MS5-P3 Structural characterization of *Helicobacter Pylori* pathogenic proteins. The case of α-carbonic anhydrase

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Helicobacter Pylori is a gram-negative, flagellated, spiral-shaped bacterium, able to colonize the human stomach, a very peculiar niche. It affects more than half of world's population. Its infection can lead to severe gastroduodenal diseases, like chronic gastritis, gastric and duodenal ulcer, gastric adenocarcinoma and mucosa-associated lymphoma. Therefore, *H. Pylori* contagion is a worldwide problem. Several bacterial species are able to transit the human stomach, but only one, *H. Pylori*, survives intragastric acidity long enough to establish a chronic colonization. There are several mechanisms involved in the acidic acclimation; particularly important in this aspect are the enzymes urease and carbonic anhydrase. Two different carbonic anhydrases (CA) are coded by Helicobacter Pylori: the alpha-CA, with periplasmic localization, and the beta-CA, with cytoplasmic localization. Carbonic anhydrases are metalloenzymes that catalyze the hydration of carbon dioxide to bicarbonate, crucial for the neutralization of the pH and the consequent survival of bacterium in the acidic environment of the stomach. The active site of the alpha-CA consists of a Zn^{2+} ion coordinated by three histidine residues. This enzyme is inhibited by many sulfonamide/sulfamate compounds and the growth of the pathogen can be blocked; thus alpha-CA represent a promising drug target for the treatment of patients infected by drug-resistant strains. *H. Pylori* alpha-CA was cloned as fusion protein with 6-His-tag in the pETit C-His vector, without the signal peptide at the N-terminus. The plasmid was transformed in the recombinant *Escherichia Coli* strain BL21(D3). The protein was purified by IMAC chromatography and gel-filtration. The His-tag was removed by incubation with TEV protease. Crystals were grown by vapor diffusion sitting drop technique. The diffraction data were collected at 1.5 Å resolution and the three-dimensional structure was solved by molecular replacement.

Keywords: Helicobacter Pylori, carbonic anhydrase, metalloenzyme, acidic acclimation

MS5-P4 NowGFP: a green fluorescent protein with an anionic tryptophan-based chromophore

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A green-emitting fluorescent variant NowGFP with the tryptophan-based chromophore (Thr65-Trp66-Gly67) was recently developed from cyan mCerulean by introducing 18 point mutations. NowGFP is characterized by bright green fluorescence at physiological and higher pH and by weak cyan fluorescence at low pH. Illumination with blue light induces irreversible photoconversion of NowGFP from a green to cyan-emitting form. We report here the X-ray structures of the intact NowGFP at pH 9.0 and pH 4.8, and of its photoconverted variant, NowGFP_conv, at 1.35, 1.18, and 2.5 Å resolution, respectively. The structure of NowGFP at pH 9.0 suggests the anionic state of Trp66 of the chromophore to be the primary cause of its green fluorescence. At both examined pH, Trp66 predominantly adopts *cis* conformation; only ~20% of *trans* conformation was observed at pH 4.8. It was shown that Lys61 is a key residue, playing a central role in the chromophore ionization; it adopts two distinct pH-dependent conformations. At high pH, the side chain of Lys61 forms two H-bonds, one with the indole nitrogen of Trp66 and the other with the carboxyl group of the catalytic Glu222, enabling an indirect non-covalent connection between them that promotes Trp66 deprotonation. At low pH, the side chain of Lys61 is directed away from Trp66 and forms an H-bond with Gln207. Here we observe that photoconversion of NowGFP is accompanied by decomposition of the Lys61 side chain. The residues Lys61, Glu222, Thr203, and Ser205 form a local H-bond network connected with the indole ring of the chromophore Trp66; replacement of any of these residues dramatically affects the spectral characteristics of NowGFP.

Keywords: fluorescent proteins, photoconversion