Poster Presentation

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Neutron crystal structure of human FPPS complexed with risedronate

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Nitrogen-containing bisphosphonates (N-BPs), such as risedronate and zoledronate, are currently used as clinical drug for bone-resorption diseases and are potent inhibitor of farnesyl pyrophosphate synthase (FPPS). The potential of N-BPs as antitumor agents has also been suggested by the several in vitro and in vivo preclinical studies. However, BP drugs limit their therapeutic use to bone-related diseases, because BPs are highly charged and water soluble molecules. X-ray crystallographic analyses of FPPS with N-BPs have revealed that N-BPs bind to FPPS with three magnesium ions and several water molecules. In order to develop a novel FPPS inhibitor, the hydrogen-bond networks formed by FPPS, BPs and water molecules are necessary to be elucidated. To understand the structural characteristics of N-BPs bound to FPPS, including hydrogen atoms and hydration by water, neutron diffraction studies were initiated using BIODIFF at the Heinz Maier-Leibnitz Zentrum (MLZ). FPPS-risedronate complex crystals of approximate dimensions 2.8 × 2.5 × 1.5 mm (~ 3.5 mm³) were obtained by repeated macro-seeding. Monochromatic neutron diffraction data were collected to 2.4 Å resolution with 98.4% overall completeness and 10.7% Rmerge. As a result of X-ray/neutron joint refinment, R and Rfree values for the neutron data were 19.6 and 23.3%, respectively. This neutron structure clearly reveals the protonation state of risedronate, hydration in the inhibitor-binding region. Furthermore, the amide H/D exchange analysis showed that there is a highly rigid region which regulate the structural change upon the binding of the ligands. Here we will discuss the detailed hydrogen-bond network and the protonation state of FPPS and risedronate.

Keywords: Neutron protein crystallography, farnesyl pyrophosphate synthase, bisphophonate