NW12A/2010G585

Crystal structures of the bacterial and protozoal A sites in complex with 6'-hydroxysisomicin

Jiro Kondo[1,*] Mai Koganei[1], Juan Pablo Maianti[2], Vu Linh Ly[2] and Stephen Hanessian[2]
[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, C.P. 6128, Succursale Centre-Ville, Montréal, Québec H3C 3J7, Canada

1 Introduction

Aminoglycoside antibiotics specifically bind to the bacterial ribosomal decoding site (A site) and disturb the fidelity of protein synthesis. Interestingly, an aminoglycoside 6'-hydroxysisomicin with a 6'-hydroxy group exhibits activity against both bacteria and protozoa (Fig. 1), whereas its parent sisomicin with a 6'-amino group lacks antiprotozoal activity. Due to the similarity in the secondary structures of the bacterial and protozoal cytoplasmic A-site RNAs, the molecular mechanism of antiprotozoal activity has been considered to be the same as that of antibacterial activity. The only difference between these A sites is found at position 1408, which is an adenine in bacteria and a guanine in protozoa. In the present study, we have solved crystal structures of 6'-hydroxysisomicin bound to the bacterial and protozoal cytoplasmic A sites in order to understand the molecular mechanisms of its antibacterial and antiprotozoal activities [1].

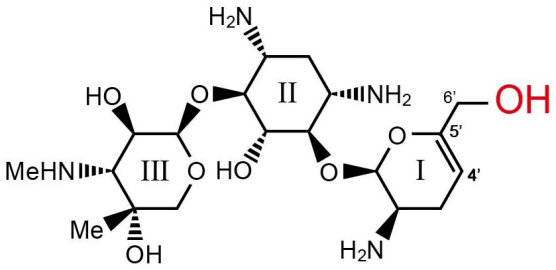

Fig. 1: Chemical structure of 6'-hydroxysisomicin

2 Experiment

RNA duplexes containing two bacterial or protozoal cytoplasmic A-site internal loops were used in the present studies. Crystallizations of the RNA-drug complexes were performed by the hanging-drop vapor diffusion method. X-ray data were collected with synchrotron radiation at AR-NW12A in the Photon Factory. Initial phases of the complexes 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 single 6'-hydroxysisomicin molecule specifically binds to the deep/major groove of the both bacterial and protozoal A sites, in which ring I is inserted into the A-site helix, stacks on the G1491 residue and forms pseudo pairs with the Watson-Crick edge of A/G1408 (Fig. 2). In the bacterial A site, two hydrogen bonds, N1(A)...H-O6' and N6(A)-H...O5', are observed between A1408 and ring I (Fig. 2a). On the other hand, two hydrogen bonds and one C-H...O interaction, N2(G)-H...O6', N1(G)-H...O5' and O6(G)...H-C1', are observed in the protozoal cytoplasmic A site (Fig. 2b).

While sisomicin is active only against bacteria, 6'-hydroxysisomicin possesses both antibacterial and antiprotozoal activity. Ring I of 6'-hydroxysisomicin forms a stable pseudo pair with G1408 of the protozoal cytoplasmic A site as described above. However, ring I of sisomicin with a 6'-NH_3^+ group cannot form a pseudo pair with G1408 because the NH_3^+ group repels both N2-H and N1-H of G1408.

It is very important to note that the secondary structure of the protozoal cytoplasmic A site is highly analogous to that of the bacterial A site with an A1408G mutation, which is the most prevalent antibiotic-resistant mutation found in clinical isolates. Therefore, the development of aminoglycosides with the 6'-OH group may also lead to useful antimicrobial activity against the A1408G antibiotic-resistant strains.

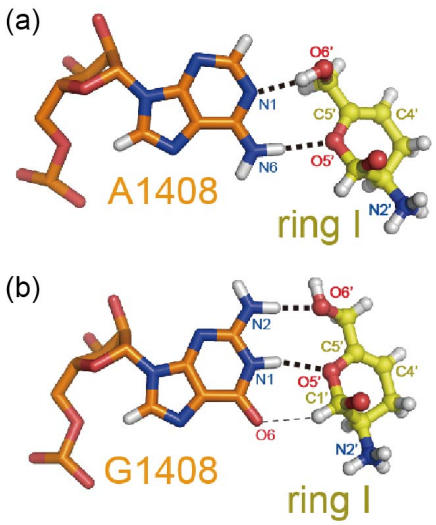

Fig. 2: Pseudo pairs between ring I and A/G1408.

References

[1] J. Kondo *et al*., *ChemMedChem*. **8**, 739 (2013).

* j.kondo@sophia.ac.jp