Antibiotic Resistance

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# A Structural Basis for the Antibiotic Resistance Conferred by an A1408G Mutation in 16S rRNA and for the Antiprotozoal Activity of Aminoglycosides**

*Jiro Kondo**

Aminoglycosides are broad spectrum oligosaccharide antibiotics acting against a variety of Gram-negative and certain Gram-positive bacteria.[1] Their antibacterial mechanism has been revealed at the atomic level by several crystallographic studies of the 30S ribosomal particles,[2] the 70S full ribosomes,[3] and model oligonucleotides[4–14] in complex with various 2-deoxystreptamine aminoglycosides. Aminoglycosides specifically bind to the A site on 16S rRNA (Figure 1a)

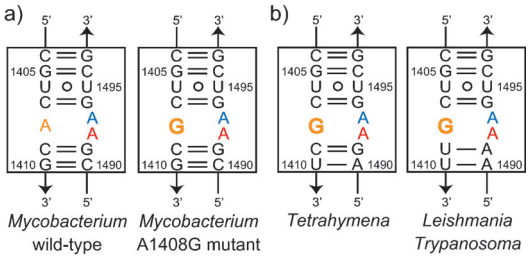


***Figure 1.*** Secondary structures of the a) bacterial and b) protozoal cytoplasmic A sites. A/G1408 orange, A1492 red, and A1493 blue. The rRNA residues are numbered according to the numbering used in *E. coli* 16S rRNA.

where a cognate tRNA is discriminated from near-cognate tRNAs based on base-pair geometries between codon and anticodon. The binding specificity is mainly conferred by ring I of aminoglycosides, which stacks on G1491 (*Escherichia coli* numbering) and makes a pseudo pair with the universally conserved A1408 in the bacterial A site (see Figure 2a right and Figure 3a right). These interactions force the A site to adopt the "on" state conformation in which A1492 and A1493 are fully bulged out and make A-minor interactions with the shallow/minor groove of the first two base pairs between mRNA codon and tRNA anticodon even when a near-cognate tRNA is delivered to the A site (Figure 2a right), thereby disturbing the fidelity of the decoding process.

While aminoglycosides have been prescribed for several bacterial infections, the major problem we have been facing for decades is the rapid increase of drug-resistant strains.[15] A single chromosomal mutation at position 1408 of 16S rRNA from A to G (see Figure 1a) has been found in clinically isolated drug-resistant strains of *Mycobacterium chelonae*,[16] *Mycobacterium tuberculosis*,[17–23] *Nocardia farcinica*,[24] and *Bartonella henselae*.[25] This mutation confers high-level resistance to aminoglycosides with an amino group at position 6' on ring I, but moderate resistance to those with a hydroxy group at the same position, such as geneticin (also known as G418), paromomycin, and lividomycin.[26–31] Interestingly, the secondary structure of the A1408G-mutant A site is highly analogous to that of the cytoplasmic A site of protozoa (Figure 1b). As evidence of this, it has been reported that geneticin and paromomycin with a 6'-OH group exhibit antiprotozoal activity[32–34] and that the protozoal cytoplasmic A site is susceptible to these aminoglycosides.[35]

Herein, the crystal structures of RNA duplexes containing two A sites of drug-resistant strains, with and without geneticin (A1408G-Geneticin and A1408G-Free hereafter), have been determined to gain insights into the antibiotic resistance conferred by the A1408G mutation and the antiprotozoal activity of aminoglycosides (Supporting Information Figure 1 ). In the A1408G-Geneticin crystal, the mutant A site adopts the "on" state conformation with fully bulged-out A1492 and A1493 (Figure 2b right). These adenine residues recognize the shallow/minor groove of consecutive G=C base pairs in a neighboring duplex through A-minor motifs. This mode of recognition perfectly mimics the A-minor interaction between the two bulged-out adenines from the A site and the codon-anticodon stem of the tRNA-mRNA complex occurring in the ribosome. The mutated G1408 residue is free from any base pair formation.

A geneticin molecule specifically binds to the deep/major groove of the mutant A site (Figure 2b right) and makes 15 direct interactions to base atoms and phosphate oxygen atoms (see Figure 4). Ring I of geneticin stacks on the G1491 residue and forms pseudo pairs with the Watson–Crick edge of G1408 (Figure 3b right). Two hydrogen bonds and a C–H···O interaction (O6′···H–$N2_{G1408}$, O5′···H–$N1_{G1408}$, and C1′–H···$O6_{G1408}$) are observed in the pseudo pair. On the opposite side of ring I, the O3′ and O4′ atoms make hydrogen bonds to the phosphate oxygen atoms of A1492 and A1493 (Figure 4), so that these adenine residues can adopt bulged-out conformations. The N3 atom of ring II makes hydrogen

[*] Dr. J. Kondo
Department of Materials and Life Sciences
Faculty of Science and Technology, Sophia University
7-1 Kioi-cho, Chiyoda-ku, 102-8554 Tokyo (Japan)
E-mail: j.kondo@sophia.ac.jp

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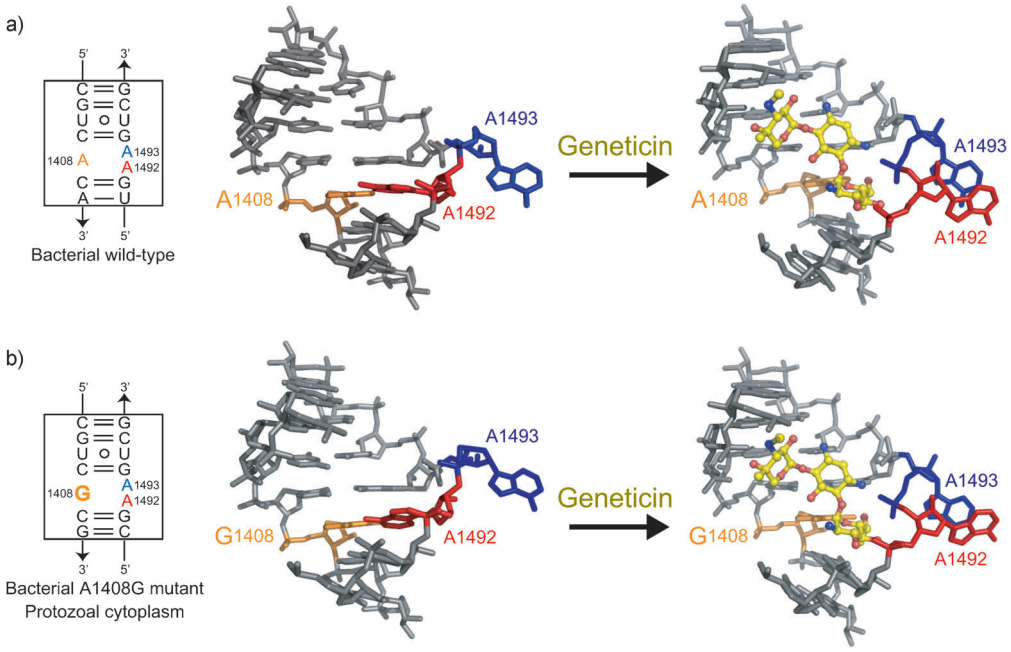


***Figure 2.*** Comparison of the two bacterial A-site molecular switches, a) the wild-type (protein data bank (PDB) code: 1T0E[11] and 1MWL[6]) and b) the A1408G mutant (present structures; PDB code: 3TD0 and 3TD1).

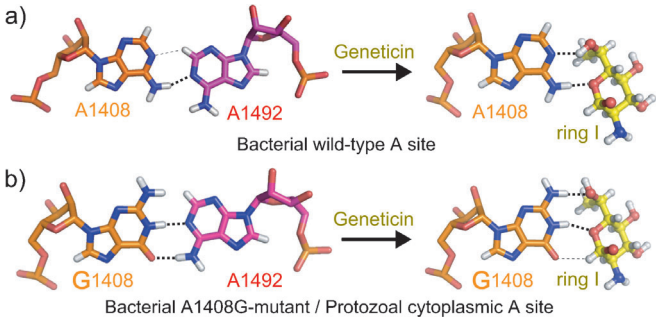


***Figure 3.*** Base pairs and pseudo pairs observed in the free and complex structures of the two bacterial A-site molecular switches, a) the wild-type (PDB code: 1T0E[11] and 1MWL[6]) and b) the A1408G mutant (present structures; PDB code: 3TD0 and 3TD1). Hydrogen atoms in white are for a better understanding of hydrogen bonds, N blue, O pink. Hydrogen bonds and C–H···O/N interactions are shown as bold and thin dashed lines, respectively.

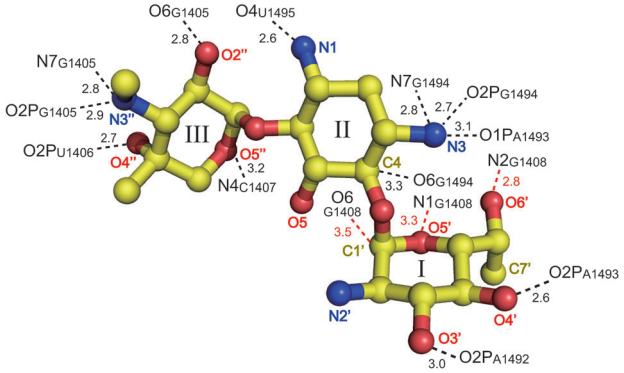


***Figure 4.*** Detailed interactions of geneticin (and ring numbering) with the bacterial A1408G-mutant A site. Direct hydrogen bonds and C–H···O interactions are represented by dashed lines with distances in Å. The interactions observed both in the bacterial wild-type and A1408G-mutant A sites are in black, and those solely in the mutant A site are in red.

bonds with the phosphate oxygen atoms of A1493 and G1494 thereby stabilizing A1493 in the bulged-out conformation (Figure 4). Ring III binds to the upper side of the A-site helix (Figure 4).

The binding mode of geneticin to the bacterial A1408G mutant A site is almost identical to that observed in the bacterial wild-type A site (Figure 2a right, Figure 2b right).[6] All interactions, except those in the pseudo pair between ring I and residue 1408, are conserved in both the wild-type and A1408G-mutant A sites. In the wild-type/geneticin complex, ring I forms a pseudo pair with A1408 through O6′–H···$N1_{A1408}$ and O5′···H–$N6_{A1408}$ hydrogen bonds (Figure 3a right). In the mutant/geneticin complex, the G1408 residue is slightly shifted toward the deep/major groove compared to

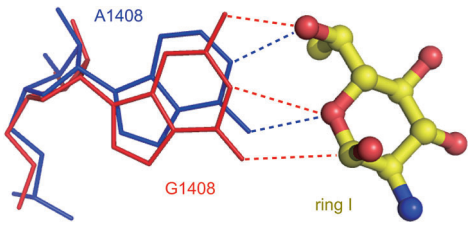


***Figure 5.*** Superimposition of pseudo pairs between A/G1408 and ring I of geneticin. Hydrogen bonds and a C–H···O interaction are represented by dashed lines.

the position of A1408 for making the pseudo pair with ring I (Figure 5). Clearly, a ring I with a 6′-$NH_3^+$ group cannot form the pseudo pair, because the $NH_3^+$ group repels N2-H of G1408. This observation agrees with the minimum inhibitory concentrations (MIC) of aminoglycosides against bacteria expressing 16S rRNA carrying the wild-type, A1408G-mutant or protozoal cytoplasmic A sites reported by Puglisi's and Böttger's groups.[26,29,31,35] The A1408G mutant strain of *E. coli* is not resistant to geneticin (the MICs of geneticin against the wild-type and the A1408G mutant strains are the same 2.5 μg mL$^{-1}$).[26] The A1408G mutant strain of *M. smegmatis* shows moderate resistance to

  

geneticin (the MICs against the wild type and the A1408G mutant are 16–32 $\mu g\,mL^{-1}$ and 64–128 $\mu g\,mL^{-1}$, respectively), but exhibits high-level resistance to gentamicin with a 6′-$NH_3^+$ group (the MICs against the wild type and the A1408G mutant are 1 $\mu g\,mL^{-1}$ and > 1024 $\mu g\,mL^{-1}$, respectively).[29,31] In addition, bacteria expressing 16S rRNA carrying the protozoal cytoplasmic A site is susceptible to geneticin (MIC = 4 $\mu g\,mL^{-1}$) but not to gentamicin (MIC > 1024 $\mu g\,mL^{-1}$).[35]

In the A1408G-Free crystal, the mutant A site takes a conformation different from that observed for the "on" state. The A1492 residue stays inside the A-site helix and forms a *cis* Watson–Crick base pair with the mutated G1408 residue through two hydrogen bonds, $N1_{A1492}\cdots H{-}N1_{G1408}$ and $N6{-}H_{A1492}\cdots O6_{G1408}$, but the A1493 residue is fully bulged out from the A-site helix (Figure 2b left, Figure 3b left). The same conformation has been observed in a crystal structure of the protozoal cytoplasmic 40S ribosomal subunit from *Tetrahymena thermophila* in complex with eukaryotic initiation factor 1 solved at 3.9 Å resolution.[36] Since one of the two adenines functioning in the decoding process is not bulged out, the conformation definitely corresponds to the "off" state of the bacterial A1408G mutant and protozoal cytoplasmic A sites. For the bacterial wild-type A site, several "off" states have been reported to date,[37] and one of them with a bulged-in A1492 and a bulged-out A1493 has been observed in crystal structures of the 70S ribosome[38] and an oligonucleotide[11] solved at 3.2 Å and 1.7 Å resolution, respectively (Only the higher resolution structure is shown in Figure 2a left, and both structures are shown in Supporting Information Figure 2). The bulged-in A1492 residue does not form a pair in the ribosome structure but forms a *cis* Watson–Crick pair with A1408 through $N1_{A1492}\cdots H{-}N6_{A1408}$ and $C2{-}H_{A1492}\cdots N1_{A1408}$ in the oligonucleotide structure (only the higher resolution structure is shown in Figure 3a left, and both structures are shown in Supporting Information Figure 2).

The structural similarities in the "off" and "on" states of the wild-type and A1408G-mutant A sites suggest that the A1408G mutation does not drastically disturb the function of the A-site molecular switch. In the "off" state, the A1492 residue forms a slightly more stable base pair with G1408 than with A1408 (Figure 3a left, Figure 3b left). Therefore, the free energy required for on/off switching of the decoding A site may be slightly higher for the A1408G mutant than for the wild type. Indeed, it has been reported that the A1408G mutation confers only a little reduction of the fitness of bacteria in the absence of antibiotics.[39]

Herein, it has been shown how bacteria acquire high-level resistance against aminoglycosides with a 6'-$NH_3^+$ group by the A1408G mutation in 16S rRNA. In addition, the structural basis of antiprotozoal activity of aminoglycosides with a 6′-OH group could be explained at the atomic level. The results may be useful for the structure-based drug design of new aminoglycosides with high activities against antibiotic-resistant bacteria and parasitic protozoa.

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