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Crystal structure of the bacterial A site in complex with axial 4'-fluoro neomycin analog

Hiroki Kanazawa[1], Stephen Hanessian[2] and Jiro Kondo[1,*]

[1]Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554, Japan, [2]Department of Chemistry, Université de Montréal, CP 6128 Succ. Centre-Ville, Montréal Québec, H3C3J7, Canada

1 Introduction

Aminoglycosides (AGs) comprise a diverse group of potent broad-spectrum natural and semi-synthetic bacterial antibiotics used in clinical practice worldwide. Recent years, one of the problems in clinical practice is resistances against AGs. One of the mechanisms is modification of AG by AG-modifying enzymes such as *O*-phosphotransferases (APHs), which phosphorylates the oxygen of AG and inactivates AG. The phosphorylation depends on the nucleophilicity of the oxygen atoms. Therefore, the phosphorylation can be prevented by reducing the nucleophilicity by introducing the fluorine nearby. Before the present study, we have designed and synthesized some fluorinated novel AGs. Compound OS174 (Fig. 1), one of these AGs, is an axially 4'-fluorinated aminoglycoside. A minimum inhibitory concentration of OS174 against *P. aeruginosa* APH(3') indicates that OS174 is not inactivated. And surprisingly, the bactericidal effects of OS174 against several bacteria are remarkably increased. In the present study, we have performed X-ray analysis of the bacterial A-site molecular switch in complex with the OS174 to reveal a role of the fluorine in the AG binding [1].

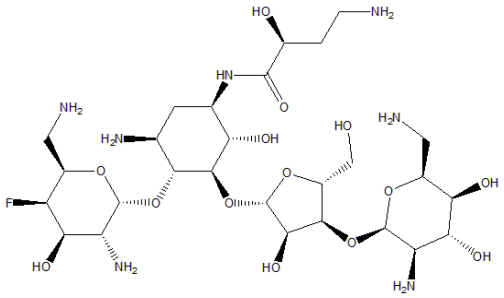

Fig. 1: Chemical structure of OS174

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/OS174 complex were performed by the hanging-drop vapor diffusion method. X-ray data were collected at 100K with synchrotron radiation at the structural biology beamline BL-17A in the Photon Factory. Initial phase of the complex crystal was derived with the molecular replacement program *AutoMR* from the *Phenix* suite. The atomic parameters of the crystal structure was refined using the program *CNS*.

3 Results and Discussion

OS174 specifically binds to the A-site switch and forces the two adenine residues to bulge out (Fig. 2a). Interactions between the AGs and the A-site are highly conserved in the Bact/OS174 complex. The axial 4'-fluorine (F4') of OS174 is accommodated by a fluorine-aryl interaction with G1491 (Fig. 3a). The F4' points toward and is in close contact with C2, N3 and C4 in G1491. The distances from F4' to these atoms are quite short compared to the sums of the van der Waals radii, and are similar to the distances from the H4' to the C2, N3 and C4 observed for the natural AG compounds[2] (Fig. 3b), suggesting that there could be non-negligible forces between these atoms. Since the F atom is a neutral electron-rich atom, it can make interactions with electron-poor atoms. Nucleic acid bases have electron-depleted π clouds. Therefore, it is reasonable that the axial F4' in OS174 can form a strong lone pair-π interaction with G1491.

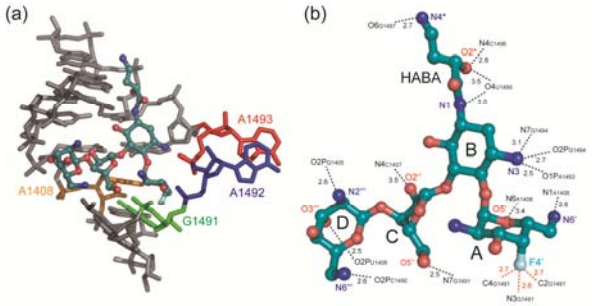

Fig. 2: Binding (a) and detailed interactions (b) of OS174 with the bacterial A site.

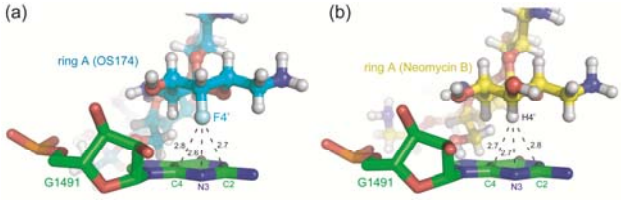

Fig. 3: Stacking interactions between ring A of (a) OS174 and (b) neomycin B [2] and G1491.

References

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[2] B. Francois *et al.*, *Nuceic Acids Res.* **33**, 5677-5690 (2005)

* j.kondo@sophia.ac.jp