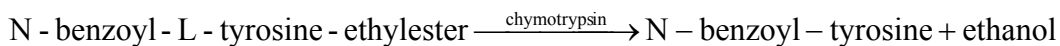
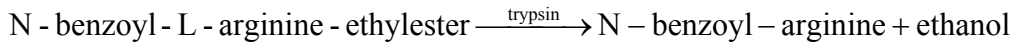


CHARACTERIZATION OF THE SUBSTRATE SPECIFICITY OF TRYPSIN AND CHYMOTRYPSIN

Trypsin and chymotrypsin are endopeptidases. Trypsin cleaves the peptide bonds formed by the carboxyl group of lysine or arginines, chymotrypsin digests the peptide bonds formed by the carboxyl groups or aromatic amino acids. Both enzymes have esterolytic activities. Trypsin and chymotrypsin catalyze the following reactions:



The carboxyl groups liberated during the reactions acidify the medium. Based on the substrate of trypsin and chymotrypsin one can judge whether an unknown protease solution contains trypsin or chymotrypsin.

Solutions

1. protease A solution
2. protease B solution
3. 0.8 mM TRIS-HCl buffer, pH 9.0
4. 2 mM N-benzoyl-L-arginine ethylester
5. 2 mM N-benzoyl-L tyrosyl ethylester
6. phenol red indicator (yellow in acidic and red in alkaline medium)

Measure the following ingredients into test tubes:

| Materials | 1 | 2 | 3 | 4 |
|----------------------------------|-----|-----|-----|--------|
| TRIS buffer | 0,3 | 0,3 | 0,3 | 0,3 ml |
| Arginine ester | 0,2 | - | 0,2 | - ml |
| Tyrosine ester | - | 0,2 | - | 0,2 ml |
| Add 50 µl indicator to each tube | | | | |
| Protease A | 0,1 | 0,1 | - | - ml |
| Protease B | - | - | 0,1 | 0,1 ml |

Mix well and incubate five minutes at room temperature. Observe in which of the test tubes do you see any change of the colour.