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Kinetics and Dynamics of DNA Hybridization

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CONSPECTUS

DNA hybridization, wherein strands of DNA form duplex or larger hybrids through noncovalent, sequence-specific interactions, is one of the most fundamental processes in biology. Developing a better understanding of the kinetic and dynamic properties of DNA hybridization will thus help in the elucidation of molecular mechanisms involved in numerous biochemical processes. Moreover, because DNA hybridization has been widely adapted in biotechnology, its study is invaluable to the development of a range of commercially important processes. In this Account, we examine recent studies of the kinetics and dynamics of DNA hybridization, including (i) intramolecular collision of random coil, single-stranded DNA (ssDNA), (ii) nucleic acid hairpin folding, and (iii) considerations of DNA hybridization from both a global view and a detailed base-by-base view. We also examine the spontaneous single-base-pair flipping in duplex DNA because of its importance to both DNA hybridization and repair.

Intramolecular collision of random coil ssDNA, with chemical relaxation times ranging from hundreds of nanoseconds to a few microseconds, is investigated both theoretically and experimentally. The first passage time theory of Szabo, Schulten, and Schulten, which determines the average reaction time of the intrachain collision, was tested. Although it was found to provide an acceptable approximation, a more sophisticated theoretical treatment is desirable.

Nucleic acid hairpin folding has been extensively investigated as an important model system of DNA hybridization. The relaxation time of hairpin folding and unfolding strongly depends on the stem length, and it may range from hundreds of microseconds to hundreds of milliseconds. The traditional two-state model has been revised to a multistate model as a result of new experimental observations and theoretical study, and partially folded intermediate states have been introduced to the folding energy landscape. On the other hand, new techniques are needed to provide more accurate and detailed information on the dynamics of DNA hairpin folding in the time domain of sub-milliseconds to tens of milliseconds.

From a global view, the hybridization of unstructured ssDNA goes through an entropy-controlled nucleation step, whereas the hybridization of ssDNA with a hairpin structure must overcome an extra, enthalpy-controlled energy barrier to eliminate the hairpin. From a detailed base-by-base view, however, there exist many intermediate states. The average single-base-pair hybridization and dehybridization rates in a duplex DNA formation have been determined to be on the order of a millisecond. Meanwhile, accurate information on the early stages of hybridization, such as the dynamics of nucleation, is still lacking.

The investigation of spontaneous flipping of a single base in a mismatched base pair in a duplex DNA, although very important, has only recently been initiated because of the earlier lack of suitable probing tools. In sum, the study of DNA hybridization offers a rich range of research opportunities; recent progress is highlighting areas that are ripe for more detailed investigation.

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Introduction

DNA hybridization/dehybridization is a fundamental process found in biology.[1] For example, DNA modifications, such as DNA methylation/demethylation,[2] deamination,[3] and the recently discovered hydroxylation,[4] all require base-flipping as a fundamental step. Many DNA-based techniques, including molecular architecture with DNA as the building blocks,[5] DNA-mediated organic synthesis,[6] DNA-guided drug delivery,[7] and DNA-based biosensors,[8,9] have been widely applied in different fields. Understanding the kinetics and dynamics of DNA hybridization is important in unraveling the molecular mechanism of these processes and improving the associated biotechnologies. In recent years, knowledge about the kinetics and the molecular mechanism of DNA hybridization has accumulated; however, some interesting and important questions remain unanswered, and new questions emerge as we advance our knowledge on DNA hybridization.

As the model system of DNA hybridization/dehybridization, oligonucleotides have attracted significant attention in recent studies, including the intramolecular collision of single-stranded DNA (ssDNA), the folding and unfolding of hairpin DNA, and the formation and dissociation of double-stranded DNA (dsDNA). The intramolecular collision of a random coil ssDNA represents a basic DNA motion which lays the foundation for all processes that involve DNA.[10−15] The folding of hairpin DNA represents an elegant model for studying the kinetics of DNA hybridization. Such a system has provided valuable information about the kinetics of DNA hybridization; however, there remain unresolved issues.[15−19] The study of kinetics and dynamics associated with the formation and dissociation of dsDNA is significant, yet difficult to characterize, because of the challenges with studying base-by-base hybridization/dehybridization.[20] Moreover, the kinetic behavior is quite different between the reaction of two random coil ssDNAs and two ssDNAs with at least one of the strands containing secondary structure elements.[21] An interesting topic, which has not been fully explored, is the dynamics of spontaneous flipping of a single base from matched or mismatched base pairs in an otherwise perfectly complementary dsDNA.

In this Account, recent progress on the kinetics and dynamics of hybridization/dehybridization of oligonucleotides will be presented, and particular questions that remain unresolved will also be addressed. Due to the journal page limitations, the selected material discussed in this Account primarily represents research interests of the authors instead of a complete review on the subject of DNA hybridization/dehybridization, and we apologize for not covering all topics and for not citing all the relevant literature.

Intramolecular Collision of Random coil ssDNA

The first passage time theory of Szabo, Schulten, and Schulten (SSS)[22] is a simple statistical theory that describes the average reaction time for diffusion controlled processes in a force field governed by a Smoluchowski-type diffusion equation. In an idealized three-dimensional polymer chain with a Gaussian end-to-end distribution, the SSS rate of the end-to-end contact formation is approximately given by

$$k_{D+} = \frac{4\pi Da}{\left(2\pi\langle r^2\rangle/3\right)^{3/2}} \quad (1)$$

where $\langle r^2\rangle$ is the equilibrium mean-squared end-to-end distance, a is the contact distance which is much smaller when compared to $\langle r^2\rangle$ for most intrachain collisions of random coil ssDNA, and D is the relative diffusion coefficient between the ends of the chain. By combining with the equilibrium constant of the intrachain collision, given by[23] $K = (4\pi a^3/3)/(2\pi\langle r^2\rangle/3)^{2/3}$, the rate of the end-to-end contact dissociation is derived as[24]

$$k_{D-} = 3D/a^2 \quad (2)$$

which indicates that the rate of end-to-end contact dissociation is length-independent, whereas contact formation is held by a scaling factor of $n^{-3/2}$ (where n is the number of segments in a chain).

The rate of intramolecular collisions in a biopolymer predicted by the SSS theory has been tested experimentally by Lapidus et al. using polypeptides[24] and by us and others using oligonucleotides.[10,11] To give an example, the intrachain collision time has been measured as 240 ns for 5′-TTTTTTTGG-3′,[25] and shorter for polypeptides of the same length. The shorter value for polypeptides reflects the lower mass in a unit segment. In the case of polypeptides, Lapidus et al. have found that the scaling law using the SSS theory is followed quite well.[24] Qu et al. selected a series of duplex DNAs with an overhang structure positioned at one end to investigate the dynamics of intrachain collision of random coil ssDNA using photoinduced electron transfer (PET) based fluorescence correlation spectroscopy (FCS) measurements.[10] To accurately extract the forward and reverse collision rates, the traditional two-state model of a bright state and a totally nonfluorescent dark state was revised to a three-state model (Figure 1). In this revised

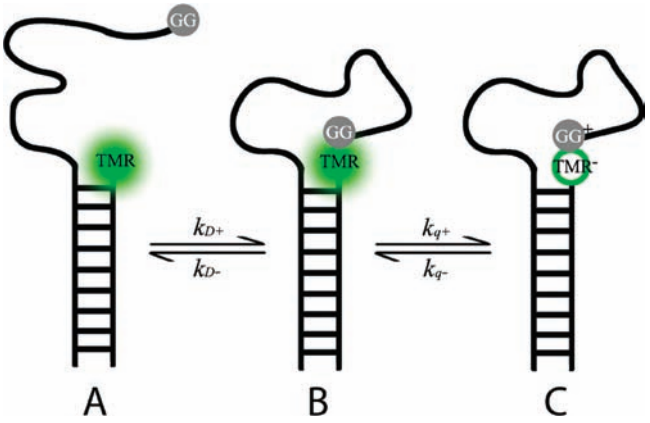

FIGURE 1. Three-state model for PET-FCS analysis. The first step involves the TMR-to-GG contact formation (A→B), and the second step is PET (B→C), which is much faster than the first step and results in fluorescence quenching.

model, an intramolecular collision leads to the formation of a collision complex between the TMR dye and a guanosine base. Laser excitation of the complex results in either fluorescence emission which gives rise to the fluorescent state (state B in Figure 1) or PET which gives rise to the nonfluorescent charge separation state (state C in Figure 1). Since the rates of fluorescence emission (k_F), photoinduced charge separation (k_{q+}), and charge recombination (k_{q-}) are much faster than the rates of forward and reverse diffusion (k_{D+} and k_{D-}), the concentration ratio of states B to C in the collision complex remains constant such that the collision complex can be viewed as a dark state with a nonzero brightness.[26,27] By varying the number of bases between the fluorescent tag (TMR) and the quencher (guanine), the length-dependence of both the forward and reverse intrachain collision rate constants (k_{D+} and k_{D-}) is obtained. The results indicate that k_{D+} is held by the $n^{-3/2}$ scaling law according to the SSS theory, whereas k_{D-} is approximately length-independent, and this observation is in agreement with the theoretical prediction.[7] In contrast, Kim et al. have also investigated the rate of end-to-end contact dissociation using random coil ssDNA carrying less than 10 bases using an FCS measurement approach.[15] The oligonucleotides used in this study were too short to follow the Gaussian chain model, and their obtained k_{D-} is quite sensitive to the end-to-end distance. Uzawa et al. studied the intrachain collision of longer ssDNAs, and they found that k_{D+} scales with $n^{-3.49}$ when the separation is >18 bases.[11] The determined stronger trend than theoretically predicted was attributed to electrostatic effects, because DNA is a highly charged polymer.[11] Clearly, our knowledge on the dynamics of intrachain collisions of ssDNA remains unresolved. More substantial experimental data and sophisticated theoretical concepts beyond the SSS theory and Gaussian chain model are required to reconcile the current discrepancies. Such efforts should provide better insights into this type of motion.

Dynamics of Hairpin Folding

From Two-State Model to Multistate Model. In earlier studies, hairpin folding was described as a predominantly two-state reaction, in which the DNA hairpin transfers between a folded base-paired state and an unfolded random-coil state along an assumed folding free energy landscape. The probed chemical relaxation time, which is attributed to the transformation between the folded and unfolded states, was found to range from several microseconds to hundreds of microseconds depending on the loop length and other related parameters.[16,17]

This two-state model has been challenged by development of techniques and new analysis models. By combining fluorescence autocorrelation/two-beam cross-correlation measurements and photon counting histogram analysis, Van Orden and Jung[18] indicated that the 62.7 ± 5.1 μs relaxation time observed was not associated with the hairpin folding/unfolding process as earlier studies had suggested. NaCl is known to stabilize the folded state,[28] and therefore, the equilibrium constant of the transformation between the folded and unfolded states of a DNA hairpin would change as the NaCl concentration is varied. However, the measured equilibrium constant, corresponding to the 63 μs chemical relaxation process, remained essentially constant when the concentration of NaCl was varied from 5 to 500 mM. This observation suggested that the 63 μs chemical relaxation probed by Van Orden's group, as well as by earlier studies, does not correspond to the transformation between the folded and unfolded states of the DNA hairpin. Therefore, they propose a "third" longer-lived intermediate state represented by the 63 μs relaxation time while the true relaxation time for the folding and unfolding process may fall in the milliseconds range or even longer.

Recent studies indicate a significantly more complicated landscape for the hairpin folding (Figure 2). Using molecular dynamics simulations, Pande's group[29,30] investigated the unfolding and refolding process of a small RNA hairpin (5′-GGGCGCAAGCCU-3′). The results of this study revealed the existence of competing unfolding/folding pathways, and intermediate states including both partially zipped and partially compacted states. However, this multistate picture for hairpin folding has only been examined by a few

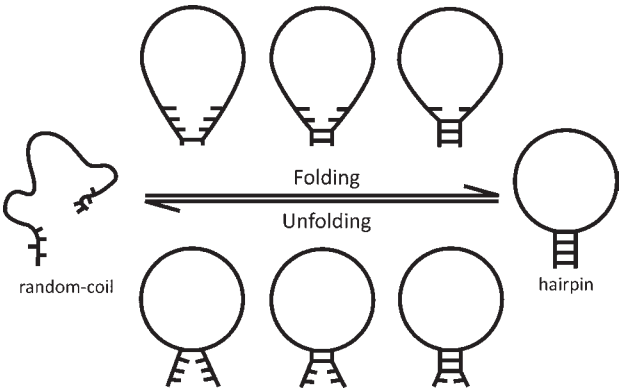

FIGURE 2. Multistate model for hairpin folding.

experimental studies, and more work is required to fill in the missing details.

The Dynamics of the Folding of Short-Stem Hairpins. Applying the PET based FCS approach, Sauer's group investigated the dynamic properties of the folding behavior of DNA hairpin molecules with a short stem structure (one or two G−C base pairs which can hybridize to form a short stem).[15] Through the analysis of the FCS data using the stretched exponential model, this study found that the FCS curve of an unstructured ssDNA (random coil DNA without a base-paired stem) contained only one kinetic component which is attributed to the intrachain collision. In contrast, a one or two base-paired DNA hairpin molecule contained more complicated kinetic components. The observed kinetic complexity in the DNA hairpin molecules indicates that not only the intrachain collision but also the base-pairing process, relaxing in a time range near that of the intrachain collision, was present in the kinetic information obtained by the FCS measurements.

Yin et al. also carried out PET based FCS experiments using oligonucleotides similar to the ones used in Sauer's study.[25] To extract accurate information about the kinetics, Yin et al. applied a model-free maximum entropy method (MEM)[31,32] instead of the physically obscure stretched exponential model for FCS data analysis, and was able to separate two processes during the folding of a short-stem hairpin (Figure 3). Guided by the MEM analysis presented in Figure 3, two major kinetic components were obtained from the FCS curves. The faster process with a ~370 ns chemical relaxation time was attributed to the intrachain collision as discussed in the section on intramolecular collision of random coil ssDNA. The slower process with a 2−3 μs relaxation time was attributed to the base-pairing process, because its pre-exponential amplitude in the FCS curve progressively increased when the number of G−C base pairs increased.

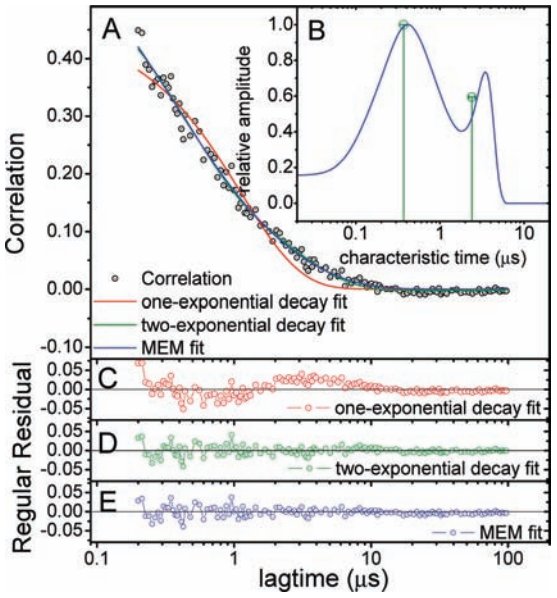

FIGURE 3. (A) Experimental FCS curve (open dots) with the corresponding fitted curves using a one-exponential decay (red), two-exponential decay (green), and MEM (blue) on the oligonucleotide 5′-CCTTTTTGG-3′. (B) Chemical relaxation time distribution from the MEM fit (blue, solid line) and the results through the double exponential fit (green circle with drop lines). (C−E) Residuals of the fits through the one-exponential decay, two-exponential decay, and MEM, respectively.

The loop formation with base-pairing was slower than the nonspecific intrachain collision because base-pair formation requires the correct orientation which will drastically reduce the reaction probability.

The Dynamics of Folding of Long-Stem Hairpins. As the number of paired bases in a DNA hairpin molecule increases, the reaction of the base-pairing stem formation, which occurs on a much wider chemical relaxation time scale, exhibits much slower folding rates than a DNA hairpin with a one or two base-paired stem. Correspondingly, the intermediate states live a relatively longer lifetime.

For DNA hairpins with a four or five base-paired stem, the formation of the stem is predicted to occur on a time scale ranging from sub-milliseconds to tens of milliseconds,[18] which indicates that the conventional FCS measurement is not suitable because it takes less than 1 ms for most biomolecules to diffuse through the FCS probe volume. Van Orden's group applied continuous-flow FCS techniques combined with dual-beam fluorescence cross-correlation spectroscopy (FCCS)[33,34] to investigate the stem formation process of a DNA hairpin with a four base-paired stem. Compared to the cross-correlation function of unstructured ssDNA, an excess contribution, single-exponentially decaying in a time range of 100 μs to 10 ms, was observed and attributed to the chemical relaxation of the formation of the

DNA hairpin stem. Additionally, a 84.2 ± 6.8 μs process was also revealed through fluorescence autocorrelation measurements and represented the lifetime of the intermediate state.

For DNA hairpins with longer stems, Schultz's group investigated the lifetime of both the unfolded random-coil state and the completely folded state by applying an immobilized single-molecule fluorescence resonance energy transfer (smFRET) approach.[19] For DNA hairpins with a stem size of seven or nine base pairs, the respective closed-state lifetimes were reported to be 45 ± 2 and 103 ± 6 ms, whereas the respective open-state lifetimes were 133 ± 6 and 142 ± 22 ms. However, because only a few events were measured (less than 100 smFRET events for both hairpins) and because of limitations with the time resolution of the immobilized smFRET method, the results of this study failed to observe any intermediate states (assuming a multistate model).

Remaining Important Questions. It appears that we already know quite a lot about the hairpin folding and unfolding process. However, detailed dynamics of the hairpin folding process remains poorly resolved. The accuracy of the rates of the folding and unfolding processes remains to be clarified. FCS is a good technique at measuring relaxation times ranging from tens of nanoseconds to hundreds of microseconds, whereas immobilized single molecule detection is suited for detecting processes with lifetimes longer than tens of milliseconds. Although both techniques indicate that the lifetimes of folding/unfolding are around tens of milliseconds for hairpins with 4−5 base-paired stems, there remains a time scale gap in the accurate determination of the reaction time between these two techniques. To unambiguously answer even a simple question of how fast the rates of hairpin folding/unfolding are, new techniques that are good at probing the reaction times between hundreds of microseconds and tens of milliseconds are required. Such methods will also allow us to probe more detailed dynamics within that time range. Microfluidic mixing is a good candidate, because the shortest mixing time is reported to reach the microsecond range.[35] Other techniques are expected to be developed that will access this important time window.

Kinetics and Dynamics of DNA Hybridization

Global Description of Kinetics of DNA Hybridization. The kinetic properties of DNA hybridization have been investigated by many researchers,[36,37] and a three-state model describing the formation of duplex DNA has been proposed. A nucleation step involves bringing two random-coil ssDNAs together to form an intermediate state, in which a few base pairs have formed, and this intermediate proceeds further to form the fully hybridized dsDNA molecule.

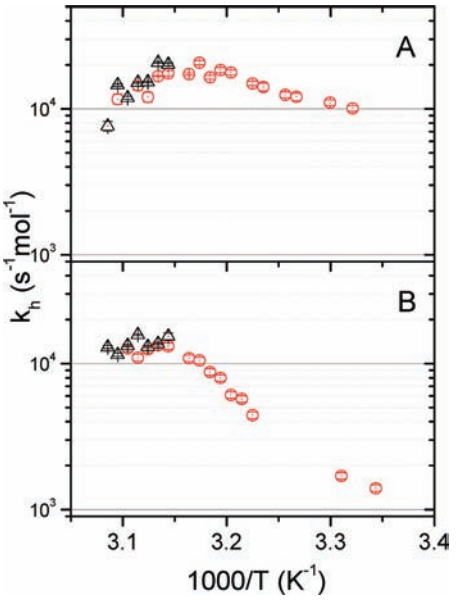

FIGURE 4. Arrhenius plots of the hybridization rate constants of a random-coil ssDNA with (A) a random-coil ssDNA and (B) a hairpin ssDNA with a 6 base-pair stem. The rates are determined from two different methods (shown in red circles and black triangles, respectively, in both the top and bottom panels).[21]

To gain a global insight into the mechanism of DNA hybridization/dehybridization, Chen et al. have investigated the kinetic properties of DNA duplex formation on the ensemble level.[21] Both unstructured ssDNA and ssDNA carrying a hairpin structure were chosen to react with their complementary ssDNA to investigate the mechanism of duplex DNA formation and dissociation. Different methods were used to investigate the kinetic properties of DNA duplex formation, through which a series of comparable hybridization/dehybridization rate constants over a wide temperature range were obtained. The results obtained by Chen et al.[21] are shown in Figure 4. The figure clearly shows that at the high temperature the apparent activation energies are negative and both the random coil and structured ssDNAs behave similarly, whereas at the low temperature the apparent activation energies are positive and the structured ssDNA has a much higher reaction barrier than the random coil ssDNA.

According to standard chemical kinetic theory, a negative apparent activation energy is an indication of the existence of a metastable intermediate in the rate-limiting step, and a barrier between this intermediate and the product. As shown in Figure 5A, at high temperatures, the negative apparent activation energy represents a nucleation barrier which acts

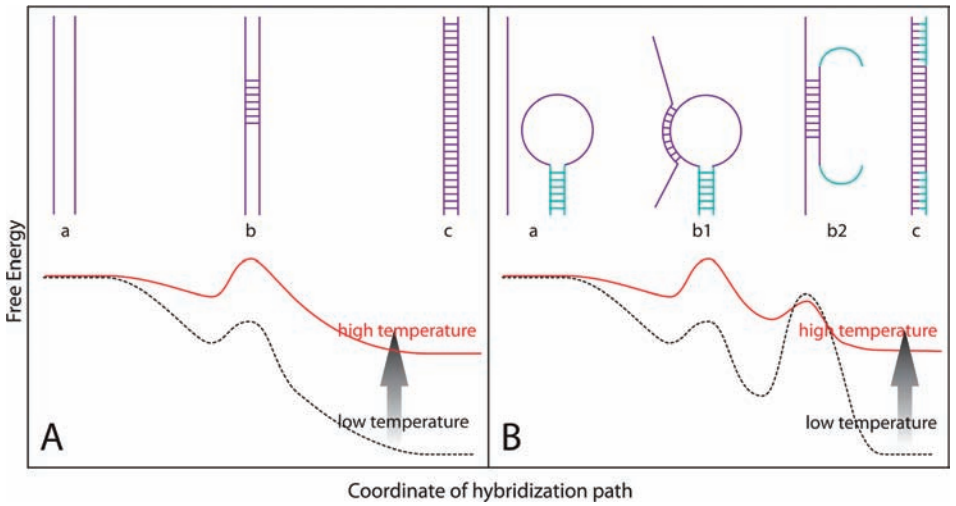

FIGURE 5. Schematic view of the profiles of free energy at high temperatures (red, solid) and at low temperatures (black, dashed) as a function of an effective reaction coordinate for the hybridization of (A) random-coil DNA and (B) structured DNA.

as the rate-limiting step. As the temperature decreases, the rate-limiting step switches to the diffusion controlled reaction[21] (Figure 5A), which agrees with earlier studies.[36,37]

Compared to the hybridization of unstructured ssDNA, the hybridization of structured ssDNA has to overcome one more energy barrier which represents the destruction of the hairpin stem[21] (Figure 5B). According to transition state theory, the kinetic rate constant is given by

$$k = k_0 \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right) \quad (3)$$

where $\Delta G^{\ddagger}$ is the activation free energy. Nucleation is an entropy-decreasing process ($\Delta S^{\ddagger} < 0$), which makes a positive contribution to the free energy barrier through $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$, where $\Delta H^{\ddagger}$ is the activation enthalpy, so that the free energy barrier of nucleation would increase as the temperature increases. Conversely, the melting of the hairpin structure is an entropy-increasing process ($\Delta S^{\ddagger} > 0$), which contributes negatively to the free energy barrier describing the destruction of the hairpin. Consequently, the free energy barrier describing the destruction of the hairpin would decrease as the temperature increases. Therefore, one would expect to see a switch in the rate limiting step from the destruction of the hairpin at a low temperature to the nucleation at higher temperatures, as illustrated in Figure 5B. At the low temperature, the rate limiting step of a random coil ssDNA is the diffusion, which would create a small but positive diffusion barrier. As the temperature increases, the nucleation becomes the rate limiting step as observed for the structured ssDNA at high temperature. We would therefore expect that the rate limiting step is switched to the nucleation (Figure 5A). The free energy profile presented in Figure 5 explains the experimentally observed variation in the apparent activation energy presented in Figure 4.

As for the dissociation of dsDNA, Chen et al. have observed similar kinetics between structured and random coil ssDNA.[21] This is understandable because the character of the hairpin structure would not appear at the early stage of dissociation. This is also compatible with the global view of the free energy landscape of hybridization/dehybridization for random-coil ssDNA and ssDNA with a secondary structure (Figure 5).

Although Figure 5 has provided a global picture about DNA hybridization and dissociation, the hybridization and dissociation processes clearly should proceed in a base-by-base manner, which would create many intermediate states in addition to what is presented in Figure 5.

Base-by-Base Dynamics in DNA Hybridization/Dehybridization. Based on extensively investigated kinetic and thermodynamic properties of short oligonucleotides and long DNA molecules, new techniques including FCS,[20] NMR,[38] and laser temperature jump approaches[39,40] have been applied to further understand the dynamic properties of DNA hybridization, and the base-by-base manner was illustrated experimentally.

From a thermodynamic viewpoint, direct evidence for the base-by-base behavior in hybridization/dehybridization of DNA is the discrepancy between melting profiles of the same duplex DNA labeled with different FRET pairs of different characteristic distances (Figure 6A, TMR-Cy5 and TMR-Dabcyl).[20] If the DNA

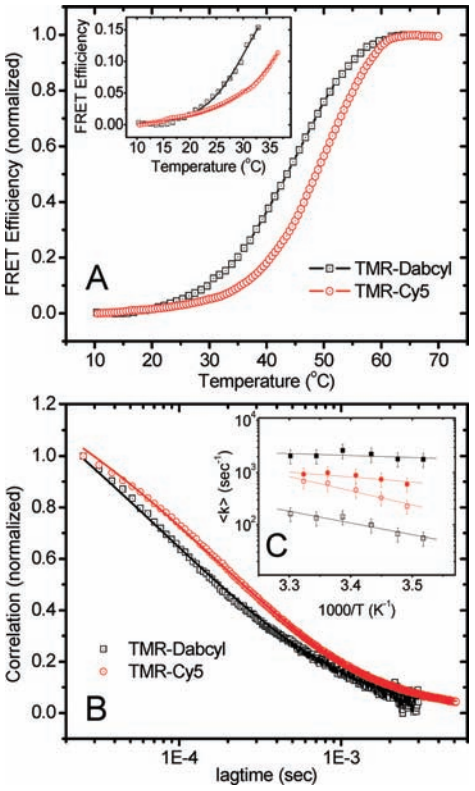

FIGURE 6. (A) Experimental melting curves obtained by FRET measurement on the same dsDNA with TMR-Cy5 labeling (red, circle) and TMR-Dabcyl labeling (black, square). Shown in the inset is part of (A) at a low temperature range together with the fitting (solid line) by the zipper model. (B) Experimental FCS data and fittings (solid line) using the SEZ model, respectively, for the TMR-Dabcyl system at 26.4 °C (black, square) and for the TMR-Cy5 system at 27.3 °C (red, circle). (C) Arrhenius plots of the averaged single base-pair rate constants from the SEZ model. Black squares are for the TMR-Dabcyl system and red circles are for the TMR-Cy5 system. Hollow points are for $\langle k_D \rangle$ and solid points are for $\langle k_H \rangle$.

hybridization would be a true two-state process, identical melting curves for different labeling schemes would be observed. In contrast to the two-state model, the observed significant discrepancies exhibited in the melting profiles indicate the presence of partially dehybridized intermediates caused by the base-by-base dissociation (the zipper model) (Figure 7). Here, FRET pairs with different characteristic distances would present different FRET efficiencies for the same partially dehybridized intermediates, and experimental data were found to fit well to the base-by-base model (the inset in Figure 6A).[20]

Based on the base-by-base zipper model in hybridization/dehybridization (Figure 7), Chen et al. have proposed the stretched exponential zipper (SEZ) model to investigate the dynamics of the terminal fluctuation in a dsDNA using FCS.[20] Because the system can populate the configurations in the vicinity of the lowest energy path statistically under a finite temperature and results in statistically distributed rate constants, the experimental FCS data are analyzed following the master equation of reaction given by

$$\begin{cases} \dfrac{dC_i}{dt} = k_{D,\mathrm{eff}}(t)C_{i-1} + k_{H,\mathrm{eff}}(t)C_{i+1} - \left(k_{D,\mathrm{eff}}(t) + k_{H,\mathrm{eff}}(t)\right)C_i \\ K = \dfrac{k_{D,\mathrm{eff}}(t)}{k_{H,\mathrm{eff}}(t)} \\ \tau = \dfrac{1}{k_{\mathrm{eff}}(t)} = \dfrac{1}{k_{D,\mathrm{eff}}(t) + k_{H,\mathrm{eff}}(t)} \end{cases} \quad (4)$$

where C_i is the concentration of the species with i dissociated bases from the end of a duplex DNA, K is the equilibrium constant of a single base-pair dissociation over formation, and τ is the characteristic chemical relaxation time. It is assumed that the dehybridization starts from the dissociation of the end base pair and propagates in a base-by-base manner with the effective time-dependent rate of single base-pair hybridization ($k_{H,\mathrm{eff}}(t)$) and dehybridization ($k_{D,\mathrm{eff}}(t)$). This time-dependent rate is taken to be identical for all base pairs located at different positions in the DNA molecule (Figure 7).

Combining the equilibrium constant K obtained from the thermodynamic study of the dsDNA melting (Figure 6A) with the dynamic fitting of the FCS data (Figure 6B), the average rate constants of single base-pair hybridization/dehybridization at different temperatures and the corresponding activation energy and pre-exponential factor are derived (Figure 6C). The successful interpretation of the experimental data by the zipper model and its derivation of the average single base-pair kinetic parameters have greatly enriched our knowledge on the molecular mechanism of DNA hybridization/dehybridization. However, the SEZ model assumes that all the base-by-base reactions start from the first base. Consequently, this model can only be applied to situations where the temperature is far below the melting temperature. In other words, the SEZ model is suitable for probing and describing the detail of the energy profile in the vicinity of product side in Figure 5. The dynamic information for the detail of the energy profile in the vicinity of the reactant side would be much richer and more important; however, at present this is not available.

Single Base-Pair Flipping in dsDNA

What if there is a mismatched base pair in a dsDNA, or what is the flipping rate of a single base pair in a perfectly complementary dsDNA (Figure 8)? Answers to these questions are not only interesting from an academic point of view but also important in applications. DNA base modification,

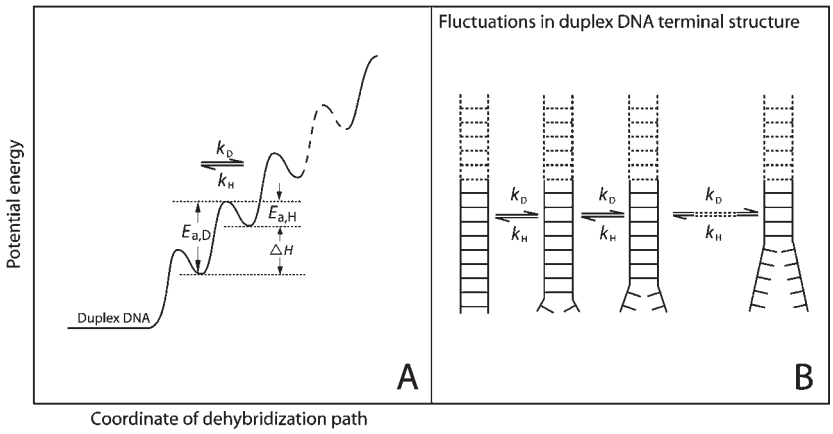

FIGURE 7. (A) Sketch of the energy profile along the dehybridization path at a dsDNA terminal. Thermodynamic and dynamic parameters for a one base-pair reaction are illustrated. (B) Scheme illustrating fluctuations in a duplex DNA terminal structure.

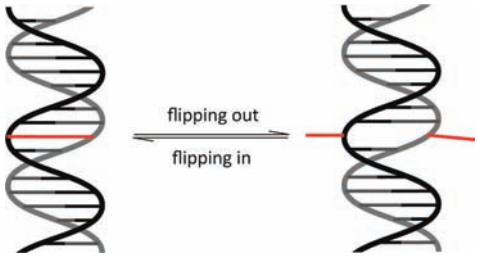

FIGURE 8. Simple view of a single base-pair flipping event in duplex DNA. Either both bases could flip out or only one of the bases flips out.

damage, and its repair, such as DNA methylation,[2] deamination,[3] and hydroxylation,[4] represent some of the most serious issues in biological sciences and technology, and elucidation of their mechanisms is vital.[2,41,42] Although those repair processes occur extra-helically with the damaged or modified base flipped out of the duplex DNA, the mechanism of how DNA repair or modification proteins recognize and locate these bases at these positions before action remains unresolved.[43] Because the majority of cytotoxic/mutagenic DNA base damage or base mismatch would result in weakened base pairing or non-Watson–Crick geometry in duplex DNA, it was possible for those damaged or mismatched bases to spontaneously flip out due to an unstable local environment, so that those "outside bases" could serve as recognition sites for DNA repair proteins to act on. The question arises on how fast does a base from either a matched or mismatched base pair flip out of the dsDNA?

To tackle such a problem, very sensitive and localized probes which do not seriously disturb the process are necessary. The dynamics and mechanism of single base-pair flipping are new research topics, and only a handful of results are available. NMR seems to be a suitable technique to study such events. Using NMR, Dallmann et al. have investigated the changes of both structure and dynamics in a duplex DNA when an adenine has been altered to a structural isomer 2-aminopurine (2AP), and reported the lifetime of each base pair.[44] However, as a probe for proton exchange rates, NMR can only provide information on base flipping indirectly, and many other possibilities have to be ruled out before the base-pair flipping rates are considered acceptable. New techniques that directly measure the conformational change induced by single base-pair flipping are highly desirable.

Conclusions and Implications

Understanding the primary mechanism of oligonucleotide hybridization could help in elucidating the molecular basis of all DNA/RNA related processes both in molecular biology and cell biology. Significant effort has been made to define the kinetics and dynamics of oligonucleotide motion, including intrachain collision, nucleic acid hairpin folding, dsDNA hybridization/dehybridization, and single base-pair flipping. The SSS theory and the Gaussian chain model appear to represent good zero order approximations of the dynamics of intrachain collision, but more elaborate theories are required to describe the intramolecular motion more precisely. A full understanding of the dynamics of the folding and unfolding of hairpin DNA is still missing, and additional efforts using both experimental and theoretical approaches are required. A global view on the free energy landscape of DNA hybridization has been established, but more accurate detail is necessary. Finally, we know very little about the dynamics of single base-pair flipping, and this is an area of research that should expand as the techniques become available.

Many biologists may feel that our endeavor to understand the dynamics and mechanistic details of biologically relevant chemical processes, such as the reactions discussed

in this Account, is irrelevant to their efforts to understand the biological world. Moreover, some chemists may feel that our efforts to gain a deeper and finer description on a biochemical reaction are redundant, for the understanding of the biochemical process based on current data appears already sufficient. The current era reminds us of the days when the field of chemical physics was just emerging. At that time, many chemical researchers would believe that the knowledge of chemical reactions was quite rich, and efforts to describe a reaction from a physicist's viewpoint were redundant. Nowadays, no one would doubt the tremendous benefit that chemistry gained from the development of chemical physics in the last 80 years. It is our belief that biology will finally share the dividends from the emerging way of thinking in chemistry and research on biological entities. The emergence of chemical biology should make a significant contribution to the development of biology in the coming years.

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FOOTNOTES

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