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Hydrolases – peptidases (pepsin, trypsin)

Exercise 1

The examination of pepsin activity in different conditions of pH

Protocol

Pipete 2 cm^3 of pepsin to tubes marked 1, 2, 3, 4. Tube no 4 heat over the burner (inactivation of pepsin). Add the following solutions to appropriate tubes:

1 - 2 cm^3 0,2 mol/dm^3 HCl
2 - 2 cm^3 1 mol/dm^3 CH_3 COOH
3 - 2 cm^3 buffer pH 7,2

Add small amount of protein to all 4 tubes. Incubate tubes in water bath for 1 hour at 37°C. Observe the tubes after incubation and give the number of tube where protein was digested. Explain obtained results.

Exercise 2

The determination of trypsin activity

Protocol

Add 1 cm^3 of 30% TCA to three centrifuge tubes and mark them 1, 2, and 3. In glass tube marked „A" prepare incubation mixture containing 4 cm^3 of 1% casein in 0,1 mol/dm^3 $NaHCO_3$ and 1 cm^3 of trypsin solution. Mix the content carefully and immediately take 1 cm3 of this mixture to centrifuge tube marked 1.

The remaining mixture of tube „A" incubate during 10 min. in temp. 37°C. After 10 min. take 1 cm^3 of this mixture and add to centrifuge tubes 2 and 3.

Leave tubes 1, 2, 3 in room temperature for 10 min. and centrifuge them for 15 min. at 2000xg. Remove the supernatant and add 1 cm^3 of 1 mol/dm^3 NaOH to each tube to resolve the precipitate. After resolving the precipitate add 4 cm^3 of cooper reagent to each tube. Mix the solution and leave it in room temperature for 20 min.

In the meantime prepare blank sample by mixing 4 cm^3 of cooper reagent and 1 cm^3 of distilled water. Read the absorbance of samples 1, 2, 3 at wavelength 540 nm against blank sample. The content of protein in examined solution can be estimated based on standard curve.