Characteristics of the HP1021 orphan response regulator as a putative redox sensor in *Helicobacter pylori*

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Abstract

Helicobacter pylori is a gram-negative, microaerophilic bacterium inhabiting the environment of the human digestive system. *H. pylori* has developed several defence mechanisms to survive in the human stomach unfavourable conditions, including exposure to oxidative stress. However, the presence of a typical oxidative stress sensor protein in *H. pylori* has not been demonstrated so far.

This work focused on the characteristics of the HP1021 orphan response regulator's regulation mechanism. It was shown that a protein probably consists of two domains, the N- and C-terminal domains. The C-terminal domain contains the HTH DNA binding motif. Notably, the HP1021 protein has 3 cysteine residues in each of the potential domains. Since cysteine residues in a protein are usually conservative, and often found in redox regulators, it was hypothesized that HP1021 is an *H. pylori* redox sensor.

In further studies, it was shown that redox conditions affect the affinity of binding HP1021 to DNA *in vitro*. Moreover, it was shown that HP1021 binds Zn^{2+} ions and that the interaction is dependent on the redox conditions and influences the affinity of HP1021 binding to DNA. Studies of the HP1021 muteins, in which cysteine residues were changed into alanine residues, indicated that the N-terminal domain may play a regulatory role and that the interaction of the two domains may be important for the regulation of HP1021 activity dependent on redox conditions and Zn^{2+} ions.

In vivo studies showed oxidation of cysteine residues in the HP1021 protein after exposure of the bacterial culture to atmospheric oxygen. Moreover, it was shown that HP1021 influences the transcription of selected genes after subjecting *H. pylori* bacteria to oxidative stress.

The obtained results indicated that the HP1021 protein acts as a sensor of oxidative stress in *H. pylori*.