A novel recombinant Lys-C protease for proteomic sample preparation

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1. Introduction

Owing to efficient proteolysis and particular advantages of trypsin-generated peptides for mass spectrometry analysis, trypsin is the most widely used proteomic protease. Recently, however, Lys-C has been increasingly used as either a trypsin alternate or as supplement. Its increasing favor is largely due to its ability to perform proteolytic digestion under protein denaturing conditions, an attribute that can greatly extend the observable proteome.

Lys-C is found in number of bacterial hosts with Lysobacter enzymogenes being used as a most popular source of commercially available Lys-C. We have now developed a recombinant form of Lys-C from Pseudomonas aeruginosa. In this study we compare performance of Pseudomonas and Lysobacter Lys-C and demonstrate benefits of Pseudomonas Lys-C for proteomic analysis.

2. Lys-C protease from Pseudomonas aeruginosa

- Highly proteolytically active Lys-C protease is a key factor of Pseudomonas aeruginosa pathogenicity
- The protease is secreted as 48 kDa inactive proenzyme, which maturates into a smaller active enzyme
- Studies have shown that Pseudomonas Lys-C demonstrates not only high proteolytic activity but also exceptional cleavage specificity
- Similarly to other Lys-C proteases, Pseudomonas Lys-C tolerates strong protein denaturing conditions. This property of Lys-C protease is extensively utilized to digest proteolytically resistant proteins
- The protease shares very limited homology with Lysobacter Lys-C. It is possible that Pseudomonas and Lysobacter Lys-C proteases use different mechanism to cleave at the carboxyl side of a lysine residue

3. Recombinant Pseudomonas aeruginosa Lys-C protease

- Expressed in E. coli to avoid the need of working with a pathogenic organism and increase the protease yield
- Has HQ tag to streamline purification
- Recombinant Lys-C undergoes natural maturation to ensure proper protein folding. MW of the matured protease is 27.7 kDa
- Purity ≥95% by HPLC
- Retains activity in a lyophilized form. The enzyme is also stable after dissolving in acetic acid, which can be used for long term storage of the enzyme in solution

4. Cleavage specificity

Digestion of a model peptide (Melittin) with Pseudomonas rLys-C and Lysobacter Lys-C proteases

Pseudomonas recombinant Lys-C (rLys-C) demonstrates high cleavage specificity while Lysobacter Lys-C is known to cleave on the amide side of the lysine residues even after long incubation of Melittin with the protease.

5. Tolerance to denaturing conditions

Lys-C tolerates increasing popularity due to its ability to perform proteolysis under protein denaturing conditions efficiently overcoming proteolytic resistance of many proteins.

6. Comparative performance in proteomic analysis

Protein identifications in total yeast protein extract digested with Lys-C and Lysobacter Lys-C protease individually and in combination

Pseudomonas recombinant Lys-C and Lysobacter Lys-C proteases provide comparable proteome coverage. The proteins identified in the digestion with either enzyme substantially overlap (~60%) indicating similar protein populations

7. Pseudomonas and Lysobacter Lys-C proteases show bias toward different lysine cleavage sites

Peptide (non-redundant) identifications in total yeast protein extract digested with Lys-C and Lysobacter Lys-C protease individually and in combination

8. Proteinolytic synergy between Pseudomonas and Lysobacter Lys-C proteases

List of identified proteins

Combined digestion with Pseudomonas and Lysobacter Lys-C proteases improves proteomic analysis

9. Conclusion

- Recombinant Pseudomonas aeruginosa Lys-C protease
  Active recombinant form of Pseudomonas aeruginosa Lys-C protease has been successfully expressed in E. coli and purified to a high degree of purity
  - Proteolytic activity
  - Recombinant Lys-C protease demonstrates high cleavage specificity, activity and tolerance to strong protein denaturing conditions (8M Urea)
  - Validated for mass spec use
  - Proteolytic analysis with use of cell total protein extract as a model system has shown that recombinant Pseudomonas Lys-C protease performs competitively as compared to native Lysobacter enzymogenes Lys-C
  - Relation to Lysobacter Lys-C
  - Pseudomonas and Lysobacter Lys-C proteases show bias toward different lysine cleavage sites. This can be used to improve proteomic analysis through combined digestion with both proteases

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