Poster Presentations

[MS5-P21] Structural basis for the site-specific chemical modification of proteins

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Recently methods were developed to genetically encode unnatural amino acids and incorporate them efficiently and site-specifically into proteins. These unnatural amino acids have various chemical, physical and biological properties, which allow the functionalisation or generation of proteins with enhanced traits for biochemical analysis, or pharmaceutical and diagnostic purposes.[1] Here orthogonal functional tRNA-synthetase tRNA pairs in an amber suppression system are used, where the tRNA recognises the amber stop codon, such as the pyrrolysyl tRNA-synthetase $tRNA_{CUA}$ pair (PylRS/$tRNA_{CUA}$) from methanogenic Archaea.[2] These Archae utilize PylRS/$tRNA_{CUA}$ to incorporate pyrrolysine (Pyl), the 22nd amino acid, in the active site of enzymes involved in their methane metabolism.[3] Next to Pyl, PylRS also accepts other lysine derivatives as substrates. Using the PylRS/ $tRNA_{CUA}$ from *Methanosarcina mazei* we in- corporated ε-N-propionyl-, ε-N-butyryl-, and ε-N- crotonyl-lysines into histone H3.[4] These lysine derivatives were recently found as novel histone modifications.[5] The so modified histones could be used to identify proteins recognising these modifications. In addition PylRS was evolved in an iterative saturation mutagenesis screen to utilise lysine derivatives with a norbonene functional head group. This PylRS tripple mutant (Y302G, Y384F, I405R= PylRS-Norb) was shown to be essential for the efficient incorporation of norbonene.[6] The target proteins site-specifically containing the norbonene can be further quantitatively, chemically modified using click-chemistry. ([6] and unpublished data). In order to shed light onto the substrate specificity of PylRS, we co-crystallised and solved the structures of wild-type enzyme from *M. mazei in complex* with ε-N-propionyl-, ε-N-butyryl-, ε-N- crotonyl- and alkyl-lysine, ranging from 2.0-2.7Å. Furthermore we present here the co-crystal structure of the PylRS-Norb in complex with the adenylated norbonene amino acid, explaining why these three mutations are essential to enable the enzyme to utilise norbonene efficiently as a substrate. Two of the mutations (Y384F, I405R) are positioned at the active site closing loop, which was shown previously to play a dual role: protection of the adenylated amino-acid in absence of the tRNA and determination of the substrate specificity in class II synthetases.[7, 8]

However the third mutation, Y306G, enlarges the active site and is necessary to accommodate the bulky functional norbonene head group, without perturbing the binding pocket. Thus PylRS-Norb can transfer *endo-*, *exo-* as well as cyclopropyl norbonene amino acids to its respect tRNA, which are than efficiently incorporated into the polypeptide chains.

In summary our data provide penetrating insights into the structural basis for the site-specific incorporation of unnatural amino acids into proteins by the PylRS/ $tRNA_{CUA}$ system from *M. mazei*.

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