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Novel lysine methyltransferases in hyperthermophilic crenarchaea

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Background

Methylation is a common post-translational modification. In proteins, it usually occurs at positively charged lysine or arginine residues and results in an increase to the residue's pK_a and therefore the strength of ionic interactions linked to this residue. Consequently, methylation can be seen as a means by which hydrophilicity, solubility and other protein characteristics can be controlled. For instance, post-translational lysine methylation has been shown to play a role in increasing protein thermostability in *Sulfolobus acidocaldarius* DNA binding protein Sac7d [1].

In eukaryotic organisms, lysine methylation is catalysed by highly specific methyltransferases (MTases). The best-studied example is histone proteins, where lysine methylation is carried out by sequence specific SET family MTases using *S*-Adenosyl methionine cofactor as a methyl group donor [2]. However, the situation in archaea is not as well understood. In *Sulfolobus solfataricus* many mono-methylated proteins have been identified with little or no consensus sequence near methylated lysine residues [3]. However, these methylated lysines were found in alpha-helices suggesting a correlation between methylation and secondary structures [3]. This leads to the belief that the species has a lysine MTase with a relaxed sequence specificity, which recognises its target from its local structure.

Aims

To clone candidate MTases of *S. solfataricus* into *E. coli* expression vectors in order to express and purify the candidates before using *in vitro* assays to determine which, if any, methylate lysine residues with relaxed sequence specificity.

Departures from the original proposal

There were no major departures from the original proposal.

Methods

Genes of 4 candidate MTases – Sso0479, Sso0554, Sso0673, and Sso2177 – were amplified by PCR from *S. solfataricus* genomic DNA and cloned into pET151 expression vector (#Invitrogen) allowing for IPTG-controlled expression of the recombinant protein in *E.coli* with an N-terminal his-tag. Small-scale expression experiments were carried out to identify optimal growth temperature, inducer concentration and *E. coli* strain to produce optimal expression conditions. Successful small-scale expressions were scaled up to 2-4 litres of bacterial culture (depending on the level of protein expression). Soluble gene products were purified by immobilised metal affinity chromatography and gel filtration. Sso0673 and Ss02177 were purified under denaturing conditions with on-column refolding. Purified candidate MTases were assayed using Sso7 and Sso1992, that are known to be methylated in *S. solfataricus in vivo*, as substrates and varying buffer components, pH and temperature for lysine methylase activity *in vitro*. The intact masses of substrates were determined by mass spectroscopy to ascertain if a post-translational modification occurred. Sso0554 and Sso2177 proteins were used in a pulldown experiment with *S. solfataricus* cellular extract as an additional interaction study. Protein partners discovered were identified by mass spectroscopy and a bioinformatic research was performed to reason these interactions.

Results and Discussion

Sso0554 was the only protein highly expressed in soluble form. Sso2177 and Sso0673 were only expressed as inclusion bodies, thus were purified under denaturing conditions in a buffer containing 8 M urea and refolded on-column. Sso0479 failed to express under conditions tested.

Under conditions used, none of the purified candidates showed obvious methylation activity. However, this could be due to several factors: a) the protein might be inactive on its own and perform its function only in a complex, b) the extensive methylation of *S. solfataricus* proteins is a co-translational modification rather than post-translational, this would imply that the MTase works together with a translational apparatus and is inactive towards a fully folded target protein, c) the *in vitro* assays require further optimisation.

Interaction study gave additional information on Sso2177 and Sso0554. Sso2177 was found to bind Sso2817 electron transfer flavoprotein alpha and beta subunit (Fig.2). Bioinformatic research revealed no literature sources regarding Sso2817 methylation, and requires further research. However, Sso0554 was shown to interact with Sso0506 (Fig. 2) a hypothetical protein, which appears to have a fold common for TPR proteins implying its role in protein-protein interactions. For the confirmation of these results the pulldown experiment should be repeated, as it could not be replicated due to lack of time.

Future directions

Further expression systems could be investigated for Sso0479 gene and additional interaction studies on all 4 candidate MTases could be carried out. If these studies do not provide any valuable information, there are two alternative ways to identify a hypothetical MTase with a relaxed sequence specificity: a) genomic studies involving a knock out of the MTase of interest and investigating phenotypic changes in the organism; b) purifying MTases from *S. solfataricus* cellular extract using a radioactively labelled S-adenosyl methionine and assaying a native protein.

Value of the Studentship to the Student

In the course of this project not only I was given an opportunity to master new laboratory techniques, but also enhanced my research skills such as time and resource planning, scientific communication, and interpretation and presentation of results. Moreover, spending time in research environment only proved that this is a suitable career path for me.

Value of the Studentship to the Lab

Kotryna worked in our laboratory for 8 weeks, and in that time managed to clone, express and purify four potential lysine methyltransferases and assay their activities. Two of these proteins were insoluble in *E. coli*, but Kotryna researched a method for on-column refolding and succeeded in rescuing these proteins. This method is now in general use in our laboratory. Although we were unsuccessful in identifying the enzyme we sought, the work Kotryna carried out has moved this project forward significantly.

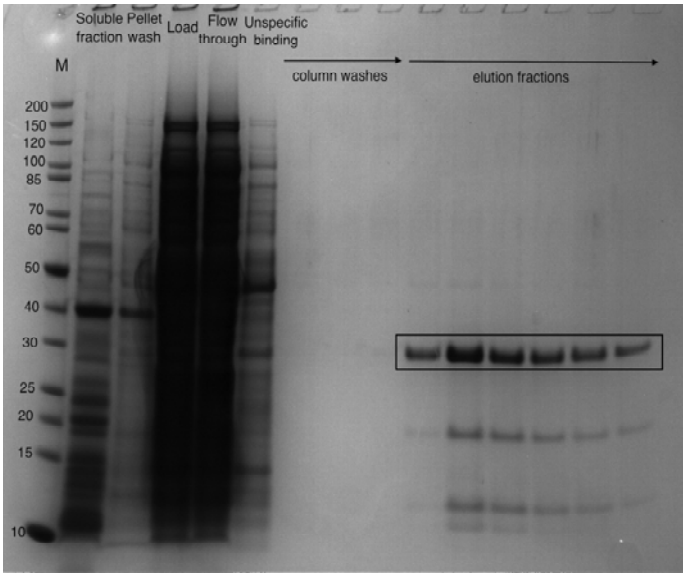

Figure 1. Purification of Sso2117 under denaturing conditions with on-column refolding. Box shows Sso2177 protein.

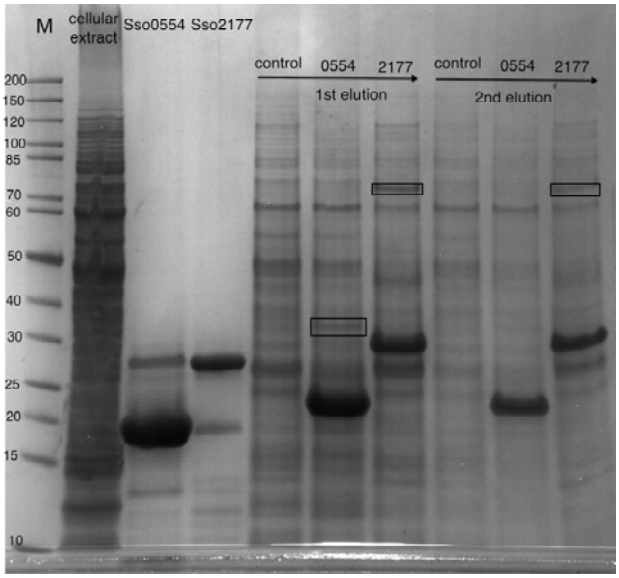

Figure 2. Interaction study of Sso0554 and Sso2177. Sso0554 pulled down 38 kDa Sso0506 and Sso2177 pulled down 67 kDa Sso2817, showed in boxes.

References:

[1] Vielle, C. and Zeikus, G. J. (2001) Hyperthermophilic Enzymes: Sources, Uses, and Molecular Mechanisms for Thermostability. *Microbiology and Molecular Biology Reviews* Vol. 65 No.1, p. 1–43.

[2] Martin C. and Zhang, Y. (2005). The diverse functions of histone lysine methylation. *Nature Reviews Molecular Cell Biology* Vol. 6, p.838-849.

[3] Botting, C. H., Paytubi, S., Talbot, P., White, M. F. (2010). Extensive Lysine Methylation in Hyperthermophilic Crenarchaea: Potential Implications for Protein Stability and Recombinant Enzymes. *Archaea* Vol. 2010, Article ID 106341.