THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

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Nucleotide Dynamics at the A-Site Cleft in the Peptidyltransferase Center of *H. marismortui* 50S Ribosomal Subunits

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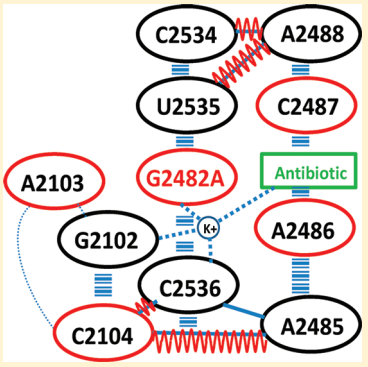
Supporting Information

ABSTRACT: Resistance mutations to antibiotics targeting rRNA can be far from the drug-binding site. Crystallography studies revealed that the antibiotic resistance mutation G2482A (G2447A in *E. coli*) in *Haloarcula marismortui* 50S ribosomes does not directly contact the drug or introduce changes to the ribosomal structure except for losing a potassium ion coordinated to a base triple at the drug-binding site. Using molecular dynamics simulations, we tested hypotheses regarding the effects of the G2482A mutation and ion coordination on the conformational dynamics of the 50S ribosome. Simulations show that the mutation enhances conformational fluctuation at the antibiotic binding site, weakens the hydrogen-bonding network, and increases flexibility at the 50S peptidyl transferase center (PTC). Our data supports the view that distant mutations can perturb the dynamic network in the ribosomal PTC, thereby raising the entropic cost of antibiotic binding. These results underscore the importance of considering conformational dynamics in rational drug design.



SECTION: Biophysical Chemistry and Biomolecules

Ribosome crystal structures have revolutionized structure-based drug design for RNA targets. Crystallographic studies of antibiotics bound to ribosomes provide a structural basis for the interpretation of decades of mutagenesis, chemical probing, and structure−activity relationships studies. Twenty-four different sites of single-nucleotide mutations across 14 different species cause resistance to A-site cleft antibiotics such as chloramphenicol, anisomycin, linezolid, and tiamulin (Tables S1 and S2, Supporting Information) that bind in the 50S ribosomal subunit peptidyl transferase center (PTC) between A2486 (2451 in *E. coli* numbering)[a] and C2487 (2452) (Figure 1). There are many cross species and species-specific mutations that confer resistance to A-site cleft antibiotics. Some of them do not occur at nucleotides that directly contact the antibiotic.[1−8] The mechanisms by which distance mutations confer drug resistance are not well understood.

Crystal structures of chloramphenicol bound to *T. thermophilus* 70S ribosomes at 3.2 Å show a nearly identical superposition of chloramphenicol and anisomycin and common potassium ion coordination.[7] In contrast, the crystal structure of chloramphenicol bound to *E. coli* 70S ribosomes at 3.5 Å shows a similar orientation of the antibiotic, an additional hydrogen bond to A2602 *E. coli* (2637 Hm), and no metal ion coordination.[8] Both 70S ribosome crystal structures show significant differences from the structure of chloramphenicol in *D. radiodurans*.[3] Although some resistance mutations can be explained rationally by observing the static crystal structures of anisomycin,[5,6] chloramphenicol,[3,7,8] or linezolid,[9,10] none of the crystal structures of A-site cleft antibiotics fully explain all 24 antibiotic resistance mutations, the species variations in antibiotic susceptibilities, or the species-specific patterns of resistance mutations.

The G2482A (2447) mutation in the 50S ribosomal subunit causes resistance for chloramphenicol, linezolid, and anisomycin, although G2482 is not in direct contact with the drugs[3,5−11] (Figure 1). The crystal structure of the mutant reveals no conformational change at the A-site cleft but only the displacement of a potassium ion coordinated by a base triple G2482 (2447)−A2486 (2451)−G2102 (2061) in the wild type.[5] The identity of K^+ was verified with rubidium soaks in *H. marismortui*.[12,13] Anisomycin interacts with K^+ and stacks with C2487 (2452), and A2488 (2453) moves to stack under C2487. The *Tetrahymena thermophila* 60S crystal structure,[14] however, did not yet identify a potassium ion at the PTC base triple but is sensitive to anisomycin. Thus, loss of the potassium ion coordination may only partially explain the loss of anisomycin binding. We hypothesized that the G2482A mutation alters the dynamics surrounding the antibiotic binding site, thus contributing to the mechanism of drug resistance.

To investigate the effects of mutation on nucleotide dynamics, we performed molecular dynamics (MD) simulations on the wild type and G2482A mutant of the 50S ribosomal subunit of *H. marismortui*. To test whether the effects of the G2482A mutation are solely due to the ion coordination or also due to the nucleotide identity at position 2482, simulation on the wild type with the displaced K^+ ion was also

Received: February 14, 2012
Accepted: March 22, 2012

   dx.doi.org/10.1021/jz3001882 | *J. Phys. Chem. Lett.* 2012, 3, 1007−1010

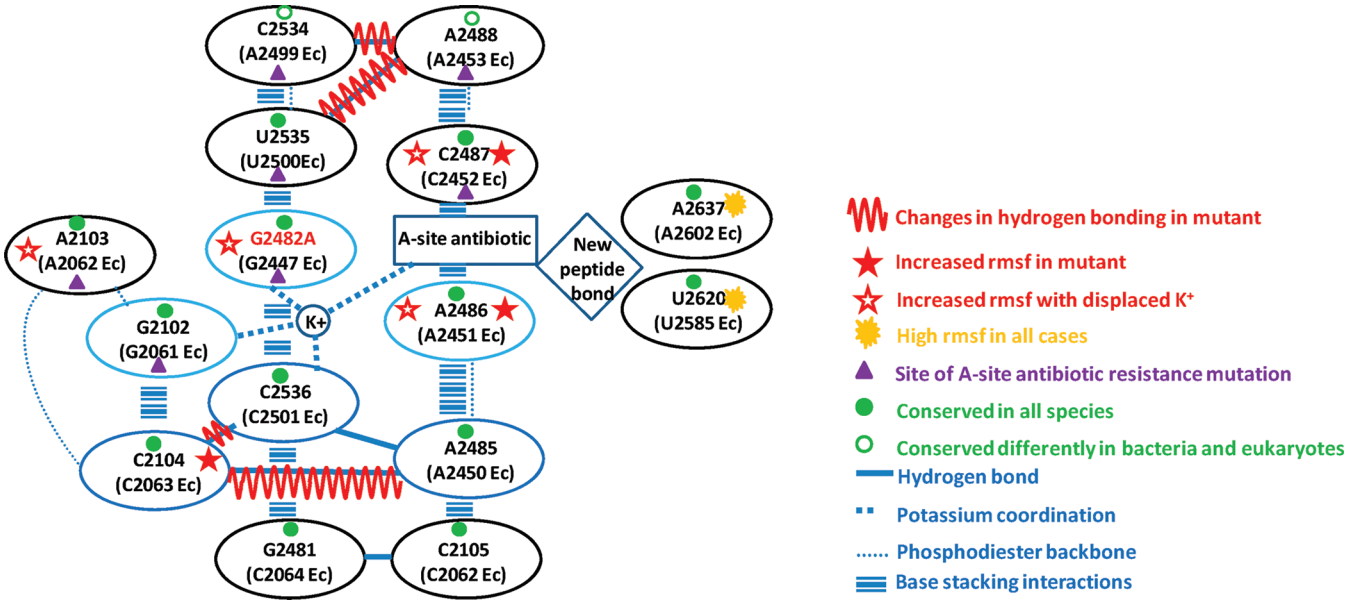


Figure 1. Schematic diagram for nucleotides in the peptidyl transferase center (PTC). Nucleotides are arranged to highlight base-stacking interactions. Dark and light blue circles indicate nucleotides in base triples. Some phosphodiester backbone connections and hydrogen bonds are omitted for clarity.

performed. These simulations reveal significant changes in the dynamic behavior of several nucleotides including A2486 (2451) and C2487 (2452), which form the antibiotic binding site. Compared to the wild type, the root-mean-square fluctuation (RMSF) for residue A2486 is increased in the simulations of the G2482A mutant and with the displaced K^+ ion (Figures 1 and 2). The RMSF for C2487 is also increased in

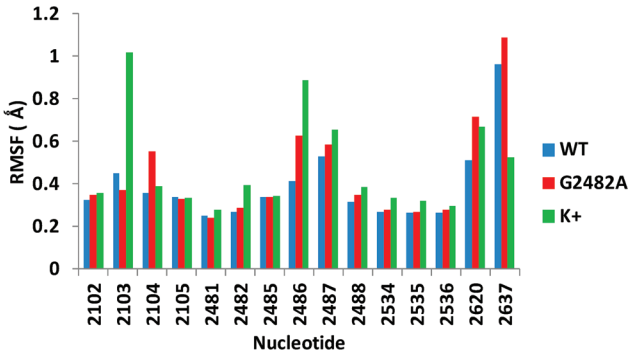


Figure 2. RMSF based on all heavy atoms in the simulations of wild type (blue), G2482A resistance mutation (red), and with a displaced K^+ ion (green).

the simulations of the G2482A mutant and with the displaced ion, although to a smaller extent than that for A2486. Interestingly, the RMSF for A2637 (2602), a flexible nucleotide that changes conformation depending on the substrate and that may form a hydrogen bond with chloramphenicol[8] is slightly increased in the simulation of the mutant but significantly decreased in the simulation with the displaced ion (Figure 2). The latter is a result of the more stable interactions with Asn110 of the ribosomal protein L10e. The RMSF for A2103 is significantly increased in the simulation with the displaced ion. A2103G is the most common chloramphenicol resistance mutation in *H. marismortui* (Table S1, Supporting Information), but electron density for the nucleotide is not observed in the crystals of 50S ribosome in the absence of bound ligands. Because A2103 is not near the A-site antibiotic cleft or the secondary chloramphenicol binding site in *H. marismortui* 50S crystals,[6] the change in the dynamics of A2103 is intriguing and may be linked to the difference in the chloramphenicol binding affinity in different species.

As compared to the wild type, simulation of the G2482A mutant shows significant changes in the hydrogen-bonding interactions in the base triple G2482 (2447)−A2486 (2451)−G2102 (2061), the adjacent base triple C2104 (2063)−C2536 (2501)−A2485 (2450), and the base pair A2488 (2453)−C2534 (2499) (Figure 1 and Table S3, Supporting Information). In the GAG base triple, while hydrogen bonding in G2482A−A2486 is significantly strengthened for both the mutant and with the displaced ion, hydrogen bonds form more frequently in G2102−A2486 during the simulation with the displaced ion. Surprisingly, hydrogen bonds are formed less frequently between A2488 and C2534 in the simulations of the mutant and with the displaced ion. Instead, A2488 more often forms hydrogen bonds with U2535. In the wild type, the movement of A2488 to stack with C2487 provides stabilization for the binding of anisomycin.[5] The A2488U mutation causes resistance to anisomycin,[5] although the nucleotide in this position is conserved as an adenine in bacteria and a uridine in eukaryotes (Table S2, Supporting Information). Reconciling these contrary biological and structural characteristics requires consideration of dynamic interactions involving A2488. The long-range effects on hydrogen bonding due to mutation of a single distant nucleotide suggest that the PTC nucleotides do not behave independently but rather as a concerted network.

To further understand the effects of mutation and ion coordination, we compare the global motion and correlation between local dynamics for the wild type, the G2482A mutant, and with the displaced K^+ ion. The principal component analysis allows us to view the collective motion projected onto the phase space. On the basis of the spread of the data points, we can see that mutation leads to sampling a larger phase space when compared to the wild type or the displaced ion simulations (Figure 3A), suggesting that the mutant exhibits a

dx.doi.org/10.1021/jz3001882 | J. Phys. Chem. Lett. 2012, 3, 1007−1010

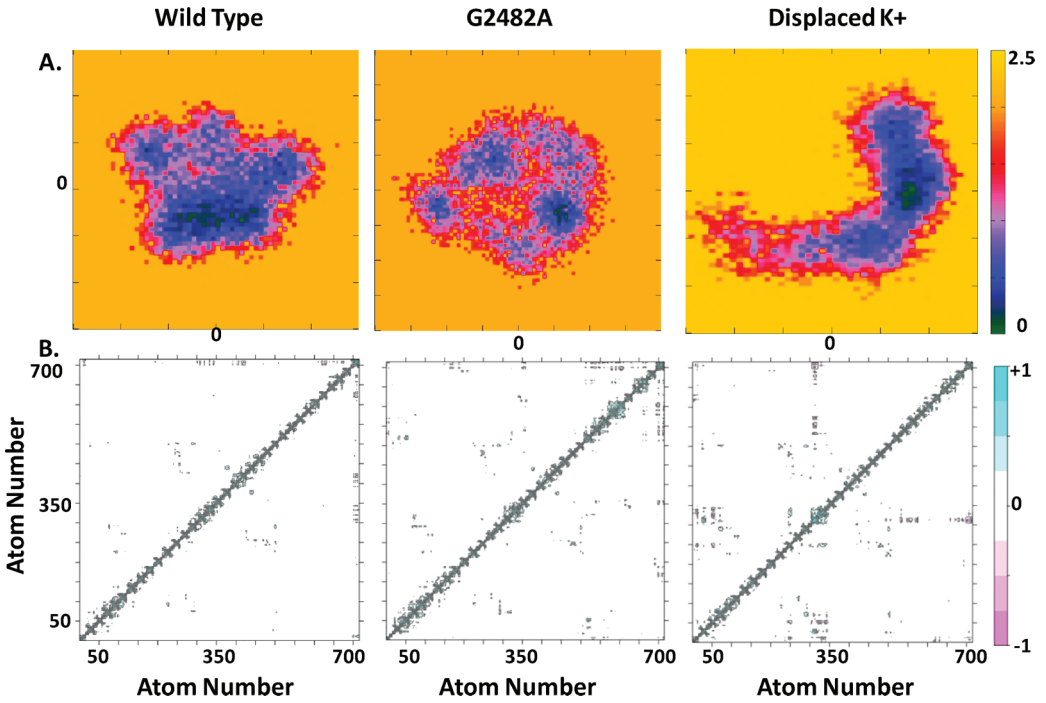


Figure 3. Collective motion and dynamic correlation between nucleotides. (A) Principal component analysis. Trajectory snapshots are projected onto the first two eigenvectors (with the largest eigenvalues). The intensity is shown as free energy (kcal/mol). (B) Dynamic cross-correlation matrix. Positive and negative correlations between different atoms are displayed in cyan and pink, respectively. Self-correlations are shown in the diagonal. Analyses were done on heavy atoms within a 20 Å sphere of A2486N3.

higher degree of flexibility in the PTC. The dynamic cross-correlation matrix reveals that the nucleotides that form the A-site antibiotic cleft exhibit different patterns of correlated motion (Figure 3B). For example, the mutant differs from the wild type in that A2486 shows a new positive correlation with G2482 but no longer shows any correlated movement with A2637 (Table S4, Supporting Information). In the simulation with the displaced potassium ion, A2486 shows new positive correlations with G2102 and G2536, and G2482 shows a very strong new correlation with C2487 (Table S4, Supporting Information). Thus, small changes in one ion or one nucleotide can have a significant effect on the dynamic behavior of many nucleotides in the PTC network.

Network dynamics has been used to explain effects of distant mutations in several protein enzymes. For example, ^{15}N NMR relaxation experiments revealed changes in the active-site and global dynamics due to mutations more than 10 Å away from the binding site in cyclophilin A.[15] Similar effects of distant mutations have been experimentally observed in dihydrofolate reductase,[16] src homology 3 domain protein,[17] and Ras signaling protein.[18] Hirsch suppressor mutations in tRNA also have long-range effects on the fidelity of decoding.[19] In *Saccharomyces cerevisiae*, the distant anisomycin resistance mutation Ψ2922C (2554 Ec and 2589 Hm) alters the chemical reactivity in U2984 (2580 Ec and 2615 Hm).[20] Mutations in protein L3 change tRNA binding and SHAPE (selective 2′hydroxyl acylation analyzed by primer extension) reactivities from far away.[21] Effects of network dynamics may contribute to the mechanisms of resistance for nucleotides distant to the A-site cleft in the ribosome PTC. The correct tRNA substrates may overcome higher entropic costs of binding a flexible A-site cleft more readily than a small-molecule antibiotic. Mutations that increase the flexibility of the binding site, thereby raising the entropic cost of binding, would have a larger effect on small-molecule antibiotic binding and thus present a mechanism for drug resistance.

In summary, the changes in the local dynamics of A2486, A2637, and A2103 and the changes in the hydrogen-bonding network for the G2482−A2486−G2102 base triple and A2488−C2534 pair confirm the importance of potassium ion coordination for the structure and dynamics of the *H. marismorti* PTC. The G2482A mutation changes the dynamics of nucleotides A2486 and C2487 in the A-site cleft, the hydrogen-bonding network involving both PTC base triples and A2488. In addition, the mutation increases the flexibility of the PTC center and alters the dynamic correlations between nucleotides. These long-range changes in the dynamic behavior due to a displaced potassium ion and the G2482A mutation are consistent with the view of the ribosome PTC nucleotides as a dynamic network. The simulation results support the hypothesis that distant mutations can change the dynamics of the antibiotic binding site. Although no single ribosome crystal structure with bound antibiotics explains all mutations that confer resistance to A-site cleft antibiotics or the species-dependent susceptibilities to antibiotics, each crystal structure captures a snapshot of important antibiotic−nucleotide interactions. A dynamic network model of the ribosome PTC may provide the synthesis for the conundrums posed by a highly conserved binding site with differing bacterial and eukaryotic antibiotic susceptibilities and long-distance resistance mutations. Thus, models including considerations of nucleotide dynamics will be essential for the development of new therapeutics that target rRNA.

■ COMPUTATIONAL METHODS

Simulations and data analyses were conducted using the GROMACS package (version 4.5.3).[22] The Amber 99 force field[23] was used with modified van der Waals parameters for K^+

dx.doi.org/10.1021/jz3001882 | J. Phys. Chem. Lett. 2012, 3, 1007−1010

and Cl^- ions[24] to avoid unrealistic ion aggregation.[25] Principal component analysis was performed based on the covariant matrix in Cartesian space using heavy atoms of nucleotides inside of the 20 Å sphere. Dynamic cross-correlation analysis was performed using Bio3D.[26] Detailed simulation protocols are included in the Supporting Information.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of antibiotic resistance mutations in different species, detailed protocols for the computational simulations, and tables for the simulations. 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 interest.

■ ACKNOWLEDGMENTS

The authors thank the Oklahoma Supercomputing Center for Education and Research (OSCER) for computing resources and Dr. Paul Whitford for information about the optimal parameters for potassium and chloride ions. Financial support is from NSF Grants MCB-1054547 (J.K.S.) and MCB-0844913 (S.J.S.), Donors of the American Chemical Society Petroleum Fund (J.K.S.), Oklahoma Center for the Advancement of Science and Technology (S.J.S.), and the University of Oklahoma.

■ ADDITIONAL NOTE

[a]The nucleotide numbering for *H. marismortui* 23 S rRNA is used throughout the text, and the number of the equivalently located *E. coli* nucleotide follows in parentheses.

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