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Amino Acid Stabilization of Nucleic Acid Secondary Structure: Kinetic Insights from Single-Molecule Studies

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Abstract

Amino acid and nucleic acid interactions are central in biology and may have played a role in the evolutionary development of protein-based life from an early “RNA Universe.” To explore the possible role of single amino acids in promoting nucleic acid folding, single-molecule Förster resonance energy transfer experiments have been implemented with a DNA hairpin construct (7 nucleotide double strand with a 40A loop) as a simple model for secondary structure formation. Exposure to positively charged amino acids (arginine and lysine) is found to clearly stabilize the secondary structure. Kinetically, each amino acid promotes folding by generating a large increase in the folding rate with little change in the unfolding rate. From analysis as a function of temperature, arginine and lysine are found to significantly increase the overall exothermicity of folding while imposing only a small entropic penalty on the folding process. Detailed investigations into the kinetics and thermodynamics of this amino acid-induced folding stability reveal arginine and lysine to interact with nucleic acids in a manner reminiscent of monovalent cations. Specifically, these observations are interpreted in the context of an ion atmosphere surrounding the nucleic acid, in which amino acid salts stabilize folding qualitatively like small monovalent cations but also exhibit differences because of the composition of their side chains.

Graphical Abstract

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1. INTRODUCTION

Interactions between amino acids and nucleic acids are biologically ubiquitous, lying at the core of numerous protein–nucleic acid interactions, such as histone-driven chromatin dynamics¹ and nucleic acid packaging into viral particles.^{2,3} Individual amino acids have been found to influence RNA functionality; for instance, many of the riboswitches discovered to date have specific binding sites for amino acids,⁴ including glycine,^{5,6} lysine,^{7,8} and arginine.⁹ RNA catalysis can also be affected by amino acids, as demonstrated in pioneering work by Yarus and co-workers, in which the self-splicing capability of the *Tetrahymena* ribozyme is inhibited by the presence of L-arginine.¹⁰ Indeed, amino acid influence over RNA catalytic properties has prompted an interesting suggestion for the mechanism by which early RNA-based life^{11,12} transitioned into the modern “protein world” via an intermediate step of amino acids acting as RNA-folding chaperones.¹³ In this hypothesis, early RNA-based structures which struggle to reach or remain in their biologically competent conformations may have benefited from the presence of amino acids, which assist in the folding and stabilization of RNA. This chaperoning could have begun with abiotically-generated amino acids, with an evolutionary path eventually leading to the biological synthesis of amino acids and small peptides, and ultimately to the protein world of today. This mechanism is speculative yet intriguing and clearly deserves further exploration.

Stimulated by such potential biological implications, crystallographic^{14–16} and computational^{16–19} studies have yielded valuable structural information on amino-acid nucleic acid interactions. However, the influence of amino acids on nucleic acid folding kinetics has remained far less well studied. Recently, single-molecule fluorescence microscopy tools have been applied to study the amino acid-based promotion or inhibition of the ubiquitous tetraloop–tetraloop receptor tertiary interaction.²⁰ Indeed, amino acids (arginine in particular) were found to *destabilize* the tertiary folding equilibrium by diminishing folding rates via competitive binding to the GAAA tetraloop. Most notably, even the simplest amino acid glycine exhibited a significant decrease in unimolecular unfolding rates for the tetraloop receptor. The present work both builds on and extends these single-molecule efforts, focusing on secondary structure rather than tertiary structure formation kinetics. One motivation for this is that research into the nucleic acid secondary structure is already quite mature, complete with the theoretical machinery for prediction of hybridization thermodynamics (e.g. nearest-neighbor models²¹), representing an area of

nucleic acid behavior that is well characterized.²² Exploring the influence of amino acids on secondary structure formation therefore allows us to isolate dynamical effects in a folding process which is better understood than tertiary folding. To perform these experiments, we employ a DNA hairpin as a simple model for nucleic acid secondary structure, which can be interrogated for its conformational status by single-molecule fluorescent resonant energy transfer (FRET) microscopy.^{23–25} In contrast to the previous studies on tertiary folding, only the charged amino acids (lysine and arginine, rather than simple zwitterionic species such as glycine) are found to alter the hairpin folding dynamics. Furthermore, these charged amino acids act to *stabilize* the folded conformation, which is exactly the opposite effect noted for tertiary structures. Implementation of temperature-dependent studies permits folding and transition-state free energies to be deconstructed into enthalpic and entropic components.²⁵ The combination of kinetic and thermodynamic data suggests an ionic screening mechanism in which charged amino acids alleviate the electrostatic cost of nucleic acid folding. This electrostatic interaction is the predominant source of amino acid-based secondary structure stabilization, though the sensitivity of the nucleic acid to the molecular detail of the side chain confers additional, amino acid-specific contributions.

2. METHODS

The DNA hairpin construct used in these studies has three components (Figure 1): (1) a 7 base-pair stem (5'-CTTCAGT-3'), (2) a loop consisting of 40 adenine nucleotides, and (3) an 11 adenine tail at the 3' terminus, end-labeled with biotin for surface attachment. This specific hairpin has no special biological significance; rather, the sequence is designed to avoid misfolding or the formation of higher-order structures, as well as to achieve kinetic rates within the current dynamic range of the instrument ($<100\text{ s}^{-1}$). For spectroscopic readout of conformation, a cyanine-based FRET fluorophore pair is used, consisting of Cy5 at the 5' terminus and internal Cy3 joining the 3' end of the loop with the stem. The complete DNA oligonucleotide (5'-Cy5-CTTCAGT-A₄₀-Cy3-ACTGAAG-A₁₁-biotin-3') is purchased from Integrated DNA Technologies.

Samples are prepared for single-molecule microscopy by biotin–streptavidin surface tethering.²⁶ Glass coverslips are cleaned by overnight acetone soaking and 30 min exposure to UV-generated ozone in air. The DNA hairpin surface attachment is achieved via sequential treatment of three solutions with 10 min incubations for each step: first, 10 mg/mL bovine serum albumin with 10% biotinylation; second, 0.2 mg/mL streptavidin; and third, 100 pM of the biotinylated DNA construct. This protocol yields a surface density of $\sim 1/\mu\text{m}^2$, which is sufficiently well resolved for optical microscopy with a high numerical aperture (NA) objective. Measurements are taken in buffer (50 mM HEPES, pH 7.6) with an enzymatic oxygen-scavenging system (2 mM TROLOX, 100 nM PCD, 5 mM PCA) to improve fluorophore photo-stability.²⁷ All experiments include a background of 100 mM monovalent ions (70 mM NaCl and 30 mM KCl for concentration-dependent studies and 100 mM NaCl for temperature-dependent studies). Other solutes are added as described below, using compounds purchased from Sigma.

Single DNA molecules are illuminated in a scanning confocal microscope,²⁸ as previously described.²⁰ Briefly, 532 nm laser light is focused through a 1.2 NA water-immersion

microscope objective to a 270 nm diffraction-limited spot. The spot is scanned over the coverslip surface by a piezoelectric stage to locate DNA molecules. Emission from fluorophores is collected by the microscope objective, split by a dichroic filter into donor and acceptor colors, and directed onto single-photon-counting avalanche photodiodes. Concentration-dependent studies are performed at 24 °C, and temperature studies are carried out using a stage heater (HSC60, Instec) and a microscope objective heating collar (Bioptechs) with 0.1 °C temperature accuracy and stability.²⁹

3. RESULTS

DNA hairpin folding in the absence of amino acids demonstrates that the construct behaves according to expectations in several ways. First, as demonstrated in Figure 2a, fluorescence trajectories depict switching between two states, indicating that the hairpin converts between an unfolded low-FRET state and a folded high-FRET state without significantly sampling additional conformations. Second, the high-FRET state has an average energy transfer efficiency of 0.88, corresponding to an interfluorophore distance of ~38 Å, which agrees well with an estimated distance of 34 Å for a 7 base-pair DNA duplex with B-form dimensions.³⁰ Third, the fractional residence time in each state can be used to compute a folding equilibrium constant ($K_{eq} = 0.74$), from which the free energy of folding ($\Delta G^\circ = -RT \ln(K)$) is determined to be 0.75 kJ mol⁻¹. This is comparable to 1.9 kJ mol⁻¹ nearest-neighbor model prediction²¹ for this hairpin at 24 °C in 100 mM Na⁺. Indeed, this ~1 kJ mol⁻¹ discrepancy is quite small and attributable to the different cation conditions in which nearest-neighbor parameters were measured.³¹ Fourth, the residence time distributions in each state are well fit to a first-order kinetic model, as seen in Figure 2b, which indicates that folding and unfolding are each governed by a single rate-limiting step, in agreement with literature observations of the folding kinetics of short hairpins.^{32,33}

Next, the influence of arginine on hairpin folding is examined. As shown in Figure 2a, the addition of 100 mM arginine hydrochloride (arginine HCl) results in the DNA hairpin spending dramatically less time in the unfolded state while remaining in the folded state for the same amount of time. These qualitative observations can be quantitatively confirmed by analysis of the residence time distributions (Figure 2b), in which the folding rate constant is increased by more than an order of magnitude from 2.6 ± 0.2 s⁻¹ in plain buffer to 26.7 ± 1.5 s⁻¹ with 100 mM arginine HCl. In contrast, the unfolding rate constant remains statistically unchanged (2.3 ± 0.1 to 2.3 ± 0.3 s⁻¹). By selectively increasing the rate of folding, arginine achieves stabilization of the DNA hairpin, therefore, by selectively increasing the rate constant for folding.

To explore the mechanism underlying stabilization of the hairpin, arginine is compared side-by-side with glycine and lysine. Glycine isolates the contribution of the zwitterionic backbone, whereas lysine represents swapping arginine's guanidinium-based side chain for an ammonium moiety. As evident in Figure 3, glycine has little influence on either the folding or unfolding of the hairpin, establishing that the zwitterionic backbone is not sufficient for stabilization of the secondary structure. Lysine hydrochloride (lysine HCl), on the other hand, is able to stabilize the hairpin, again primarily by increasing the folding rate with only a minor slowing of unfolding, in a similar fashion to arginine. However, the

magnitude of lysine's influence is not as large: 100 mM arginine HCl speeds up folding over nine-fold, compared to the four-fold increase in 100 mM lysine HCl. Thus, the positively charged side chain in lysine is sufficient to stabilize the hairpin, but the guanidinium group in arginine clearly provides an additional stabilizing contribution.

The net positive charge of arginine and lysine at the experimental pH suggests that the mode of interaction with DNA may be primarily electrostatic. To evaluate this possibility, hairpin folding measurements have been carried out in the presence of sodium chloride, a simple monovalent cation (Figure 3, dashed line; note that the concentration represents additional sodium chloride beyond the 100 mM NaCl present in all samples). Sodium chloride exhibits hairpin stabilization via the same kinetic signature as the amino acid salts, viz. increasing the folding rate with a small effect on the unfolding rate. This is in agreement with previous studies of the monovalent cation influence on secondary structure formation.³⁴ However, the net stabilization (η) illustrated by the equilibrium constants in Figure 3b was different for each species, resulting in an order of stabilization: $\eta_{\text{ArgHCl}} > \eta_{\text{NaCl}} > \eta_{\text{LysHCl}}$. Though their effects are different in magnitude, all three cations display a similar kind of influence on folding kinetics for the DNA hairpin, in general corroborating the heuristic connection between amino acid charge and hairpin stabilizing power.

Further insight comes from temperature-dependent measurements, which permit the deconstruction of free energies into entropic and enthalpic contributions. The van't Hoff plot in Figure 4a displays the influence of temperature on the DNA hairpin's folding equilibrium constant with and without 100 mM arginine HCl, lysine HCl, and NaCl over the range of 24–29 °C. Linear fits to these data yield the entropy (ΔS°) and enthalpy (ΔH°) of folding, according to the van't Hoff equation (eq 1)

$$\ln(K) = -\frac{\Delta G^\circ}{RT} = -\frac{\Delta H^\circ}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^\circ}{R} \quad (1)$$

where T is the absolute temperature and R is the gas constant. The enthalpies and entropies of folding, derived from these slopes and intercepts respectively, are displayed in Figure 4b. Folding of the hairpin without any additional solute (leftmost column) is driven by significant enthalpic release ($\Delta H^\circ = -138 \pm 21 \text{ kJ mol}^{-1}$) offset by a large entropic penalty ($\Delta S^\circ = -470 \pm 70 \text{ J mol}^{-1} \text{ K}^{-1}$), in agreement with previous observations of hairpin formation.^{35,36} Each of the added amino acids *enhances the enthalpic favorability* (average $\Delta H^\circ = -46 \pm 23 \text{ kJ mol}^{-1}$; dashed line in the upper panel of Figure 4b), while also *increasing the entropic penalty* associated with folding (average $\Delta S^\circ = -130 \pm 80 \text{ J mol}^{-1} \text{ K}^{-1}$). The net result between these opposing effects is increased stabilization ($\Delta G^\circ < 0$), that is, the enthalpic benefit provided by each of the solutes is stronger than the additional entropic cost incurred. However, any differential effect between these three species is statistically indistinguishable from zero within experimental uncertainty (see Figure 4b).

Similarly, the temperature dependence of the *rate* constants for folding may be used to extract thermodynamic parameters for approaching the folding transition state. To achieve

this, the transition state theory expression for the rate constant may be recast into a more convenient, activated complex form

$$\ln(K) = \ln(v) + \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{R} \left(\frac{1}{T} \right) \quad (2)$$

where v is the attempt frequency along the reaction coordinate, and $\Delta S^\ddagger$ and $\Delta H^\ddagger$ are changes in entropy and enthalpy, respectively, required to reach the transition state. In a plot of $\ln(k)$ against $1/T$, the slope of a linear fit reveals $\Delta H^\ddagger$ unambiguously, whereas the intercept reflects information on both $\Delta S^\ddagger$ as well as v . Because the attempt frequency v is not known *a priori*, the Arrhenius plot does not provide the absolute activation entropy. However, if v is assumed to be constant over the range of conditions in this study, then the solute-induced *change* in the activation entropy ($\Delta S^\ddagger$) can be determined unambiguously. Figure 5a reveals activated complex theory Arrhenius plots for the folding rate constant of the DNA hairpin, with the least-squares fitting results summarized in Figure 5b. Similar to what was noted in the above van't Hoff analysis, it is difficult to distinguish the more detailed differences between arginine HCl, lysine HCl, or NaCl. However, all three salts do act in the same general fashion: the enthalpic requirement to reach the transition state is significantly diminished (average $\Delta H^\ddagger = -54 \pm 15 \text{ kJ mol}^{-1}$), while the transition state becomes entropically less accessible (average $\Delta S^\ddagger = -160 \pm 50 \text{ J mol}^{-1} \text{ K}^{-1}$). Notably, the thermodynamic parameters of the folding reaction are similarly impacted by arginine and lysine (Figure 5b), which in turn account for their comparable influences on the folding kinetics.

4. DISCUSSION

Previous work has shown the ability of amino acids, especially L-arginine, to alter ribozyme activity.¹⁰ The suggestion that the origin of such inhibitory behavior lies in amino acid modification of nucleic acid folding properties has prompted this series of single-molecule investigations into the kinetic behavior of nucleic acids in amino acid environments. Prior efforts examined an isolated tertiary interaction, the tetraloop–tetraloop receptor, revealing the ability of glycine, lysine, and especially arginine to destabilize the tertiary structure, each in an amino acid-specific manner.²⁰ The current study aims to bring the same analysis to bear on secondary structure formation kinetics, modeled by a DNA hairpin.

First, we have explored the ability of arginine, lysine, and glycine to influence the secondary structure. Of these three amino acids, both arginine and lysine are found to stabilize the hairpin formation, whereas glycine has little or no effect. The insensitivity of hairpin folding to the presence of glycine suggests that the amino acid zwitterionic backbone is relatively unimportant in the mechanism by which arginine and lysine promote the folded state. Rather, it is the side chain which determines the effectiveness of the promoting interaction. Arginine HCl and lysine HCl have qualitatively similar influences on the hairpin folding kinetics (Figure 3a) and thermodynamics (Figures 4 and 5), but the *magnitude* of these effects is different, with arginine offering more pronounced stabilization. That arginine and lysine have a shared kinetic and thermodynamic signature suggests that they also have a

shared *mechanism* for stabilization, with the overall effect modulated by subtle differences in residue composition. In particular, arginine and lysine share the structural feature of a charged side chain, suggesting that charge state is the primary source of stabilization. If charge determines stabilizing power, then the addition of other monovalent cations should affect hairpin folding in a similar fashion. Indeed, sodium chloride titrations produce kinetic and thermodynamic modifications comparable to those of the amino acid salts.

The ability of monovalent cations to promote nucleic acid folding is well known^{34,37} and can be explained by considering the role of the ionic atmosphere in folding.³⁸ Nucleic acids are highly charged polyanions which recruit electrolytes from solution to form an ionic cloud surrounding the polynucleotide, known as the “ionic atmosphere.” A nucleic acid undergoing a conformational change will concomitantly experience reorganization in its ionic atmosphere.^{39,40} Importantly, folding events typically make the nucleic acid more compact, resulting in the net recruitment of ions from the bulk.^{34,41} Consequently, increasing salt concentration serves, by mass action, to shift the equilibrium to favor more compact, folded states. Our kinetic observations can be explained by examining the ionic atmosphere of the transition state. According to a “kinetic zipper” picture,^{32,42} the transition state for the DNA hairpin ought to be structurally similar to the folded state, with the loop already formed (and entropic costs already paid) and held together by a few nascent base pairs. Therefore, the transition state and the folded state will have similar ionic atmospheres, which would explain why the unfolding rate is found to be relatively insensitive to salt levels. On the other hand, the initial folding process requires net uptake of ions to reach the transition state, so the folding rate constant is anticipated to be strongly dependent on salt and/or charged amino acid concentration.

This salt-mediated promotion of the secondary structure is partially opposed by an additional entropic cost arising from the salt dependence of the polynucleotide flexibility. Repulsive forces between phosphates along the nucleic acid backbone tend to straighten the polynucleotide, limiting the conformational phase space sampled by the polymer. Increasing salt levels improve ionic screening, which diminishes phosphate repulsion down the backbone and decreases the polymer persistence length.^{43–45} In turn, the nucleic acid can sample a greater region of conformational phase space, thereby increasing its entropy. For the DNA hairpin, both the folded and unfolded states enjoy this entropic increase; however, the effect is greater in the unfolded state which has a longer single-stranded region with unconstrained ends. Therefore, the folding reaction suffers a net entropic cost due to the increase in bulk salt concentrations. The present temperature-dependent experiments have confirmed the prediction of a salt-induced entropic cost in the folding of the hairpin ($\Delta S^\circ = -130 \pm 80 \text{ J mol}^{-1} \text{ K}^{-1}$, 100 mM added salt). Furthermore, the structural similarity of the folded state and the transition state implies that there should be a similar cost in the *activation* entropy for folding, and indeed, the Arrhenius analysis reveals an activation entropy increase of equal magnitude ($\Delta S^\ddagger = -160 \pm 50 \text{ J mol}^{-1} \text{ K}^{-1}$). It is worth noting that the salt dependence of DNA *duplex* formation has been historically observed to be primarily entropic in origin⁴⁶ (i.e. $\Delta S > 0$; $\Delta H \approx 0$), though recent studies have identified nonnegligible enthalpic contributions.^{34,47} That the DNA hairpin in these studies responds to monovalent salt levels in a thermodynamically distinct fashion ($\Delta S < 0$; $\Delta H < 0$) is a reminder of the ongoing need for research into the thermodynamic properties of the salt-

mediated nucleic acid folding (e.g. bimolecular vs intramolecular processes, long vs short hairpin loops, and long vs short stems). In particular, we anticipate the entropic effect to be different in hairpins with shorter loops, which are known to be less sensitive to salt overall.^{41,48}

Though arginine and lysine influence hairpin folding in a way that is qualitatively similar to a monovalent cation, the additional stabilizing ability of arginine over lysine suggests that the molecular detail of the side chain further modulates these amino acid–nucleic acid interactions. To isolate the effects of the amino acid side chain without the backbone, we have also explored DNA hairpin folding in the presence of guanidine hydrochloride (GuHCl), which imitates arginine's guanidinium group and ammonium chloride, which imitates lysine's ammonium group (Figure 6). Both salts are observed to stabilize the hairpin, with GuHCl having a stronger effect than NH_4Cl . This is unsurprising, given guanidine's known strong interactions with nucleotides due to hydrogen bonding.^{49,50} In addition, GuHCl and NH_4Cl were both found to have greater stabilizing power than their respective amino acid counterparts. Interestingly, the kinetic source of GuHCl's greater stabilization of the hairpin is depression of the unfolding rate constant (3-fold at 100 mM GuHCl) beyond what any of the other salts in this study achieve, so the guanidinium ion stabilizes the folded state relative to the transition state more effectively than any other cation in these experiments.

These observations lead to a simple picture explaining the relative stabilizing power of lysine, arginine, ammonium, and guanidium based on the monovalent cation size^{41,51} and hydrogen bonding ability. Lysine interacts with the nucleic acid via a mechanism similar to that of an ammonium cation but with additional molecular bulk which prevents lysine from approaching the hairpin as closely as ammonium. Lysine therefore cannot contribute to the ionic atmosphere as efficiently as ammonium, resulting in a restricted ability to stabilize the secondary structure ($\eta_{\text{LysHCl}} < \eta_{\text{NH}_4\text{Cl}}$). Arginine, on the other hand, interacts with the hairpin like guanidinium, which is dominated by the molecule positive charge but made even stronger because of the hydrogen-bonding abilities of the guanidinium moiety ($\eta_{\text{GuHCl}} > \eta_{\text{NH}_4\text{Cl}}$; $\eta_{\text{ArgHCl}} > \eta_{\text{LysHCl}}$). However, arginine suffers from the same steric hindrance as lysine, so arginine cannot stabilize the secondary structure of the nucleic acid as effectively as guanidinium ($\eta_{\text{GuHCl}} > \eta_{\text{ArgHCl}}$).

Several control tests of this model have been carried out. First, we examined the role of amino acid chirality in imparting stability to the hairpin. If the dominant mode of interaction between amino acids and nucleic acids is electrostatic, then the folding of the hairpin should be insensitive to the chirality of the amino acid. In addition, the guanidinium group in arginine is achiral, so the hydrogen-bonding ability of arginine should not depend on the chirality of the distant α -carbon. As shown in Figure 7, smFRET experiments comparing L- and D-reagents reveal no difference between the enantiomers of arginine HCl or lysine HCl, in agreement with the model. Second, we tested to see if *negatively*-charged amino acids can participate in secondary structure stabilization. Because nucleic acids are highly negatively-charged, a negative amino acid like aspartate would not be expected to interact strongly with the hairpin. However, the negative amino acid must be accompanied by a positive counter-ion, which should participate in the same stabilizing interactions as other cations. To test

this, we compared the impact of sodium aspartate and sodium chloride on the folding kinetics of the DNA hairpin, depicted in Figure 8. The data confirm that the effect of sodium aspartate is indistinguishable from that of sodium chloride, supporting our model for nucleic acid-induced secondary structure stabilization.

5. SUMMARY AND CONCLUSION

In this work, single-molecule analysis has been used to examine the ability of amino acids to influence the formation of secondary structure in nucleic acids. Positively charged amino acids (arginine and lysine) are found to stabilize the secondary structure, while the zwitterionic amino acid glycine has no significant effect. The kinetic origin of the stabilization is primarily a large increase in the folding rate constant, with only a minor change in the unfolding rate. Thermodynamic analysis reveals that both arginine and lysine increase the reaction exothermicity ($\Delta H^\circ < 0$) while also imposing a slight entropic penalty ($\Delta S^\circ < 0$), yielding a net stabilizing effect ($\Delta G^\circ < 0$). Furthermore, the barrier to folding is modified and characterized by a transition state which is enthalpically more accessible ($\Delta H^\ddagger < 0$) but entropically less approachable ($\Delta S^\ddagger < 0$). The kinetic and thermodynamic signature of arginine and lysine is shared by other monovalent cations, suggesting that the mechanism of secondary structure stabilization is predominantly electrostatic. A model is proposed to explain these observations, by examining how amino acids contribute to a nucleic acid's ionic atmosphere. In an evolutionary context, significant stabilization effects were observed only at relatively high amino acid concentrations (tens of millimolar), which disfavor secondary structure stabilization as a major driving force for amino acid incorporation into biology. However, secondary structure formation could prove to be far more selectively sensitive to small di- and tripeptides, which could serve as an interesting direction for further experiments.

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Figure 1.
DNA hairpin construct used in folding studies.

Figure 2.

(a) Single-molecule time traces for hairpin folding with and without 100 mM arginine HCl, exhibiting two-state behavior. Green and red lines correspond to donor (Cy3) and acceptor (Cy5) fluorescence, respectively. Residence durations are used to construct survival time distributions for (b) folding and (c) unfolding. Exponential fits are used to extract rate constants.

Figure 3.

Effects of amino acids on DNA hairpin folding. (a) The folding rate constant is strongly increased, whereas (b) the unfolding rate constant is slightly decreased. (c) The equilibrium constant shows little sensitivity to glycine, while positively charged amino acids stabilize the secondary structure. Sodium chloride (dashed line) is shown for reference.

Figure 4.

(a) van't Hoff plot of the hairpin folding equilibrium's temperature dependence. Colored symbols have 100 mM solute. Black squares have sodium chloride added to make the same ionic strength as colored squares, that is, 200 mM total [NaCl]. Linear fits are used to extract H and S contributions, shown in (b). Dashed lines indicate average H and S for added solutes.

Figure 5.

(a) Arrhenius plot for hairpin folding rate coefficient as a function of temperature. Linear fits yield activation enthalpies and entropies in (b). Dotted line indicates averaged enthalpies and entropies, not including “no amino acid.”

Figure 6.

Comparison of positively charged amino acids with their side chain moieties, for (a) folding rate constants, (b) unfolding rate constants, and (c) equilibrium constants. Arginine HCl and guanidine HCl have similar effects on folding kinetics, but guanidine HCl has a stronger influence on unfolding rates. NH₄Cl has a slightly larger influence on both folding and unfolding over lysine HCl.

Figure 7. Hairpin folding is insensitive to amino acid chirality. (a) Concentration dependence of folding (filled) and unfolding (unfilled) rate constants for enantiomers of arginine HCl. (b) van't Hoff plots for arginine HCl (red) and lysine HCl (green) enantiomers.

Figure 8. Negative amino acids behave according to their positively charged counterion. Sodium chloride and sodium aspartate have indistinguishable effects on hairpin folding kinetics.