

Misfolded Loops Decrease the Effective Rate of DNA Hairpin Formation

In a recent Letter [1], Goddard *et al.* claim that the dynamics of short single-stranded DNA (ssDNA) are inconsistent with that of a flexible polymer. They find that, for hairpins with poly(A) loops, the apparent activation energies ΔH_c for the closing times τ_c increase with increasing loop length L . This result runs counter to what is expected for a flexible polymer for which the energetic cost of forming a loop should decrease as $\sim 1/L$. Furthermore, their ΔH_c values are positive (≥ 5 kcal/mol), in apparent contradiction with negative ΔH_c (~ -11 kcal/mol) obtained from kinetics measurements following temperature jumps [2]. In this Comment, we show that apparent activation energies cannot be interpreted as the energetic cost of forming loops and present a configurational diffusion model that reconciles the change in sign of ΔH_c .

Prior to the nucleation step in hairpin formation that leads to subsequent “zipping,” the ssDNA can be transiently trapped in conformations with mismatched stems and “non-native” loops [2]. This trapping leads to a decrease in the effective diffusion coefficient in the preexponential for the closing step, $D \sim D_0 \exp[-(\Delta E/k_B T)^2]$, where ΔE is the “roughness” in the free energy from transient trapping [3]. Hence, ΔH_c in an Arrhenius plot has two contributions: one from the enthalpy of the transition state relative to the random coil and another from the temperature dependence of the diffusion coefficient. The configurational diffusion model leads to deviations from a simple Arrhenius dependence for the closing times; τ_c is expected to be small below the melting temperature T_m because of trapping in misfolded conformations and again small at high temperatures because of the uphill climb in free energy [2]. Deviations from an Arrhenius dependence are, in fact, observed for τ_c in the measurements of Goddard *et al.* Their ΔH_c values are determined primarily from data at $T \lesssim T_m$ where the dynamics are more sensitive to configurational diffusion among the traps and not to the enthalpy of the transition state. In the temperature-jump measurements ($T \sim T_m$) the roughness ΔE is expected to decrease, yielding ΔH_c values closer to the enthalpy of the transition state [2].

We have calculated τ_c by solving the diffusion equation on free energy profiles obtained from an equilibrium “zip-

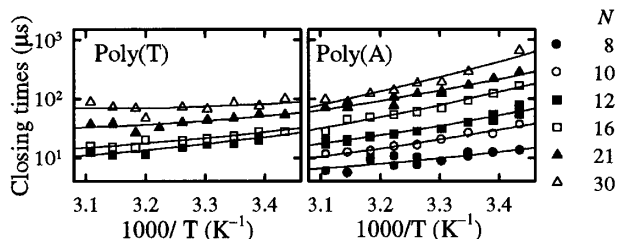


FIG. 1. Closing times versus temperature. Data are from Ref. [1] for hairpins with N bases in the loop.

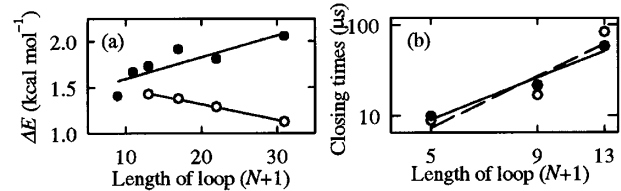


FIG. 2. (a) The roughness in the energy landscape versus the length of the loop for the hairpin of Fig. 1. (b) Closing times for another hairpin versus the length of the loop.

per” model and with D_0 and ΔE as parameters [2]. The calculated values reproduce the measured τ_c including the slight deviations from an Arrhenius dependence (Fig. 1). The anomalous loop-size dependence of ΔH_c for poly(A) loops in this model arises from an increase in the parameter ΔE as the poly(A) length increases (Fig. 2a). Poly(A) loops have a greater propensity to stack, or “misstack,” as the intervening chain length increases, thus increasing the roughness from trapping. A prediction of the diffusion model with transient traps is that the kinetics at low temperatures should deviate from a single exponential. Non-exponential kinetics have been observed for hairpins with long poly(A) loops [4]. Finally, we report new data where τ_c near T_m scales as $L^{2.0 \pm 0.2}$ for both poly(A) and poly(T) loops, in good agreement with the simplest polymer theories (Fig. 2b). In conclusion, we argue that the dynamics of short ssDNA chains are consistent with that of a flexible polymer that can be transiently trapped in misfolded loops and do not find any strong reasons for rejecting the polymer model.

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