CHYMOTRYPSIN

Determination of chymotrypsin with S-2586.

Measurement Principle

The chymotrypsin activity is determined by its amidolytic effect on the substrate MeO-Suc-Arg-Pro-Tyr-pNA (S-2586). The rate at which p-nitroaniline (pNA) is released is measured photometrically at 405 nm. This can be followed on a recorder (initial rate method) or read after stopping the reaction with acetic acid (acid stopped method). The correlation between the change in absorbance per minute (\(\Delta A/\text{min}\)) or absorbance (A) and the chymotrypsin activity is linear in the 0.05-1.0 \(\mu\text{kat}/\text{l}\) or 3-60 U/l range. The amidolytic activity of different chymotrypsin preparations does not necessarily parallel the protease activity.

Reagents

1. S-2586, 25 mg
   - Art. No. S820894
   - Reconstitute the substrate S-2586 (MW: 705.3) with 60 ml of distilled water.
2. Tris/Calcium Buffer, pH 8.3 (25°C)
   - Tris 12.1 g (100 mmol/l)
   - NaCl 56.2 g (960 mmol/l)
   - Distilled water 800 ml
   - Adjust the pH to 8.3 at 25°C by adding approximately 50 ml of 1 mol/l HCl. Add 10 ml of 1 mol/l CaCl2 solution. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for two months at 2-8°C.
3. Acetic acid, 20%
   - Acetic acid is used in the acid-stopped method.

Equipment

1. Spectro- or filter photometer, 405 nm
2. Semi-microcuvettes, 1 cm.
3. Thermostat, 37°C
4. Stop watch
5. Disposable plastic tubes
6. Photometer with cuvette housing, thermostated at 37°C (for the initial rate method)

Sample

The sample containing chymotrypsin is dissolved in or diluted with 1 mmol/l HCl to a concentration of 0.1 g/l. This stock solution is stable for more than two weeks at 2-8°C. Before assay, the solution is diluted 1:200 with 1 mmol/l HCl. If the sample is a pure protein, it is advisable to use 0.1% Carbowax 6000 (Union Carbide, NY) or 1% albumin (previously checked for amidolytic activity) to avoid adsorption to surfaces.

Method

1. Transfer the sample immediately to a 1 cm semi-microcuvette (preheated to 37°C) for measurement of the absorbance change in a photometer at 405 nm and at 37°C. Calculate \(\Delta A/\text{min}\).

2. Read the absorbance (A) of the sample against a water or sample blank in a photometer at 405 nm. The colour is stable for at least 4 hours.

Calculation

Calculate the chymotrypsin activity of the stock solution from the following formulas:

- Initial rate method: \(\mu\text{kat}/\text{l} = 5.19 \times \Delta A/\text{min} \times 200\)
- Acid stopped method: \(\mu\text{kat}/\text{l} = 2.31 \times A \times 200\)

Bibliography

