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Dissect Conformational Distribution and Drug-Induced Population Shift of Prokaryotic rRNA A-Site

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Supporting Information

ABSTRACT: The dynamic behavior of the rRNA A-site plays an important functional role. We have employed femtosecond time-resolved spectroscopy to investigate the nature of the conformational dynamics. In the drug-free state, the A-site samples multiple distinct conformations. Drug binding shifts the population distribution in a drug-specific manner. Motions of bases on nanosecond and picosecond time scales are differentially affected by the drug binding. Our results underscore the importance of understanding the detailed dynamic picture of molecular recognition by resolving dynamics in the distinct picosecond time regime and facilitate development of antimicrobial drugs targeting dynamic RNAs.

During protein synthesis, the dynamic behavior of rRNA A-site is believed to play a critical role in ensuring the accuracy of decoding via a kinetic discrimination mechanism.[1−3] The crystal structure of the 30S ribosomal subunit showed that the electron density for the two functionally critical adenine bases A1492 and A1493 (Figure 1A) is not consistent with a single conformation,[4,5] and they sample an equilibrium between intrahelical and extrahelical states.[2,6,7] These observations are consistent with solution NMR evidence that also indicated mixed sugar conformations[8,9] and nanosecond time-resolved fluorescence spectroscopy that revealed multiple conformations.[10−13] The precise nature of these dynamics, however, has not been adequately characterized. We resort to femtosecond time-resolved spectroscopy to investigate the dynamic characteristics of the A-site to paint a more complete picture of the complexity of the functional element.

Our previous works have demonstrated the experimental strategies of femtosecond time-resolved probing of RNA dynamics.[14,15] In accordance, we obtained prokaryotic rRNA A-site constructs (Figure 1A) with 2AP labeled individually at two positions A1492 and A1493 (*Ec*-P1492 and *Ec*-P1493) here.

Figure 1B shows the femtosecond fluorescence decay profiles for *Ec*-P1492 and *Ec*-P1493 in the drug-free states. Three decay components were needed to adequately account for the decay dynamics (Figure S1 and Table S1). These multiphasic decay profiles suggest that the RNA indeed samples various degrees of heterogeneous conformations depending on the positions. For *Ec*-P1492, the 9.2 ps decay component (τ_1) is consistent with fluorescence quenching by the stacked G1491 in a major

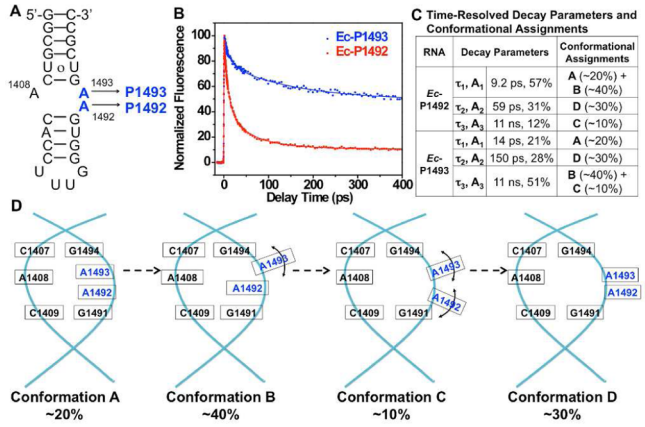

Figure 1. (A) Model *E. coli* A-site with 2AP labeled at 1492 (*Ec*-P1492) and 1493 (*Ec*-P1493). (B) Fluorescence decays of *Ec*-P1492 (red) and *Ec*-P1493 (blue). (C) Decay parameters and conformational assignments. (D) Ensemble model. Double arrows indicate motions.

subpopulation (57%). The intermediate decay component (τ_2, 59 ps) represents stacking by A1493 in another subpopulation (31%). The slow decay term on ~11 ns (τ_3) is due to lack of any direct stacking (unstacked) in a minor subpopulation (12%). For *Ec*-P1493, the fastest decay component (τ_1, 14 ps, 21%) represents a low subpopulation that this base stacks on G1494, and the intermediate decay component (τ_2, 150 ps, 28%) represents the reciprocal stacking with A1492 in a minor subpopulation. Half of the population (τ_3, 11 ns, 51%) for base 1493 is in a totally unstacked state.

The earlier studies on nanosecond time-resolved probing of prokaryotic and eukaryotic A-site with 2AP at position 1492[10−12] or 1493[13] revealed three decay components within the sub-nanosecond to nanosecond time regime, with the sub-nanosecond component being dominant. The τ_1 and τ_2 from our femtosecond probing further resolved this sub-nanosecond component into two separate distinct picosecond components thereby revealing new information on the subpopulations and their specific base stacking interactions as the origin of quenching dynamics. Thus combining time-resolved spectros-

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copies with different time-resolutions can provide a more complete picture of the total decay dynamics.

Given the ultrafast dynamic data presented here as well as existing information available in the literature,[6,8−12] we proposed an ensemble model (Figure 1D) for the dynamic conformational landscape of prokaryotic rRNA A-site in the drug-free state. Conformation A (∼20%, estimated from amplitude A_1 for *Ec*-P1493) represents the closed state, where both A1492 and A1493 stack intrahelically. Conformation B (∼40%) features a stacked 1492 but 1493 is flipped out. Both conformations A and B (total ∼60%) contribute to the fastest decay component (τ_1, 57%) for *Ec*-P1492. In conformation C (∼10%), both 1492 and 1493 are flipped out without stacking on each other, and it is the only conformation that leads to the slow decay for *Ec*-P1492 (τ_3, 11 ns, 12%), while both conformations B and C (total ∼50%) contribute to the slow decay component for *Ec*-P1493 (τ_3, 51%). In conformation D (∼30%), the two adenines are flipped out but also stacked on each other. Such extrahelical base stacking gives rise to the intermediate decay component due to mutual adenine stacking (τ_2, 28−31%) for both bases. The model fits well to the decay time scales and amplitudes, underscoring the power of ultrafast dynamics probing in resolving complex conformational ensemble.

Overall, base 1492 has a 60:40 distribution of stacked vs unstacked populations, while 1493 samples more flipped-out conformation (20:80). These results mirror those from crystallographic studies.[6] These population data can be used to calculate the free energy differences between the substates and range mostly within ∼0.2−0.8 kcal/mol at 22 °C (calculation formula in Supporting Information), with the population of the highest occupancy, conformation B, representing the overall ground state.

Aminoglycosides target the A-site with varying degrees of specificity[16,17] and can significantly affect the fidelity of translation,[3] and thus they are an important class of therapeutics. Structures of ribosome model A-site complexed with aminoglycosides have revealed some of the molecular detail of the antibiotic actions.[2,5−9,18−22] These studies suggested that drug-induced base destacking of A-site is critical for their antimicrobial activities. How the dynamics of A-site are altered by the various aminoglycosides has not been fully elucidated and is important for understanding the detailed mechanisms of the actions of these compounds.

The steady state fluorescence response at 1492 and 1493, which is a sensitive indicator of base stacking,[6,10−13,23] is dependent on the specific aminoglycosides (Figure S2 and Table S2). For *Ec*-P1492 (Figure 2A), with either neomycin or paromomycin, the amplitude of the ultrafast decay component (19%/Paro, 29%/Neo) is smaller compared to those of the free A-site, with a concomitant increase in the amplitude of the nanosecond slow component (71−81%). These results suggested that binding of drugs shifts the population toward the unstacked state, but there is still detectable subpopulation of the stacked state. For *Ec*-P1493 (Figure 2B), the nanosecond component also dominates the decays (∼88−94%), with small amplitude of ultrafast component (6−12%). The nanosecond component most likely represents a conformationally gated charge transfer event that occurs on such a time scale. Motions of base 1493 on such a time scale upon neomycin B and paromomycin binding have in fact been observed.[13] Taken together, there is also an equilibrium of conformations in the drug-bound prokaryotic A-site, but with distributions shifted

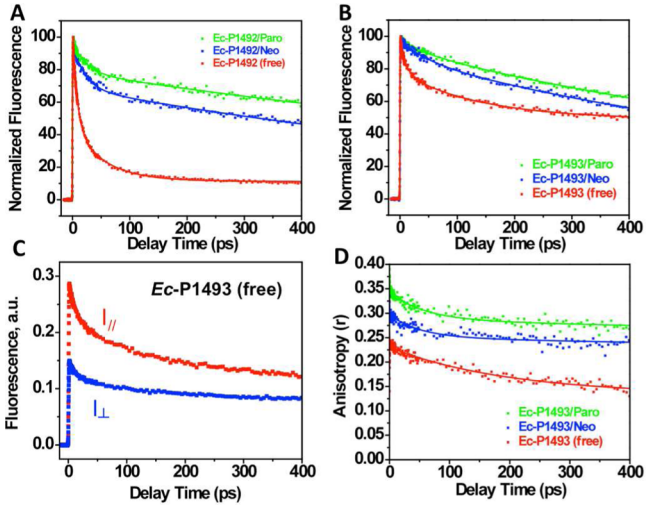

Figure 2. (A) Magic angle decay profiles of aminoglycoside binding to *Ec*-1492. (B) Magic angle decay profiles of aminoglycoside binding to *Ec*-1493. (C) Parallel (red) and perpendicularly (blue) polarized decay profiles for free *Ec*-1493. (D) Anisotropy decays of free *Ec*-1493 and complexes.

mostly toward the unstacked state. These results support that aminoglycosides recognize A-site most likely via the conformational capture mechanism.[24,25]

Time resolved anisotropy with nanosecond time resolution showed that aminoglycoside-induced reduction in the mobility of bases 1492[12] and 1493[13] can also be an important determinant of antimicrobial activities. Those earlier studies showed that time scales of base motion increases from ∼0.5 ns in the drug-free state to 1−2 ns in the drug-bound complexes in order of their antibacterial activities. It is necessary to characterize if altered mobility on ultrafast time scales will further contribute to antimicrobial functions of those drugs.

Here we sought to expand the probing of base motion into faster time regime using femtosecond time-resolved anisotropy measurements. Figure 2C,D shows the anisotropy decay dynamics of *Ec*-P1493 in different states. In the drug-free state, we detected anisotropy decay with a 184 ps lifetime (Table S3), suggesting hindered internal motion of the base on this time scale.[14,15] Such motion may also act to conformationally gate the charge transfer reactions and contribute to the intermediate term (τ_2, 150 ps) of the magic angle experiments. The rest of the anisotropy decays on a nanosecond time scale defined by the earlier nanosecond study,[13] suggesting they sample a range of rotation angles in the flipped-out conformations and may transiently collide on a 2−3 ns time scale that gives rise to the decay observed in the nanosecond probing. Upon drug binding, our results demonstrated that the nanosecond component apparently becomes slower, consistent with the previous report.[13] Interestingly, the ultrafast anisotropy decay component of drug-bound state in fact becomes faster (66 ps/Neo, 58 ps/Paro) than the drug-free state. This finding implied that the reduction in the nanosecond motion reported earlier and the increased mobility on picosecond motion discovered here are different modes of base dynamics that are differentially affected by drug binding.

Taken together, these results contribute to a more complete dynamic ensemble picture of the complexity of the rRNA A-site and its conformational transitions upon drug binding. A drug-specific model for the mechanism of recognition will help guide

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the efforts of modifying drug leads to improve their efficacy of targeting dynamic RNAs.[26−28] Furthermore, our femtosecond dynamics approach can be very powerful in addressing fine details of binding modes. Therefore, we are currently conducting a more detailed analysis on how differences in the chemical structures of drugs differentially affect the dynamics of individual adenine bases in the A-site to further expand the spectrum of this field.

■ ASSOCIATED CONTENT

Supporting Information

Methods and tables of parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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