Synthesis of signaling nucleotides [(p)ppGpp and (p)ppApp] by an RSH enzyme from Methylobacterium extorquens AM1

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The bacterial stringent response is a widespread regulatory phenomenon accompanying their adaption to changing environmental conditions. It is mediated by two nucleotide analogs of GDP and GTP, i.e. the ppGpp and pppGpp alarmones, collectively called (p)ppGpp. Long ago it was discovered that nutritional and other stresses trigger a rapid accumulation of (p)ppGpp, which causes a global cellular response that diverts resources away from growth to promote stress survival instead. Accumulation of (p)ppGpp potentially is medically important because it is essential for pathogens to survive the stress of host cell defenses and to viably persist despite the presence of antibiotics.

The first goal of the study presented here was identification and purification of a protein responsible for (p)ppGpp synthesis in *Methylobacterium extorquens* AM1. By using bioinformatics methods, one potential RSH enzyme was identified. State-of-the-art methods were employed together with the construction of a protein fragment containing only the N-terminal (p)ppGpp hydrolase and synthetase domains, without the C-terminal regulatory domain. Such an approach yielded a homogenous and active protein preparation. After successful purification, the RSHMex 1-352 enzyme was characterized biochemically *in vitro*. For efficient alarmone synthesis, the RSHMex 1-352 enzyme requires cobalt cations, which is uncommon for all so far biochemically characterized RSH enzymes. By using two-dimensional chromatography, it was determined here that the purified enzyme synthesizes pppGpp, ppGpp, and pppApp nucleotides, both *in vivo* and *in vitro.* This is first documented case of pppApp synthesis by an RSH enzyme. RSH_{Mex}1-352 has a much higher affinity for ATP than for GTP which is exclusive to this enzyme. In addition, this protein is also active when expressed from a plasmid in *E. coli* cells (with no active host (p)ppGpp synthetase present). In addition, the final results in this dissertation prove that pppApp is synthesized by *Methylobacterium extoquens* AM1 and what is extremely intriguing, also in E. *coli* cells. This may suggest that a new potential alarmone (pppApp) is involved in the stringent response.