/mol).

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Evidence for the Stochastic Nature of Base Pair Opening in DNA: A Brownian Dynamics Simulation

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Abstract: The time dependence of the opening of the central thymine base in a B-DNA $(dA)_5\cdot(dT)_5$ oligomer has been simulated by Brownian dynamics using a previously developed opening model where the base is allowed to rotate toward the major groove of the double helix around an axis perpendicular to the base plane and passing roughly through the center of the sugar moiety. From an analysis of the considerable variations of this rotation angle as a function of time, it is possible to estimate the lifetime of the base pair as well as the activation energy for the opening process. These values are found to be in good agreement with the corresponding experimental measurements obtained by hydrogen exchange. This study thus points to the stochastic character of the base pair opening process in double-stranded nucleic acids.

It is now well-acknowledged that the helices formed by double-stranded nucleic acids are not rigid entities, but present rather a high degree of flexibility characterized by numerous thermally excited motions of atoms or groups of atoms. These motions are generally very fast; for example, the motions detected by NMR spectroscopy involving the phosphodiester backbone or the torsional motions localized in the base pairs revealed by fluorescence depolarization studies have relaxation times in the nanosecond time range.[1-6]

In contrast, the so-called opening reaction, leading to a state with disrupted base pairs, is surprisingly slow and occurs on a millisecond time scale.[7] The existence of this open state has been inferred principally from hydrogen-exchange experiments where the exchange occurs between water protons and base imino protons. In the native conformation these protons are completely shielded from the water molecules by being hydrogen bonded within Watson-Crick base pairs, and thus, for the exchange to occur, the formation of a transient open state where these protons become accessible to the solvent, is required.

Despite many experimental hydrogen-exchange studies, which have successfully delineated the base pair opening time range[7,8] and the overall reaction scheme of the proton-exchange process in double-stranded nucleic acids,[9] a detailed description, as well as a basic theoretical understanding, of the opening reaction is still lacking. One aspect of this understanding involves the conformational features of the open state which remain completely unknown (the low stability of the open state compared to the native state precludes any direct measurements by NMR spectroscopy or X-ray crystallography). Most notably, nothing is known about either the molecular opening trajectory or the underlying mechanism by which the energy barrier involved in breaking the base pair can be overcome.

In order to improve our fundamental understanding of the opening reaction in double-stranded nucleic acids, we have recently undertaken a theoretical study on a B-DNA $(dA)_5\cdot(dT)_5$ oligomer by molecular modelling techniques.[10,11] Using an algorithm specifically designed for nucleic acid energy optimization,[12] the energetics of the different opening pathways available for the central AT base pair have been computed. These energies were found to be comparable to the experimental activation energies determined from hydrogen-exchange experiments, thus indicating that this single base pair opening model could account correctly for the energies involved in the real DNA opening process.

In the present work we address more specifically the question of the time dependence of the opening reaction, with the aim of finding, at the molecular level, a mechanism which can explain the surprisingly slow opening kinetics. Therefore, as a first step in this direction, we have simulated the opening kinetics of the central thymine in the $(dA)_5\cdot(dT)_5$ oligonucleotide using Brownian dynamics. The results of this study are found to be in good agreement with experimental values, thus supporting the stochastic character of the opening reaction mechanism and the basic premises of the model we have employed.

Methods

The present Brownian dynamics simulation of opening the central thymine in the $(dA)_5\cdot(dT)_5$ oligonucleotide refers to the molecular opening model described by Ramstein and Lavery.[10] According to this model, based on the results of preliminary investigations by molecular

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(1) Hogan, M. E.; Jardetzsky, O. *Biochemistry* **1980**, *19*, 3460-3468.
(2) Bolton, P. H.; James, T. L. *J. Am. Chem. Soc.* **1980**, *102*, 25-31.
(3) Genest, D.; Wahl, P. *Biochim. Biophys. Acta* **1978**, *521*, 502-509.
(4) Genest, D.; Mirau, P. A.; Kearns, D. R. *Nucl. Acids Res.* **1985**, *13*, 2603-2615.
(5) Thomas, J. C.; Allison, S. A.; Appellof, C. J.; Schurr, J. M. *Biophys. Chem.* **1980**, *12*, 177-188.
(6) Millar, D. P.; Robbins, R. J.; Zewail, A. H. *J. Chem. Phys.* **1982**, *76*, 2080-2086.
(7) Gueron, M.; Kochoyan, M.; Leroy, J. L. *Nature* **1987**, *328*, 89-92.
(8) Englander, S. W.; Kallenbach, N. R. *Q. Rev. Biophys.* **1983**, *16*, 521-655.
(9) Hartmann, B.; Leng, M.; Ramstein, J. *Biochemistry* **1986**, *25*, 3073-3077.
(10) Ramstein, J.; Lavery, R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7231-7235.
(11) Ramstein, J.; Lavery, R. *J. Biomol. Struct. Dynam.* **1990**, *7*, 915-933.
(12) Lavery, R. *DNA Bending and Curvature* In *Structure and Expression*; Olson, W. K., Sarma, M. H., Sarma, R. H., Sundaralingam, M., Eds.; Adenine Press: New York; Vol. 3, pp 191-211.

0002-7863/91/1513-2490$02.50/0

mechanics, the central thymine base is allowed to rotate around an axis perpendicular to the base plane, pointing in the 5'-3' strand direction and passing roughly through the center of the sugar moiety (see Figure 1 in ref 10). The rotation angle θ of the base around this axis, relative to the rest of the oligomer (starting from 0° at the equilibrium position), is termed the opening angle. θ is thus an internal coordinate describing base opening; positive values correspond to a rotation into the major groove and negative values to a rotation into the minor groove.

Thus considering the Brownian dynamics description of this opening model, the function expressing the rotation angle as a function of time obeys the Langevin differential equation:

$$I\mathrm{d}^2\theta/\mathrm{d}t^2 = -\zeta\mathrm{d}\theta/\mathrm{d}t + \Gamma_{eq} + \Gamma_{ran} \quad (1)$$

where I is the moment of inertia of the rotating base with respect to the rotation axis defined above, ζ is the corresponding frictional coefficient, Γ_{eq} is the systematic equilibrium restoring torque, and Γ_{ran} is a random torque.

To solve this equation we have used the method described by Ermark and McCammon.[13] If $\theta(t)$ is the opening angle at time t and $\Gamma_{eq}(\theta)$ the corresponding equilibrium restoring torque, then after an elapsed time interval, Δt, the opening angular increment $\Delta\theta$ of the base is given according to Ermak and McCammon by

$$\Delta\theta = (D/kT)\Gamma_{eq}(\theta)\Delta t + R(\Delta t) \quad (2)$$

where D represents the rotational diffusion constant, k is the Boltzmann constant, and T is the absolute temperature. $R(\Delta t)$ is a random increment of the opening angle with a Gaussian distribution characterized by a mean $\langle R\rangle = 0$ and a variance $\langle R^2\rangle = 2D\Delta t$.

In order for eq 2 to be valid, the selected time increment Δt must satisfy the condition $\Delta t \gg DI/kT$, and, simultaneously, Δt has to be small enough to ensure that all the time functions, with the exception of the random torque, are nearly constant during Δt.

The equilibrium restoring torque is related to the opening potential energy $V(\theta)$ by

$$\Gamma_{eq}(\theta) = -\delta V(\theta)/\delta\theta \quad (3)$$

where $V(\theta)$ was derived by Ramstein and Lavery.[10] In the case of an oligonucleotide with a rigid linear helical axis, this potential can satisfactorily be approximated by $V(\theta) = C\theta$ with $C = 0.4 \times 10^{-15}$ J·deg^{-1} in the range $0° < \theta < 15°$. This 15° upper limit roughly corresponds to the opening angle leading to the breaking of the hydrogen bonds in the base pair[10] and is thus taken as the opening criteria for the central thymine. Consequently, as soon as θ exceeds this value, thymine is considered as open and the simulation is halted.

Finally, we have completed the description of the opening potential energy by two additional features. Firstly, we assume the existence of an infinite potential for negative values of θ, which precludes any excursion of the thymine residue toward the minor groove. Secondly, because the base is not subjected to a systematic torque at its equilibrium position, we have set $C = 0$ for $\theta = 0$ so that only the random torque can remove the base from its resting conformation. While this latter condition results from the definition of an equilibrium state, the assumption of an infinite potential for negative values of θ relies on the existence of steric hindrance between the adenine and thymine base as either base opens toward the minor groove.[11]

The rotational diffusion coefficient D was deduced from the frictional coefficient ζ according to the relation

$$D = kT/\zeta \quad (4)$$

Several authors have measured the value of ζ in aqueous solution, mainly by transient fluorescence anisotropy.[5,14,15] Here we took the value of $\zeta = 2 \times 10^{-29}$ m^2·kg·s^{-1},[15] which according to relation 4 corresponds to $D = 2 \times 10^8$ s^{-1} for $T = 300$ K. For the other temperatures investigated in this work (T = 273, 288, and 318 K), the corresponding diffusion constants were deduced from the above D value, assuming that D is proportional to T/η, η being the coefficient of viscosity of water.

To compute the momentum of inertia I of the opening base, we have neglected the slight roll and tilt angles of the thymine and assumed that during the opening process the base always stays perpendicular to the helix axis. In this way, a value of $I = 2.3 \times 10^{-44}$ kg·m^2 was obtained. This imposes the condition $\Delta t \gg 1.5 \times 10^{-15}$ s for all temperatures. The upper limit of Δt was found empirically by preliminary runs using different values for the product D·Δt which is the relevant parameter of the simulation. These values ranged between 4×10^{-5} and 2×10^{-3}. It was found that up to D·$\Delta t = 6 \times 10^{-4}$ all the simulations gave similar results, whereas, at higher values, deviations appeared. Finally, we took $\Delta t = 2 \times 10^{-12}$ s which satisfies all the relevant conditions for the temperatures considered in this work. The number of steps was 6×10^9.

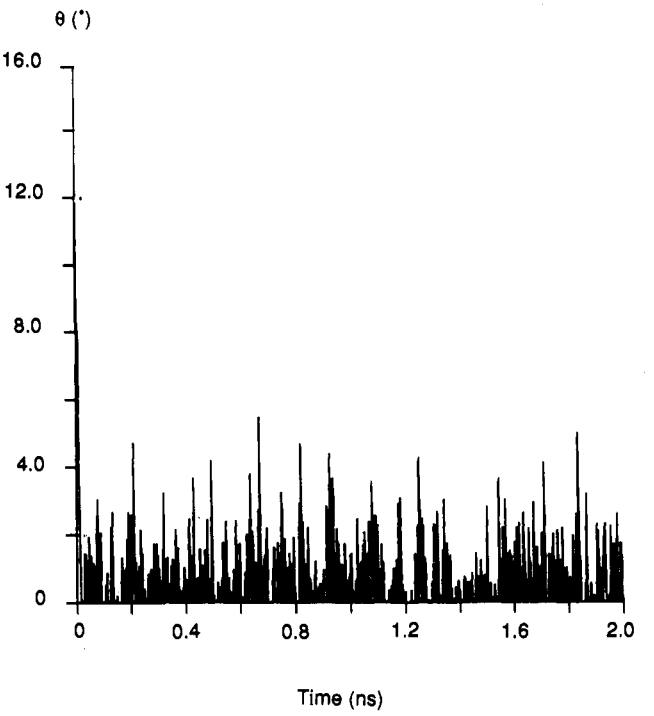

Figure 1. Fluctuation of the thymine opening angle θ as a function of time. Total time range is 2 ns.

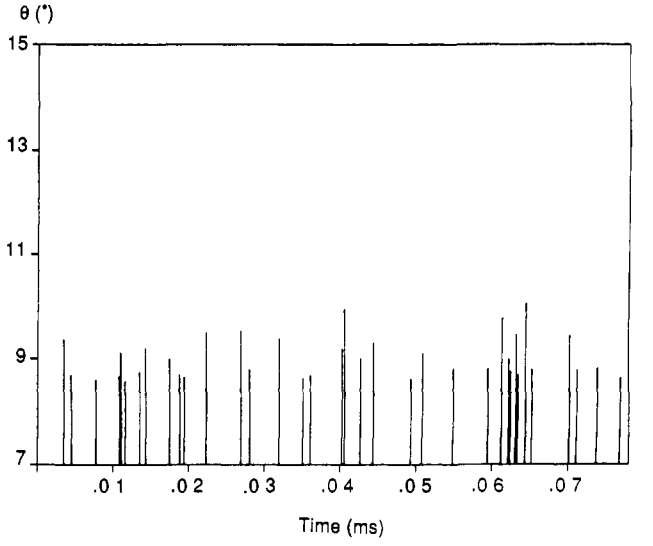

Figure 2. Fluctuation of the thymine opening angle θ (exceeding 8°) over a total time range of 0.08 ms.

It should be remarked that the present opening dynamics is simulated with a potential energy deduced from an overall minimization of the molecular conformational energy where the thymine is constrained in an open position. This corresponds to the so-called adiabatic approximation. In this approximation it is assumed that the relaxation times of all the degrees of freedom involved in the opening process are small when compared to the time range of the opening dynamics and are thus always at equilibrium along the opening pathway. If these requirements are not met, the use of the present potential energies would lead to an underestimation of the potential energies relevant for the dynamics of the opening process. It should be noted that a similar technique has been used by McCammon et al. to study the hinge bending motions of lysozyme.[16]

(13) Ermak, D. L.; McCammon, J. A. *J. Chem. Phys.* **1978**, *69*, 1352–1360.
(14) Barkley, M. D.; Zimm, B. H. *J. Chem. Phys.* **1979**, *70*, 2991–3007.
(15) Genest, D.; Wahl, P.; Erard, M.; Champagne, M.; Daune, M. *Biochimie* **1982**, *64*, 419–427.
(16) McCammon, J. A.; Gelin, B. R.; Karplus, M.; Wolynes, P. G. *Nature* **1976**, *262*, 325–326.

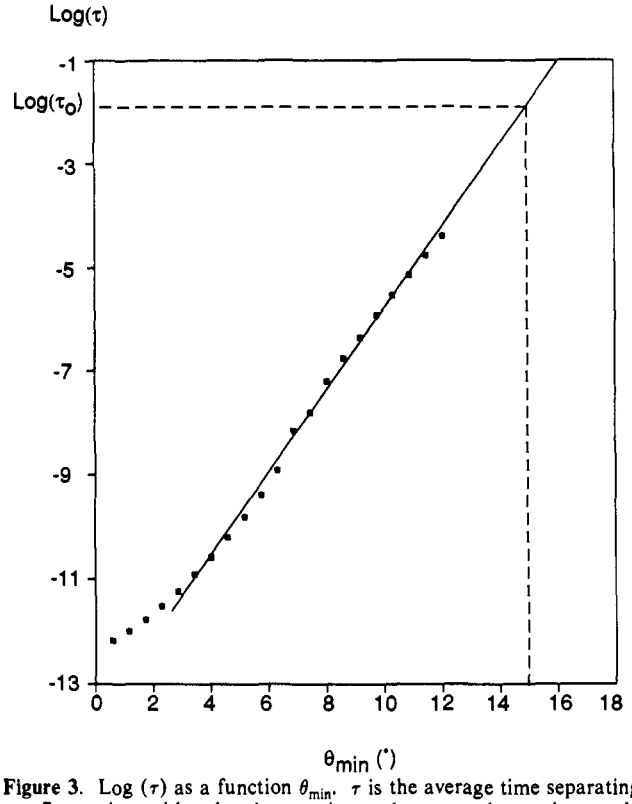

Figure 3. Log (τ) as a function θ_{min}. τ is the average time separating two fluctuations with a thymine rotation angle greater than a given angle θ_{min}. τ_0 is the time between two fluctuations of more than 15°, corresponding to breaking of the base pair hydrogen bonds, and can be considered as the base pair lifetime.

Table I. Base Lifetime τ_0 as a Function of the Absolute Temperature T

T (K)	τ_0 (ms)
318	2.2
300	15
288	180
273	1600

Results

In order to picture the motion of the thymine base obtained by Brownian dynamics simulation over a time range of 2 ns, the whole set of fluctuations of the opening angle θ for a typical run performed at 300 K are presented in Figure 1. These results may be compared with those in Figure 2 corresponding to longer time range of 0.08 ms, where, for clarity, only those fluctuations exceeding 8° are shown. This latter figure indicates clearly that fluctuations of the opening angle can have sizable amplitudes in the millisecond time range.

From these results it is straightforward to deduce the average time τ separating two fluctuations with a rotation angle greater than a given value θ_{min}. The logarithm of the τ values as a function of θ_{min} for T = 300 K are presented in Figure 3. In order to keep computational times within acceptable limits, it was possible to determine τ with good accuracy only up to an angle of 12°. However, owing to the linearity of the curve τ, the value of τ_0 corresponding to the breaking of the hydrogen bonds, which occurs for an opening angle of 15°, could be confidently determined by a simple linear extrapolation. At 300 K we found τ_0 = 15 ms.

Linear curves identical with the one shown in Figure 3 were obtained for temperatures ranging from 273 to 313 K, and the corresponding τ_0 values are summarized in Table I. The activation energy determined from the slope of the Arrhenius plot, shown in Figure 4 is about 20 kcal/mol.

Discussion and Conclusion

This theoretical study allows us to describe the time-dependent behavior of an opening model referring to an oligonucleotide

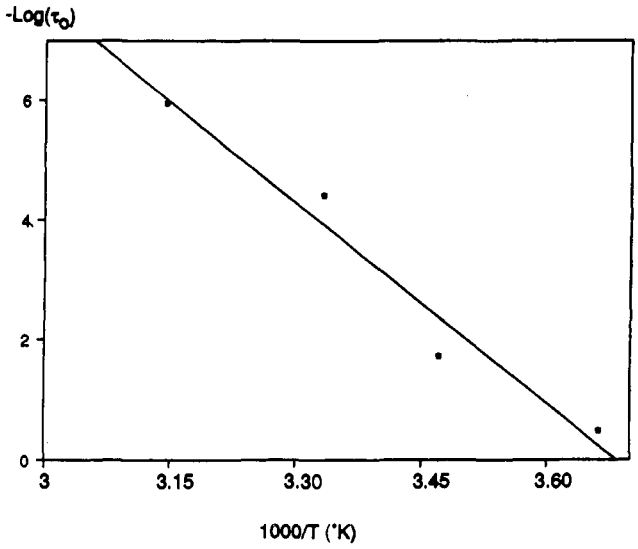

Figure 4. Arrhenius plot of the inverse of the base pair lifetime $1/\tau_0$.

$(dA)_5 \cdot (dT)_5$ where the central thymine can swing out toward the major groove around a rotation axis passing through the center of the sugar ring and oriented perpendicular to the opening base plane. The potential energy along this opening pathway was calculated by internal coordinate molecular mechanics, and it was found that the energetic cost of forcing thymine out of the double helix was comparable to the activation energy obtained from hydrogen-exchange measurements. From a structural point of view it is also important to mention that along this opening trajectory, except for the central base pair, only minor changes of the conformation were detected in the rest of the duplex, and, in particular, the hydrogen bonds in all the other base pairs were maintained.[10]

By introducing the time variable, the present study considerably extends the scope of this model. It has to be remarked, nevertheless, that this simulation of the thymine opening relies on several approximations which are either inherent to the method, for example, the adiabatic approximation, or which were introduced to keep the model at a reasonable level of sophistication with respect to the phenomenological nature of the Brownian dynamics approach. Our model thus ignores any possible dynamic coupling of the thymine opening rotation with other types of conformational fluctuation of the base pair position, such as, for example, motions along the helical axis, or to other deformations in the oligomer. Furthermore, the potential energy we used refers to an oligonucleotide with a rigid helical axis and thus neglects energetic coupling between bending and base opening which has been demonstrated to be important.[10,11] Consequently, this stochastic dynamics simulation should be considered as a simplified representation of the real opening process. However, if the quantitative predictions of this model can be shown to be in good agreement with experimental values, this approach should nevertheless be helpful in delineating the physical processes which underlie base pair opening in double-stranded nucleic acids.

In order to test the simulation against the measured time behavior of the base pair opening, we have estimated the base pair lifetime τ and found τ_0 = 15 ms at 300 K. It should be noted that this lifetime was defined as the average time interval between two successive breakings of the adenine–thymine hydrogen bonds, which has been shown to occur for a rotation angle of about 15°.[10] An experimental base pair lifetime can be obtained from hydrogen-exchange kinetic measurements of the thymine imino proton and was also found to lie in the millisecond time scale (for example, in the case of poly(rA)·poly(rU) at 293 K, the lifetime obtained by NMR measurements is about 5 ms[17]). In addition, to these results, there is also a good agreement between the ac-

(17) Leroy, J. L.; Broseta, D.; Gueron, M. *J. Mol. Biol.* **1985**, *124*, 165–178.

tivation energy of the opening process estimated in this work (20 kcal/mol) and the experimental activation energies of several duplexes containing either A-T or A-U base pairs (in the case of poly(rA)·poly(rU), an activation energy of about 20 kcal/mol was measured by NMR[17]). It thus appears that as far as these two parameters are concerned, the simple thymine rotation opening model, in conjunction with Brownian dynamics, describes quite satisfactorily the real opening process.

Let us now examine what we can learn from this study about the physical mechanism underlying the large scale base motion leading to the transient open state. This process is still poorly understood and two fundamental questions remain largely unanswered. The first question concerns the low opening frequency of the bases. As we have already seen this event occurs in the millisecond time range, whereas other motions involving the base pairs generally exhibit relaxation times in the nanosecond range. Two possible explanations can be put forward: either the motion of the base along the opening trajectory is intrinsically slow or base pair opening is simply a rare event because it involves rotation through a random walk process which is very time consuming owing to the large scale motion necessary to reach the open state.

The second question concerns the mechanism by which a given base pair manages to concentrate enough energy to spontaneously open. In this respect, an interesting model involving the slow migration of delocalized excitations several base pairs long, termed solitons, has been invoked in the case of polymeric nucleic acids.[18] Such an explanation, however, seems improbable since it is now known that oligomeric duplexes have hydrogen-exchange rates similar to those of polynucleotides. It is also important to mention here the existence of a strong energetic coupling between DNA bending and base pair opening which may facilitate the opening of base pairs in biological complexes where the DNA is constrained to a curved pathway.[10,11,19,20] Nevertheless, in the case of oligomeric or polymeric duplexes a more general mechanism must be acting to concentrate the energy in the opening base pair. The simplest mechanism would be the one mentioned previously where the energy of the base pairs is fluctuating statistically and slowly builds up to a critical opening value.

Such a stochastic opening mechanism is, in fact, the essence of the model which we have implicitly dealt with when stimulating the thymine opening rotation by Brownian dynamics. In this type of simulation it is assumed that the rotating base is subjected to three different forces. One is the systematic equilibrium restoring force which is a consequence of the potential energy appearing when the base pair is perturbed. In addition, there are two rapidly fluctuating forces, the first taking into account all the stochastic interactions of the base with its surroundings, for example, the collisions occurring between the bases and solvent molecules, while the second accounts for the damping effect produced by the solvent viscosity while the base is rotating. The simulated opening dynamics thus reflects the interplay of the random forces that govern the opening rotational diffusion and the systematic equilibrium restoring force that controls the conformation of the duplex. The fact that this stochastic model can account reasonably well for the experimental data gives credence to the stochastic nature of the base pair opening process in double-stranded nucleic acids.

In conclusion, this Brownian dynamics study gives a better insight into the physics of the base pair opening mechanism. It suggests that the bases are continuously subjected to considerable and rapidly fluctuating deviations from their equilibrium positions. Over longer periods these fluctuations can add up statistically to produce states where the base pair hydrogen bonds are broken and the base protons are fully accessible to the solvent. According to this viewpoint the slow opening of the base pair measured experimentally is not related to inherently slow motions, but rather reflects the stochastic nature of the base rotation which parallels the buildup of energy necessary to break the base pair. Finally, the present dynamic modelling, in agreement with our previous studies,[10,11] does not favor the existence of a single open state, but instead points to a continuum of open states, each being characterized according to our opening model by a given value of the opening angle θ. Consequently the measured lifetimes must be considered as an average over the lifetimes of the whole set of open states where the thymine imino proton is accessible to the solvent.

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(18) Englander, S. W.; Kallenbach, N. R.; Heeger, A. J.; Krumhamsl, J. A.; Litwin, S. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7222–7226.
(19) Manning, G. S. *Biopolymers* **1983**, *22*, 689–729.
(20) Manning, G. S. *Cell Biophys.* **1985**, *7*, 57–89.