UKE Paper of the Month September 2020

Identification of targets of AMPylating Fic enzymes by co-substrate-mediated covalent capture

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ABSTRACT:
Various pathogenic bacteria use post-translational modifications to manipulate the central components of host cell functions. Many of the enzymes released by these bacteria belong to the large Fic family, which modify targets with nucleotide monophosphates. The lack of a generic method for identifying the cellular targets of Fic family enzymes hinders investigation of their role and the effect of the post-translational modification. Here, we establish an approach that uses reactive co-substrate-linked enzymes for proteome profiling. We combine synthetic thiol-reactive nucleotide derivatives with recombinantly produced Fic enzymes containing strategically placed cysteines in their active sites to yield reactive binary probes for covalent substrate capture. The binary complexes capture their targets from cell lysates and permit subsequent identification. Furthermore, we determined the structures of low-affinity ternary enzyme–nucleotide–substrate complexes by applying a covalent-linking strategy. This approach thus allows target identification of the Fic enzymes from both bacteria and eukarya.

STATEMENT:
Identifying targets of enzymes from bacterial pathogens is a general challenge in elucidating the molecular mechanisms of infections. Here, we established an interdisciplinary concept for capturing bacterial targets that are modified with adenosine monophosphates (a process referred to as AMPylation) using a combination of biochemistry, chemistry, mass spectrometry and structural biology. Our work presents a completely new avenue for identifying and elucidating mechanisms of bacterial infections.

BACKGROUND:
This work was a collaborative project between the UKE (Prof. Itzen and Prof. Schlüter) and Umeå University (Prof. Hedberg, Sweden). The work will serve as a basis for future projects, such as a DFG-funded research training group recently applied for by Prof. Martin Aepfelbacher. The project was supported by the collaborative research centre SFB1035 (B05). Dr. Burak Gulen as the first author was funded by the Alexander von Humboldt Foundation and the Technical University of Munich Foundation Fellowship during the project. All authors have a strong research interest in the field of post-translational modifications of host proteins by bacterial pathogens.