Enhancement of Penzyme effects by natural products

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1. Introduction

This project is part of a PhD study that focuses on **Penzyme** activity. **Penzyme** [1] is trypsin isolated from cod viscera and has very high activity at low temperatures compared to similar enzymes from mammals [2]. Other enzymes that can be isolated from the cod are chymotrypsin and elastase.

Trypsin, chymotrypsin and elastase are all proteases that belong to the S1 family of serine proteases [3]. Even though these enzymes do not have high sequence identity, the tertiary structure is highly conserved. The enzymes are endopeptidases and are found in the digestive system where they contribute to the digestion of proteins. Even though trypsin, chymotrypsin, and elastase all cleave peptide chains, have similar structures and mechanisms, they display very different specificities. **Trypsin** cleaves peptides on the carboxyl side of arginine or lysine. On the other hand, **chymotrypsin** prefers to cleave on the carboxyl side of aromatic residues, (i.e. phenylalanine and tyrosine). **Elastase** is not as specific as the other two it prefers to cleave peptides at the carboxyl side of small, neutral residues.

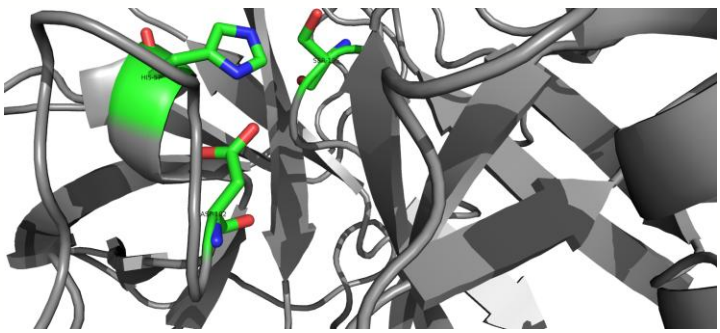


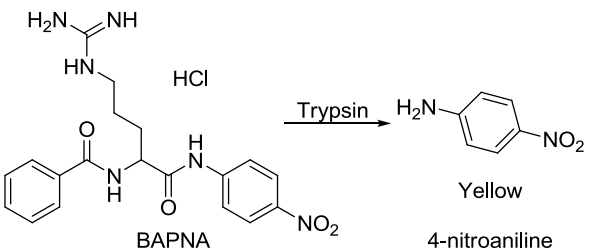
Figure 1. Cleavage site of typsin. His-57, Asp-102 and Ser-195.

2. Methods

The aim is to develop an activity assay for Penzyme, chymotrypsin, and elastase proteolytic activity using 96-well microtiter plates. Currently the Penzyme proteolytic activity is tested through a chromogenic substrate assay with a spectrophotometer using single cuvettes. In order to speed up the assay, to enable high throughput screening of the many natural products Penzyme activity will be screened with, a chromogenic substrate test using a 96-well microtiter plate and a 96-well plate reader will be developed.

The natural product that will be tested are e.g. menthol, thymol, camphor and eucalyptol. The effect of antibiotics will also be researched..

Trypsin cleaves BAPNA reagent on the carbonyl site of the arginine giving of the 4-nitroaniline, which absorbs light at 410 nm. Using Beer's law it is easy to calculate the activity of the enzyme compared to the concentration of 4-nitroaniline.



Scheme 1. BAPNA reagent for trypsin activity measurments

3. Acknowledgements

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4. Reference

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