Poster Presentation

BM.P37

Structural study of the Schiff base copper(II) chelates as thrombin inhibitor

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Thrombin is a trypsin-like serine protease that plays a critical role in the blood coagulation cascade. Thus, studies on thrombin-specific inhibitor are useful for the design of clinical useful compounds. We have previously reported of the Schiff base metal chelates carrying benzamidine and amino acid moieties. More than 50 kinds of Schiff base copper(II) chelates were synthesized from *p*- or *m*-amidinosalicylaldehyde and various L- or D-amino acids. These chelates have effective inhibitory activity for trypsin and thrombin (K_i = 10^{-5} ~ 10^{-6} M). In this series of inhibitors, the copper(II) chelate derived from *p*-amidinosalicylaldehyde and D-tryptophan (chelate **1g'** in original paper) have shown exceptionally potent inhibitory activity against thrombin (K_i = 2.7×10^{-8} M). To elucidate the structural basis of this high efficient inhibition, we were planning to determine and compare with four protein-inhibitor complex structures, chelate **1g'** or **2g'** bound to trypsin or thrombin (chelate **2g'** is derived from *m*-amidinosalicylaldehyde and D-Trp). The crystals of trypsin binding the chelate **2g'** was obtained by sitting-drop vapor diffusion method by mixing 2.5 µL of 20 mg/mL protein-chelate complex solution with 2.5 µL of 0.1 M HEPES, pH 7.4, containing 0.1 M $Li_2(SO_4)$, 25.0% PEG 3350. In the crystal structure, the imidazole nitrogen of His57 is coordinated with the copper(II) ion (1.94 Å). This close contact is made possible by conformation change of the His57. The indole ring of the chelate **2g'** simultaneously interacts with the copper(II) and the His57 by cation-π and π-π stacking interaction, respectively. In addition to trypsin-chelate **2g'** complex structure, we will report on the other three complex structures of trypsin-chelate 1g', thrombin-chelate **1g'** and thrombin-chelate **2g'**.

[1] *E. Toyota, H. Sekizaki, Y. Takahashi, K. Itoh, K. Tanizawa, Chem Pharm Bull (Tokyo), 2005, 53(1), 22-6*

Keywords: enzyme-inhibitor complex, thrombin inhibitor, medicinal chemistry