Poster Presentation

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Crystal structure of the M14R mutant of oryctin in complex with trypsin

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Oryctin is a 66-amino-acid protein purified from the larval haemolymph of the coconut rhinoceros beetle Oryctes rhinoceros, which shows no sequence similarity to any other protein known. We determined the solution NMR structure of oryctin, and found that oryctin had a similar backbone fold to the turkey ovomucoid domain 3, OMTKY3, a Kazal-type serine protease inhibitor [1]. Based on the structural similarity, we tested the serine protease inhibitory activity of oryctin, and found that oryctin does inhibit some serine proteases, such as α-chymotrypsin, endopeptidase K, subtilisin Carlsberg, and leukocyte elastase [1]. However, oryctin cannot inhibit trypsin at all. In this study, we have introduced point mutations to the putative inhibition loop of oryctin to obtain oryctin mutants that can inhibit trypsin. Then, we have solved the crystal structure of such an oryctin mutant, M14R-oryctin with a Ki value of 3.4 ± 0.8 nM, in complex with trypsin to reveal how it binds to and inhibits trypsin. As predicted, the putative inhibition loop lay on the substrate binding cleft of trypsin. Particularly, the side chain of R14 fit into the S1 pocket of trypsin by forming hydrogen/ionic bonds with D191, S192 and G216 at the bottom of the S1 pocket and G195, D196, S197 and S212 at its entrance. In addition, R65 located in the C-terminal α-helix of M14R-oryctin formed hydrogen bonds with S40 and F44 of trypsin. The latter interaction, which is unique to oryctin, enhances its binding affinity to trypsin.

[1] *Horita S. et al. (2010). J. Biol. Chem. 285, 30150-30158.*

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