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Direct Revelation of Multiple Conformations in RNA by Femtosecond Dynamics

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The intrinsic dynamics of RNA structures occur on a wide range of length and time scales,[1] implying structural flexibility or heterogeneity. The role of conformational dynamics in molecular recognition and functions, however, is not well understood. Despite significant advances in traditional and new spectroscopic methodologies, it is still difficult to extract information on both the exact nature of the heterogeneous conformations and their quantitative distributions. Here we demonstrate a femtosecond dynamics approach for probing the multiple conformations coexisting in RNAs. We focus primarily on mapping the base stacking patterns. The observed base stacking/unstacking rates in the single strand are typically in the range of 100−500 ns.[1] This means that, for a time window much shorter than the nanosecond regime, the distributions of stacking patterns are static and can potentially be analyzed by ultrafast spectroscopy.

The family of GNRA tetraloops has been shown to be well structured,[2−4] where the last three bases of the loop are mostly stacked on the 3′-side of the loop. There is also strong evidence that the loop structures are not rigid and undergo conformational dynamics and transitions.[3,5] In particular, a structural model has been proposed where a 5′-stacked form coexists with the 3′-stacked form with similar populations.[5] The precise nature of the alternative stacking patterns, however, has been difficult to characterize due to the fast interconversion.

To probe the distributions of stacking patterns using the ultrafast dynamics approach, $G_1N_2R_3A_4$ tetraloop RNAs, including GAAA, GCAA, and GAGA, are labeled with 2-aminopurine (2Ap, Figure 1A). Previously, we have shown that charge transfer (CT) is the main channel of 2Ap excited state decay. All nucleobases can act as quenchers with unique ultrafast quenching dynamics.[6] Because efficient quenching via CT requires that the quenchers are stacked with 2Ap, capturing the ultrafast CT dynamic processes can thus provide direct information on the stacking interactions. However, using the natural bases as quenchers can produce ambiguity in the interpretation of structural information in terms of observed quenching dynamics. First, site-specific information is difficult to obtain due to the presence of natural bases surrounding the 2Ap probe. Second, without other independent evidence, direct CT with one stacked base may not be easily distinguished from conformation-gated CT[7] with another base.

In our approach, some of the RNAs are also labeled by 7-deazaguanine (Z, Figure 1A). Z quenches 2Ap fluorescence on a 1 ps time scale, a rate that is much faster than those observed for all natural bases.[6] Incorporation of Z provides site-specific structural information. On the 1 ps time scale, relevant RNA structures are static. Thus observation of such ultrafast decay dynamics gives unambiguous information on the stacking interaction at the site, without complications from any other dynamic processes. Furthermore, the amplitude of the 1 ps component of the decay profile provides quantitative information on the relative population of the RNA where the two bases (2Ap and Z) are in a base-stacked geometry. The remaining much slower decay components represent all other conformations where the two bases are not stacked. Therefore the stacked versus unstacked populations are resolved. It has been shown that 2Ap substitution at the second or third positions does not significantly destabilize the GAAA tetraloop.[5] Our UV melting experiments show that the melting temperatures (T_m) for all constructs (Figure 1B) vary within 3−4 °C compared to their respective unlabeled RNAs (Figure S1 and Table S1), thus confirming that neither a single 2Ap substitution nor a 2Ap/Z double substitution is destabilizing.

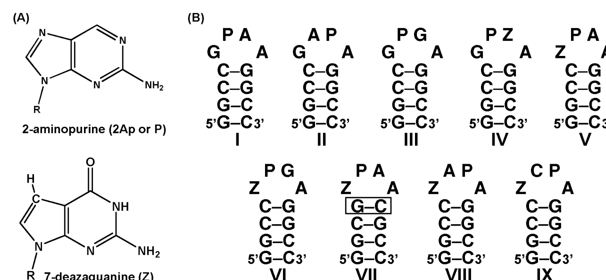

Figure 1. (A) Chemical structures of 2-aminopurine (2Ap) and 7-deazaguanine (Z). (B) Secondary structure representations of the GNRA tetraloop constructs used in this study. 2Ap is denoted as P in the sequences. Constructs I−III are only labeled by 2Ap; constructs IV−IX are labeled with both 2Ap and Z. The GC closing base pair in VII is boxed.

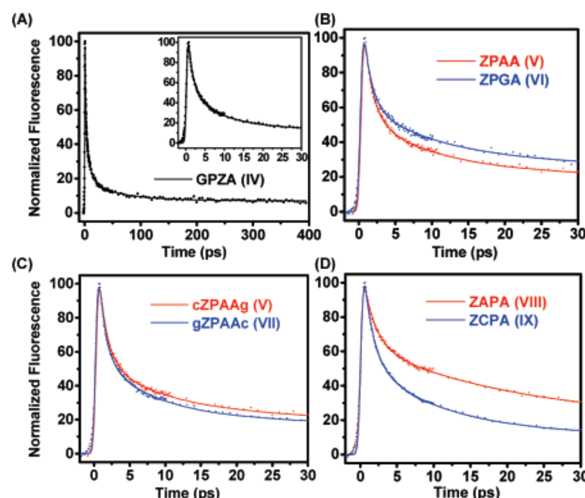

Figure 2. Femtosecond time-resolved fluorescence decay profiles for constructs (A) GPZA (IV) (inset: same profile for 30 ps window), (B) ZPAA (V, red) and ZPGA (VI, blue), (C) cZPAAg (V, red) and gZPAAc (VII, blue), and (D) ZAPA (VIII, red) and ZCPA (IX, blue).

Femtosecond time-resolved fluorescence decay transients for the double-labeled RNAs are shown in Figure 2. As expected, none of the control constructs (I−III) shows 1 ps dynamics as they lack the Z quencher in the loop (Figure S2 and Table S2). When Z is introduced together with 2Ap, a 1 ps decay component with varying amplitudes can be observed (Table S2). For construct IV (GPZA),

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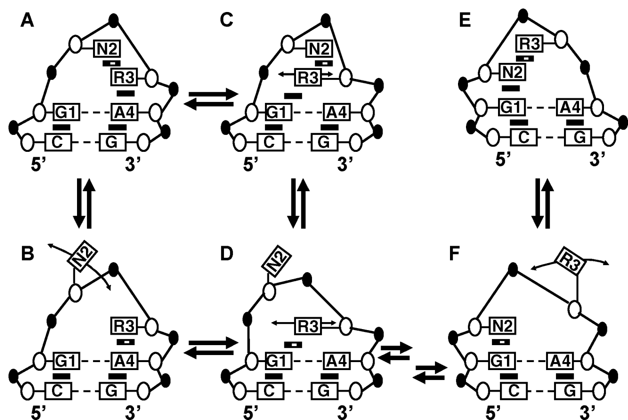

Figure 3. The dynamic multiconformation model for GNRA tetraloops. Phosphate, sugar, and bases are represented by filled circles, open circles, and rectangles, respectively; dotted lines indicate hydrogen bonds; black bars represent stacking. Double arrows indicate conformational equilibrium and possible transitions. Thin curved arrows indicate the possible motions of bases. Thin straight arrows indicate the sliding motion for R3 over the G1−A4 pair.

the amplitude of this component is 55% (Figure 2A), consistent with the fact that the second and third nucleotides in the GNRA tetraloops are directly stacked. However, clearly there are other populations in which the two bases are not directly stacked, by virtue of this component being significantly less than 100%. Constructs V (ZPAA) and VI (ZPGA) were designed such that Z and 2Ap are at positions 1 and 2. In the population that is in the 3′-stacked state, these two bases are relatively far apart and do not directly interact[2−4] and therefore should not contribute to a 1 ps decay component. If the 5′-stacked conformation does exist in a subpopulation where the two bases are directly stacked as proposed,[5] a 1 ps dynamics is expected. Indeed, there is 1 ps dynamics with significant amplitude for both constructs (Figure 2B). This is clear and direct spectroscopic evidence that a 5′-stacked conformation indeed exists.

We also studied the potential effect of the loop closing base pair. Our UV optical melting experiments show that switching the CG closing base pair to GC lowers the T_m by 16−17 °C for both the unlabeled GAAA and the labeled ZPAA tetraloops (Figure S1 and Table S1), consistent with previous findings by others.[8] Comparison of the decay dynamics between constructs V (cZPAAg) and VII (gZPAAc) indicates that again the 5′-stacked state is present in gZPAAc (Figure 2C). The similar decay dynamics for V and VII suggests that the orientation of the closing base pairs does not significantly affect the total population of the 5′-stacked state of the GAAA tetraloop. Interestingly, the decay profiles for VIII (ZAPA) and IX (ZCPA) (Figure 2D), where Z and 2Ap are separated by one nucleotide in sequence, also showed an ultrafast dynamic component (1.6 ps, 43% for VIII; and 1.3 ps, 55% for IX). Although somewhat slower than the typical rate of 1 ps, this can only be due to quenching by Z because no other mechanism can produce such ultrafast rates. The CT quenching rate has been found to be very sensitive to the distance or intervening bases between donor and acceptor.[9] The slightly slower rates suggest that the stacking interactions between Z1 and 2Ap3 in these constructs may not represent the optimal stacked conformation, but likely a partially stacked pair.

On the basis of our femtosecond dynamics probing as well as findings by others,[2,3,5,10] we propose a dynamic multiconformation model for the GNRA tetraloop motif (Figure 3). Clearly, a GNRA tetraloop structure should be characterized as an ensemble of conformations. Conformation A represents the 3′-stacked structure that is predominantly observed for the isolated motif[2,3] or in the complexes with tetraloop−receptor.[4,11] It appears that this conformation and its close variants account for about half of the total population at equilibrium. We propose that the conformation A undergoes conformational exchange with conformation B, where the second base (N2) unstacks from the third base (R3). Conformation B, in fact, has been observed, particularly for the GCAA tetraloop.[3] This is partially due to the weaker tendency for a pyrimidine base to be stacked. We propose that this equilibrium between conformations A and B exists in all GNRA tetraloops and could be an important pathway for transitioning to other conformations. The dynamic component observed for constructs VIII and IX indicates that, in some of the populations, the first base (G1) and the third base (R3) may be at least partially stacked. Conformations C and D account for these observations, where R3 can slide over the G1−A4 sheared base pair, switching from stacking on A4 to stacking on G1. In a molecular dynamics simulation of the GCAA tetraloop, a conformation similar but not identical to C was observed and proposed to be the precursor of transition to the 5′-stacked conformation.[10] Note that conformation D somewhat resembles the base-stacking topology of the UUCG tetraloop.[12] The unstacking of N2 from R3 makes it possible for N2 to stack on the 5′-side of the loop (possibly via multiple steps, indicated by multiple arrows in the model) to form conformation F, which can lead to a totally 5′-stacked conformation (E). Both conformations E and F likely contribute to the observed 1 ps dynamic component in constructs V, VI, and VII. Our data suggest that these 5′-stacked forms may account for about half of the total population, in agreement with an earlier estimate.[5]

These observations underscore the notion that RNA structures are intrinsically dynamic, which forms the basis for the understanding of the collective behavior of the conformations of a molecule. It remains to be investigated how the observed structural distributions may be altered by docking with the tetraloop receptor,[4,11] such that one conformation is selectively captured from the ensemble in favor of the complex formation.[13]

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Supporting Information Available: Experimental methods, UV melting curves and parameters, femtosecond transients for constructs I−III, and parameters for all constructs. This material is available free of charge via the Internet at http://pubs.acs.org.

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