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

Jiro Kondo1,*, Mai Koganei1, Juan Pablo Maianti2, Vu Linh Ly2 and Stephen Hanessian2

1Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554, Japan, 2Department 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\textsubscript{3}+ group cannot form a pseudo pair with G1408 because the NH\textsubscript{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

* j.kondo@sophia.ac.jp