Controlling and Predicting the Stability of a Protein Against Degradation by Proteases

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a method of using the last three amino acid residues of the C-terminus of a polypeptide to predict and control protein stability in prokaryotes such as Mycobacterium smegmatis.

OVERVIEW

The pathogen Mycobacterium tuberculosis causes tuberculosis, one of the world’s deadliest diseases. Because the desaturase DesA3 is essential for survival of M. tuberculosis, this enzyme is a potential drug target for the treatment of tuberculosis, although little is known about its function.

To study mycobacterial pathogenicity, M. smegmatis, a non-pathogenic strain that grows faster than pathogenic mycobacteria and transforms efficiently, has been used widely as a host for the expression of target genes and proteins like DesA3. Many genes from pathogenic mycobacteria yield folded proteins and active enzymes when expressed in M. smegmatis but not in E. coli.

THE INVENTION

UW–Madison researchers have developed methods for predicting and controlling the stability of expressed polypeptides in prokaryotes, particularly mycobacteria like M. smegmatis. They previously showed that DesA3 expressed in M. smegmatis with a modified C-terminal sequence had higher catalytic activity and stability than with the natural C-terminal sequence. The researchers found that the identity of the last two or three amino acid residues of the C-terminus is a predictor and determinant of protein stability and resistance to proteolytic degradation.

Specifically, altering one or more of the last three amino acid residues at the C-termini of polypeptides can make the proteins more stable during heterologous expression in mycobacterial hosts. Identifying the last three residues also can be used to predict the relatively stability of proteins against degradation by proteases.
APPLICATIONS

• Predicting and controlling stability of expressed polypeptides, including DesA3
• Identifying amino acid residues and sequences that increase or decrease protein stability and resistance to proteolytic degradation
• Modifying heterologous protein sequences to improve production of important bioactive targets
• High throughput screening assays for DesA3 and the human desaturase SCD1

KEY BENEFITS

• Provides improvements to high throughput screening assays for DesA3
• Increases the stability of DesA3, an essential enzyme in *M. tuberculosis* that cannot be successfully expressed in *E. coli*
• Provides a novel antimycobacterial drug target

STAGE OF DEVELOPMENT

The researchers found that DesA3 variants with the last three amino acids substituted from LAA to either DKD or LEA showed up to 13-fold greater stearoyl-CoA Δ9 desaturase activity as compared to DesA3 alone.

ADDITIONAL INFORMATION

Publications

Tech Fields
Research Tools - Protein interactions & function
Research Tools - Gene expression

CONTACT INFORMATION

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854.