Structural basis for the recognition of muramyltripeptide by *Helicobacter pylori* Csd4, a D,L-carboxypeptidase controlling the helical cell shape

Hyerry Jeon[a], Hyoun Sook Kim[a,b,c], Ha Na Im[a,b], and Se Won Suh[b]

[a]National Cancer Center, Gyeonggi 410-769, [b]Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, [c]Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Helicobacter pylori infection causes a variety of gastrointestinal diseases, including peptic ulcers and gastric cancer. Its colonization of the gastric mucosa of human stomach is a prerequisite for the survival in the stomach. Colonization depends on its motility, which is facilitated by the helical shape of the bacterium. In *H. pylori*, crosslinking relaxation or trimming of peptidoglycan muropeptides affects the helical cell shape. Csd4 has been identified as one of the cell shape-determining peptidoglycan hydrolases in *H. pylori*. It is a Zn^{2+}-dependent D,L-carboxypeptidase that cleaves the bond between the γ-D-Glu and *m*DAP of the uncrosslinked muramyltripeptide (muramyl-L-Ala-*γ*-D-Glu-*m*DAP) of the peptidoglycan to produce the muramyldipeptide (muramyl-L-Ala-*γ*-D-Glu) and *m*DAP. Here we report the crystal structure of *H. pylori* Csd4 (HP1075 in strain 26695) in three different states, i.e., the ligand-unbound form, the substrate-bound form, and the product-bound form. *H. pylori* Csd4 consists of three domains: (i) the N-terminal D,L-carboxypeptidase domain of the typical carboxypeptidase fold, (ii) the central β-barrel domain of a novel fold, and (iii) the C-terminal immunoglobulin-like domain. The D,L-carboxypeptidase domain recognizes the substrate by interacting primarily with the terminal *m*DAP moiety of the muramyltripeptide. It undergoes a significant structural change upon binding either *m*DAP or the *m*DAP-containing muramyltripeptide. We also show that Csd5, another cell-shape determinant in *H. pylori*, is capable of interacting not only with *H. pylori* Csd4 but also with the dipeptide product of the reaction catalyzed by Csd4.