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Crystal Structure and Specific Binding Mode of Sisomicin to the Bacterial Ribosomal Decoding Site

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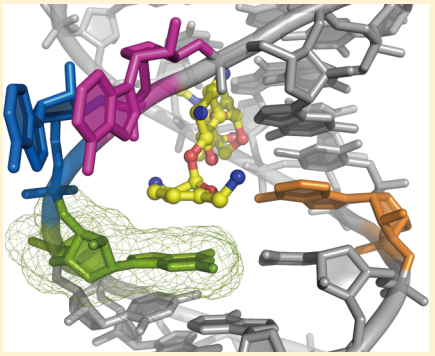
Supporting Information

ABSTRACT: Sisomicin with an unsaturated sugar ring I displays better antibacterial activity than other structurally related aminoglycosides, such as gentamicin, tobramycin, and amikacin. In the present study, we have confirmed by X-ray analyses that the binding mode of sisomicin is basically similar but not identical to that of the related compounds having saturated ring I. A remarkable difference is found in the stacking interaction between ring I and G1491. While the typical saturated ring I with a chair conformation stacks on G1491 through CH/π interactions, the unsaturated ring I of sisomicin with a partially planar conformation can share its π-electron density with G1491 and fits well within the A-site helix.



KEYWORDS: *aminoglycoside, decoding, ribosome, stacking interaction, X-ray analysis*

Sisomicin is an aminoglycoside antibiotic with broad spectrum activity against Gram-negative and some Gram-positive bacteria.[1] Like other aminoglycosides, it specifically targets the ribosomal decoding site (A site) and causes misreading of the mRNA codon during protein synthesis.[2] Sisomicin belongs to the 4,6-disubstituted class of aminoglycosides, and its chemical structure most closely resembles that of gentamicin C1a, but it differs from others by having unsaturated sugar ring I (Figure 1).[3−5] Interestingly, it has been reported that sisomicin is more effective than other structurally related aminoglycosides, such as gentamicin, tobramycin, and amikacin, against several bacterial species.[1] Therefore, the difference found in ring I is structurally small but functionally significant. Sisomicin is also an attractive compound as a starting material for designing semisynthetic aminoglycosides due not only to its high antibacterial activity but also to its ability to protect itself from several aminoglycoside-modifying enzymes, such as APH(3′) and ANT(4′), by lacking the 3′- and 4′-OH groups in ring I.[6] In the present study, we have solved crystal structures of the bacterial A site in complex with sisomicin to provide insight into the binding mode of the aminoglycoside at the atomic level.

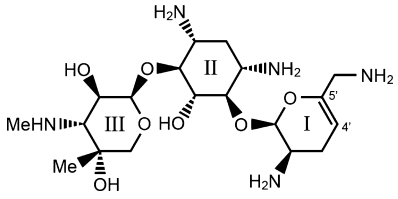


Figure 1. Chemical structure of sisomicin.

Two asymmetrical internal loops of the bacterial A site were inserted between Watson−Crick base pairs in a sequence designed to fold as a double helix. Such RNA fragments have been used as successful models in a series of crystallographic studies.[7−18] The RNA oligomer was chemically synthesized by Dharmacon (Boulder, CO) and FASMAC (Japan), purified by 20% polyacrylamide gel electrophoresis under a denaturing condition containing 8 M urea, and desalted by ultrafiltration or reversed-phase chromatography. Prior to crystallization, RNA solutions containing 1 mM RNA, 2 mM sisomicin sulfate, and 50 mM sodium cacodylate (pH 7.0) were prepared. Crystallizations were performed by the hanging-drop vapor diffusion method at 20 and 30 °C by mixing 1 μL of RNA solution and 1 μL of crystallization solution containing 50 mM sodium cacodylate (pH 7.0), 1 mM spermine tetrahydrochloride, 1% (v/v) 2-methyl-2,4-pentanediol, and 10−750 mM monovalent or divalent cation-chloride. Two types of crystals, called Siso-NH_4^+ and Siso-Na^+ hereafter, were obtained in conditions containing ammonium chloride and sodium chloride, respectively. The final optimized conditions are shown in Table 1 in the Supporting Information.

X-ray data of Siso-NH_4^+ and Siso-Na^+ were collected at 100 K with synchrotron radiation at the structural biology beamlines AR-NW12A in the Photon Factory (Japan) and BL38B1 in the SPring-8 (Japan). Data sets were processed with

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the program CrystalClear (Rigaku/MSC). The obtained intensity data were further converted to structure-factor amplitudes using TRUNCATE from the CCP4 suite.[19] The statistics of data collections and the crystal data are summarized in Table 1. Initial phases of Siso-NH_4^+ and Siso-Na^+ were

Table 1. Crystal Data, Statistics of Data Collections, and Structure Refinements

crystal code	Siso-NH_4^+	Siso-Na^+
	crystal data	
space group	$C2$	$P2_12_12$
unit cell (Å)	a = 115.1, b = 28.8, c = 43.7	a = 31.7, b = 107.7, c = 48.9
(°)	β = 97.2	
Z^a	1	1
	data collection	
beamline	AR-NW12A of PF	BL38B1 of SPring-8
wavelength (Å)	1.0	1.0
resolution (Å)	18.3−2.0	44.5−2.8
of the outer shell (Å)	2.1−2.0	2.9−2.8
unique reflections	9679	4352
completeness (%)	98.1	97.3
in the outer shell (%)	97.7	99.8
$R_{merge}{}^b$ (%)	3.0	7.3
in the outer shell (%)	27.1	49.4
redundancy	3.5	5.8
in the outer shell	3.5	6.8
	structure refinement	
resolution range (Å)	18.3−2.0	44.5−2.8
used reflections	9679	4349
R factor^c (%)	25.8	22.6
$R_{free}{}^d$	26.8	26.5
no. of RNA atoms	920	900
no. of aminoglycosides	2	2
no. of waters	63	1
rmsd bond length (Å)	0.005	0.006
rmsd bond angles (°)	0.9	1.0

[a]Number of dsRNA in the asymmetric unit. [b]$R_{merge} = 100 \times \Sigma_{hkl}|I_{hkl} - \langle I_{hkl}\rangle|/\Sigma_{hkl}\langle I_{hkl}\rangle$. [c]R factor = $100 \times \Sigma||F_o| - |F_c||/\Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are optimally scaled observed and calculated structure factor amplitudes, respectively. [d]Calculated using a random set containing 10% of observations that were not included throughout refinement.[31]

derived with the molecular replacement program AutoMR from the Phenix suite[20,21] using the coordinates of the bacterial A site in complex with tobramycin (PDB code: 1LC4).[8] The molecular structures were constructed and manipulated with the programs O and Coot.[22,23] The atomic parameters of the crystal structures were refined using the program CNS through a combination of simulated-annealing, crystallographic conjugate gradient minimization refinements, and B-factor refinements.[24] The statistics of structure refinements are summarized in Table 1. Molecular drawings were made using PyMOL.[25] The atomic coordinates of Siso-NH_4^+ and Siso-Na^+ have been deposited in the Protein Data Bank (PDB) with the ID codes 4F8U and 4F8V, respectively.

Crystal structures of both Siso-NH_4^+ and Siso-Na^+ are shown in Figure 2. The overall conformations of the RNA duplexes in both Siso-NH_4^+ and Siso-Na^+ crystals are almost identical to each other. In both structures, a single sisomicin molecule specifically binds to the deep/major groove of each A site. The root-mean-square deviations among the four independent copies of the A site complexed with sisomicin are in the

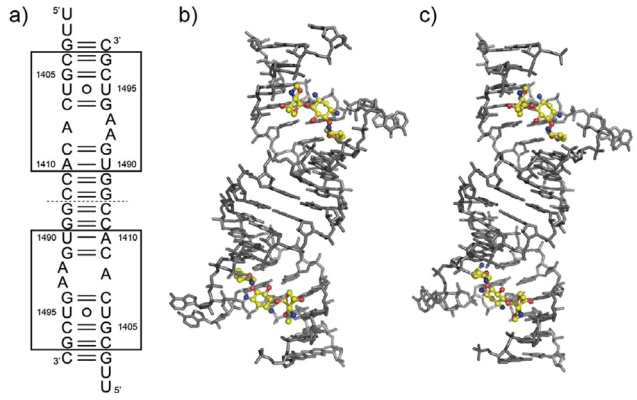

Figure 2. Secondary structure of the RNA duplex used in this study (a) and crystal structures of Siso-NH_4^+ and Siso-Na^+.

range of 1.0−1.2 Å. Differences are found mainly in the conformations of the bulged-out A1492 and A1493 residues (see Figure 1 in the Supporting Information). This indicates that the complexed binding pocket is rigid, but the switching residues are flexible. Hereafter, only the better defined A site (the one in the Siso-NH_4^+ crystal, which is of higher resolution) is used to represent the binding interaction with sisomicin.

The bacterial A site takes the "on" state with fully bulged-out A1492 and A1493 (Figure 3a). Because these adenine residues

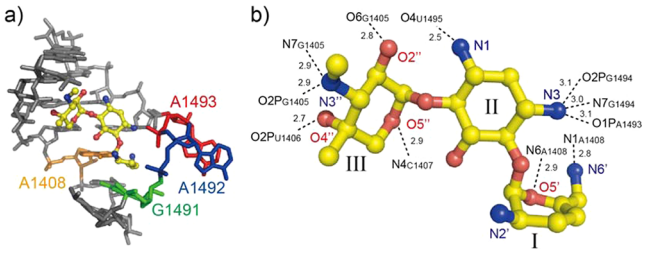

Figure 3. Binding (a) and detailed interactions (b) of sisomicin with the bacterial A site. Hydrogen bonds are represented by dashed lines with distances in Å. The A1408, G1491, A1492, and A1493 residues are colored in orange, green, blue, and red, respectively. The rRNA residues are numbered according to the numbering used in *E. coli* 16S rRNA.

recognize the shallow/minor groove of consecutive G═C base pairs in a neighboring duplex through A-minor motifs,[26] the crystal-packing interaction perfectly mimics the A-minor recognitions between the A site and the codon−anticodon stem occurring in the ribosome (see Figure 2 in the Supporting Information). At both ends of the A-site internal loop, five canonical Watson−Crick base pairs and a bifurcated U1406oU1495 pair are formed. The A1408 residue is free from any base pair formation.

A sisomicin molecule specifically binds to the deep/major groove of the bacterial A site (Figure 3a) and makes 11 hydrogen bonds to base and phosphate oxygen atoms (Figure 3b). Ring I of sisomicin is inserted into the A-site helix, stacks on the G1491 residue, and forms pseudo pairs with the Watson−Crick edge of A1408 (Figure 4). Two hydrogen bonds, N6′-H···$N1_{A1408}$ and O5′···H−$N6_{A1408}$, are observed in the pseudo pair. The N3 atom of ring II makes hydrogen bonds with the phosphate oxygen atoms of A1493 and A1494 so that A1492 and A1493 can take bulged-out conformations (Figure 3). Ring III binds to the upper side of the A-site helix through

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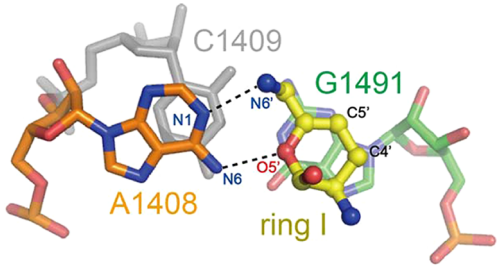

Figure 4. Pseudo pair and stacking interaction observed between ring I of sisomicin and RNA residues of the bacterial A site.

four hydrogen bonds (Figure 3). All hydrogen bonds observed between the sisomicin and the bacterial A site are identical to those observed in the complex between the A site and the gentamicin C1a (Figure 3 in the Supporting Information).[11]

Remarkable differences between sisomicin and gentamicin C1a are found in the structure and binding mode of ring I. Because sisomicin has a double bond between the C4′ and the C5′ atoms, ring I has a partially planar conformation (Figure 4). On the other hand, ring I of gentamicin C1a has a chair conformation as observed in other aminoglycosides (Figure 3 in the Supporting Information). Therefore, a characteristic stacking interaction between ring I and G1491 is observed for sisomicin. As observed in all crystal structures of complexes between the A site and the aminoglycosides,[7−12,16] the saturated carbohydrate ring I of gentamicin C1a with a chair conformation stacks on the aromatic G1491 ring through CH/π interactions (Figure 5b). Recent thermodynamic measurements and NMR studies confirmed the structural observations.[27] On the other hand, the unsaturated ring I of sisomicin can share its π-electron density with G1491 and fits well within the A-site helix, since the C4′═C5′ double bond in sisomicin ring I is parallel to aromatic ring of G1491 (Figure 5a).

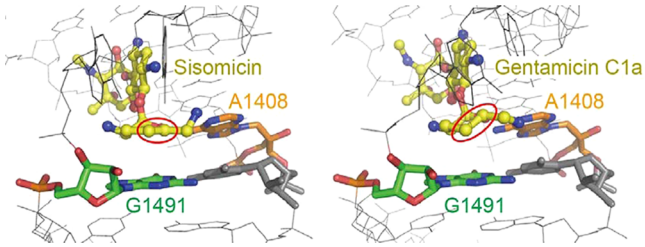

Figure 5. Stacking interactions between G1491 and ring I of sisomicin (a) or gentamicin C1a (PDB code: 2ET3) (b). The double and single bonds between C4′ and C5′ are highlighted by red circles.

Herein, the characteristic structure and binding mode of sisomicin have been revealed by X-ray crystallography. Sisomicin has been used as a lead compound for the development of next-generation aminoglycosides. For example, a sisomicin derivative ACHN-490 (Achaogen, San Francisco, CA), which has a hydroxylethyl group at position 6′ of ring I and a HABA group at position 1 of ring II, displays antibacterial activity against several aminoglycoside-resistant strains.[28,29] In addition, a sisomicin analogue with a substitution from 6′-NH_3^+ to 6′-OH group is found to possess antiprotozoal activity.[30] The structure information obtained in this study would be applicable for further drug design.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Table 1 and Figures 1−3. 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.

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