

W0188

Cephalosporin Acylase: A Protein with Two Chemistries. Jin kwang Kim, In Seok Yang, Hye Jung Shin, Ki Joon Cho, Eui Kyung Ryu, Sun Hwa Kim, Sung Soo Park, Kyung Hyun Kim, Dept. of Life Sciences & Biotechnology, School of Life Sciences & Biotechnology, 5-1, Anam-dong, Sung-buk ku, Seoul 136 701, SOUTH KOREA.

Cephalosporin acylase (CA), a member of the N-terminal nucleophile (Ntn) hydrolase family, is activated through sequential primary and secondary autoproteolytic reactions with the release of a pro-segment. We have determined crystal structures of four CA mutants. Two mutants are trapped after the primary cleavage, and the other two undergo secondary cleavage slowly. These structures provide a first look at the pro-segment conformation during activation in Ntn hydrolases. The highly-strained helical conformation of the precursor pro-segment is transformed into a relaxed loop conformation in the intermediates, suggesting that the relaxation of structural constraints drives the primary cleavage reaction. The secondary autoproteolytic step has been proposed to be intermolecular. However, our analysis provides evidence that CA is processed in two sequential steps of intramolecular autoproteolysis involving two distinct proteolytic mechanisms, the first mediated by a serine residue and the second by a glutamate.