

Time-Resolved β -lactam Cleavage by L1 Metallo- β -Lactamase

Andrzej Joachimiak¹, Mateusz Wilamowski², Darren Sherrell³, Youngchang Kim⁴, Alex Lavens⁵, Robert Henning⁶, Krzysztof Lazarski⁶, Michael Endres⁷, Natalia Maltseva⁸, Gyorgy Babnigg⁹, Shawn Burdette¹⁰, Vukica Srajer¹¹

¹Argonne National Lab/University of Chicago ²Center for Structural Genomics of Infectious Diseases, ³N/A, ⁴Argonne National Lab, ⁵Argonne National Lab, ⁶N/A, ⁷Argonne National Lab, ⁸Center for Structural Genomics of Infectious Diseases, ⁹Center for Structural Genomics of Infectious Diseases, ¹⁰Argonne National Lab, ¹¹WORCESTER POLYTECHNIC INSTITUTE, ¹²CARS, Univ of Chicago
andrzejj@anl.gov

The time course and corresponding binding and chemical transformations during enzyme catalysis were concealed for many years. Advances in structural biology such as room temperature serial x-ray crystallography provide opportunity to uncover binding events, and subsequent chemical conversions occurring during enzymatic reaction. Here we reveal the structure of intact β -lactam moxalactam antibiotic bound to wild-type L1 metallo- β -lactamase (MBL) from *Stenotrophomonas maltophilia*. Using time-resolved (TR) serial synchrotron crystallography (SSX) we showed the time course of β -lactam cleavage and assembled molecular movie with 10 time points spanning 4 seconds. Starting with crystals of L1 MBL with "empty" active site, the process was initiated by laser pulse releasing Zn²⁺ ions from a UV-labile zinc-photocage. Two Zn²⁺ ions bind to active site, followed by binding of moxalactam. In the shortest data point (20 ms) two bound Zn²⁺ ions and partial electron density for moxalactam are observed. For 100 ms after photolysis the intact β -lactam ring is seen. At 150 ms an opened β -lactam ring of moxalactam is detected and the part of the ligand is significantly displaced. The reaction product conformational adjustments continue until reaching steady state at 2000 ms corresponding to the relaxed state of enzyme. Strikingly very small changes are observed in positions of metal ions and the active site amino acids. The structural arrangement of L1 active site is the most common in MBLs, therefore, captured here mechanistic details should be generalized to other enzymes. These observations can aid development of more effective inhibitors against emerging pathogens with MBL activity.