

Chulabhorn Research Institute

Special Lecture

*“ Characterization of the Indirect Pathway for Asn-tRNA(Asn)
Biosynthesis Used by the Human Pathogen Helicobacter pylori ”*

By

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1st Floor Conference Room, Chemistry Research Building

Characterization of the indirect pathway for Asn-tRNA^{Asn} biosynthesis used by the human pathogen *Helicobacter pylori*

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Abstract

The genome of the pathogenic bacterium *Helicobacter pylori* (*H. pylori*) lacks genes encoding for asparaginyl-tRNA synthetase (AsnRS) and glutaminyl-tRNA synthetase (GlnRS); this bacterium relies on Asp-tRNA^{Asn}/Glu-tRNA^{Gln} amidotransferase (Asp/Glu-Adt) activity in order to synthesize Asn-tRNA^{Asn} and Gln-tRNA^{Gln} from mischarged Asp-tRNA^{Asp} and Glu-tRNA^{Gln}. Furthermore, in order to generate these two misacylated aminoacyl-tRNAs as substrates for Asp/Glu-Adt, the tRNA specificities of two *H. pylori* AARSs are modified: This organism's non-discriminating aspartyl-tRNA synthetase (ND-AspRS) generates Asp-tRNA^{Asp} and Asp-tRNA^{Asn}, and *H. pylori*'s glutamyl-tRNA synthetase 2 (GluRS2) generates Glu-tRNA^{Gln}, specifically.

Here we report the first characterization of the ND-AspRS from *H. pylori*. This enzyme aminoacylates both tRNA^{Asp} and tRNA^{Asn}, however the cognate tRNA^{Asp} is a better substrate. Overexpression of this ND-AspRS in *E. coli* results in a toxic phenotype, which can be rescued by co-expression of *H. pylori* Asp/Glu-Adt. Kinetic analyses of wild-type *H. pylori* AspRS as well as two mutations in the anticodon binding domain demonstrate that this toxicity is correlated to the specificity ratio of tRNA^{Asp}/tRNA^{Asn}. The determination of the mechanism behind the toxicity of this enzyme utilizing an *E. coli* *trpA34*, a tryptophan auxotroph, suggests that this ND-AspRS is generating Asp-tRNA^{Asn} *in vivo* and aspartate is being erroneously introduced into proteins at asparagine codons.

The heterotrimeric Asp/Glu-Adt from *H. pylori* was cloned and purified to homogeneity. This enzyme was shown to be catalytically active using a newly developed thin layer electrophoresis (TLE) technique.

Finally, Hp0100, a protein with unknown function, has been successfully cloned, overexpressed, and purified to homogeneity. This protein enhances the deacylation of both Asp-tRNA^{Asp} and Asp-tRNA^{Asn} *in vitro*. This enhancement is even more pronounced in the presence of *H. pylori* ND-AspRS, an enzyme that is not known to have deacylation activity.

