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Method Sheet S-2288

Determination of Proteolytic Activity in Plasma, Serum or Euglobulin Fractions

PRINCIPLE

$$\text{H-D-Ile-Pro-Arg-pNA} + H_2O \xrightarrow[\text{activity}]{\text{proteolytic}} \text{H-D-Ile-Pro-Arg-OH} + \text{pNA}$$

Several proteases with arginine specificity readily split the substrate H-D-Ile-Pro-Arg-pNA · 2HCl (S-2288).

The proteolytic activity is thus determined by the rate at which p-nitroaniline (pNA) is released. The formation of pNA can be followed spectrophotometrically at 405 nm by using a recorder (initial rate method).

The correlation between the change in absorbance per minute (ΔA/min) and the proteolytic activity is usually linear in the 0.05—0.5 μkat/l or 3—30 U/l range. If possible the linearity of the assay should be checked for each individual type of sample. This can be done by serial dilution of the sample. In several instances the proteolytic activity may originate from α_2-macroglobulin enzyme complexes.

EQUIPMENT

1. Spectro- or filter-photometer, 405 nm, with cuvette housing, thermostated at 37°C.
2. Semi-microcuvettes, 1 cm.
3. Water bath, 37°C.
4. Stop-watch.
5. Disposable plastic tubes.
6. Recorder.

REAGENTS

1. Substrate S-2288 (H-D-Ile-Pro-Arg-pNA · 2HCl) mol.wt. 577.6.

S-2288 from KabiVitrum AB, Sweden.

The substance (25 mg) is dissolved in 7.2 ml of distilled water. If prepared with sterile water and not contaminated by microorganisms, the 6 mmol/l solution is stable for at least two months at 2 to 8°C.

2. Buffer pH 8.4 (25°C)

Tris	12.1 g (100 mmol/l)
NaCl	6.2 g (106 mmol/l)
Distilled water	800 ml

Adjust the pH to 8.4 at 25°C by adding an appropriate amount (approximately 44 ml) of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, is stable for six months at 2 to 8°C.

PROCEDURE

Sample

Dilute the plasma, serum or euglobulin fraction with buffer (Reagent 2) to a proteolytic activity of 0.05—0.5 μkat/l or 3—30 U/l.

Methods

Initial rate method		Volume
Buffer	μl	200
Thermostate at 37°C (2—4 min)		X
Sample dilution (20—25°C)	μl	200
Mix and thermostate at 37°C (2—4 min)		X
S-2288 (37°C)	μl	200
Mix		X

Transfer 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 △A/min.

Calculation

The proteolytic activity in the sample is calculated from the following formulas:

μkat/l = △A/min × 5.21 × F
U/l = △A/min × 313 × F
F = dilution factor (e.g. 10 if the sample is diluted 1:10 before initial rate determination).

Note

For some enzymes with low K_m less substrate can be used.

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