Towards protein N-terminal acetyltransferase with broad substrate specificity

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Introduction

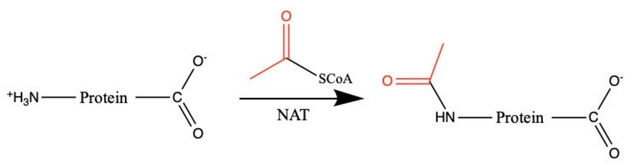
Among the various types of protein modification, acetylation is critical for proper function. N-terminal acetylation plays essential roles in the stability, activity, and targeting of proteins in eukaryotes. Most proteins expressed in bacteria are not acetylated, although the N-terminal acetylation is critical for the activities of a handful of biologically important proteins. Therefore, it is of practical significance to control N-terminal acetylation of recombinant proteins in bacteria. This study is aimed to alter the substrate specificity of RimJ, a protein N-terminal aminotransferase (NAT) that is known to acetylate a few recombinant proteins including the Z-domain in E. coli. We created RimJ variants, so that the active site becomes larger to accommodate substrate proteins containing varying N-terminal amino acid residues. Then, the substrate specificity of RimJ was investigated by co-expressing two Z-domain variants T2I and S3K, which were not acetylated by the wild type RimJ. The expressed Z-domain variants were purified by immobilized metal affinity chromatography and subsequently analyzed by mass spectrometry, by which a 42-Da mass increment indicates the presence of N-terminal acetyl group.

Background

N-terminal acetylation by RimJ is dependent upon:

1. Initiator methionine cleavage
2. N-terminal amino acid sequence

Protein N-terminal acetylation:



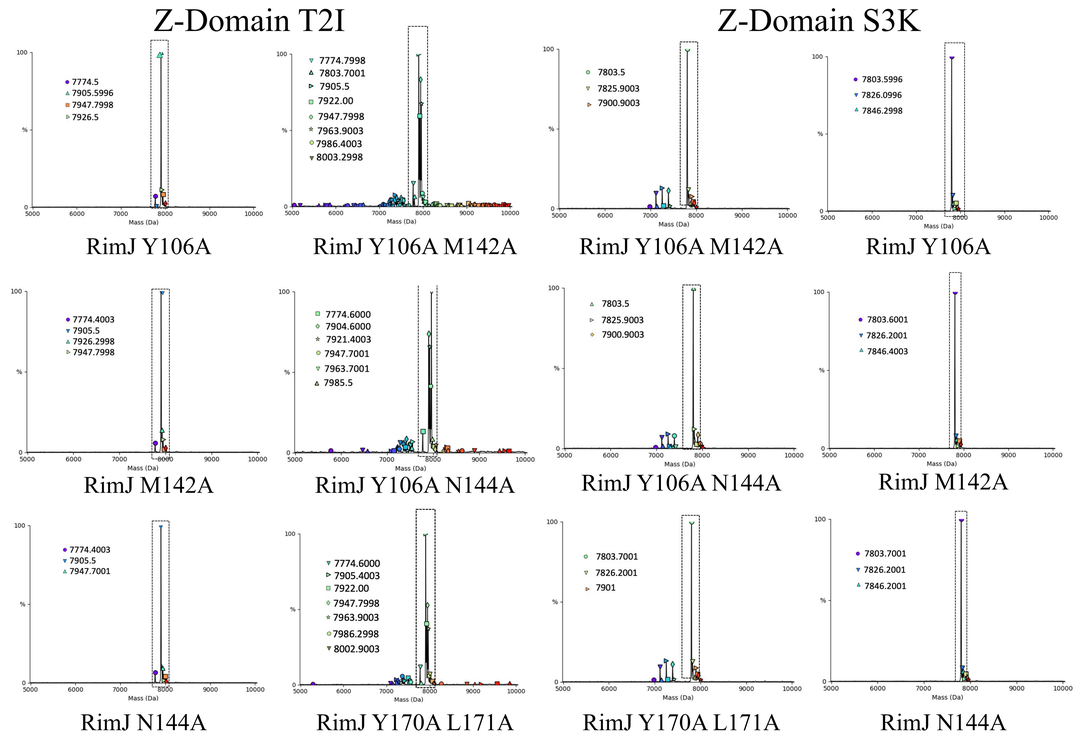
Method

1. Site-Directed Mutagenesis of RimJ
2. Co-expression of Z-Domain variants, T2I and S3K
3. Purification of protein by Ni-NTA affinity chromatography
4. Analysis of Z-Domain acetylation by ESI-Mass Spectrometry

RimJ

Single Mutation	Double Mutation
Y106A	Y106A M142A
M142A	Y106A M142A
N144A	Y170A L171A

Mass Spectrometry Results



7905.69 Da (non-acetylated)
7947.63 Da (acetylated)

7804.54 Da (non-acetylated)
7845.57 Da (acetylated)

Conclusion

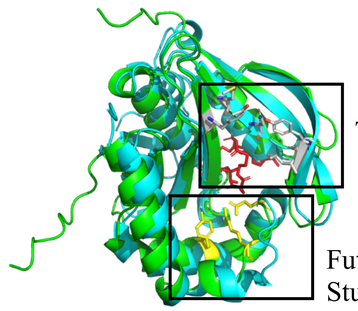
- RimJ single mutants such as N144A, M142A, and Y106A showed little acetylation on both T2I and S3K Z-domain variants.
- The RimJ double mutants, Y106A M142A, and Y106A N144A, and Y170A L171A showed higher acetylation rates on the Z-domain T2I variants.
- Little acetylation was observed for the Z-domain S3K variant by any of these double mutants.

Future Directions

Broaden the substrate specificity of RimJ.

Mutations on the other side of the active site.

Y35A
E46A
R49A



References

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Perez, L. and Ryu, Y. (2015) RimJ-Catalyzed Sequence-Specific Protein N-Terminal Acetylation in *Escherichia coli*. *Advances in Bioscience and Biotechnology*, **6**, 182-193.

Acknowledgments

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