Crystal structures of the bacterial A site in complex with sisomicin

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1 Introduction
Sisomicin is an aminoglycoside antibiotic with broad spectrum activity. Like other aminoglycosides, it specifically targets the bacterial ribosomal decoding site (A site) and causes misreading of the mRNA codon during translation. Chemical structure of sisomicin most closely resembles that of gentamicin C1a, but it differs by having unsaturated sugar ring I (Fig. 1). Interestingly, it has been reported that sisomicin is more effective than other structurally-related aminoglycosides against several bacterial species. Therefore, the difference found in ring I is structurally small but functionally significant. In the present study, we have determined crystal structures of the bacterial A site in complex with sisomicin in order to provide insight into the binding mode of the aminoglycoside at the atomic level [1].

Fig. 1: Chemical structure of sisomicin

2 Experiment
The RNA oligomer designed to fold as a double helix containing two asymmetrical internal loops of the bacterial A site was chemically synthesized. Crystallizations of the RNA-sisomicin complex were performed by the hanging-drop vapor diffusion method. X-ray data were collected at 100K with synchrotron radiation at the structural biology beamlines AR-NW12A in the Photon Factory. Initial phases of the complex crystals were derived with the molecular replacement program AutoMR from the Phenix suite. The atomic parameters of the crystal structures were refined using the program CNS.

3 Results and Discussion
A sisomicin molecule specifically binds to the deep/major groove of the bacterial A site (Fig. 2a) and makes 11 hydrogen bonds to base and phosphate oxygen atoms (Fig. 2b). 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 (Fig. 3). All hydrogen bonds observed between sisomicin and the bacterial A site are identical to those observed in the complex between the A site and gentamicin C1a.

Fig. 2: Binding (a) and detailed interactions (b) of sisomicin with the bacterial A site.

Fig. 3: Pseudo pair between ring I and A1408.

Fig. 4: Stacking interaction between ring I and G1491.

References
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