Urease (URE)

from jack bean

Urea amidohydrolase, EC 3.5.1.5

$$\text{Urea} + H_2O \longrightarrow CO_2 + 2\ NH_3$$

SPECIFICATION

Appearance	white lyophilizate
Activity	≧100 U/mg lyophilizate
Storage	at −20°C

PROPERTIES

Molecular weight	*ca*. 480 kDa (gel filtration)	
Structure	8 subunits (containing nickel)	
Isoelectric point	5.0–5.1	
Michaelis constant	1.05×10^{-2} M (urea)	
pH Optimum	*ca*. 8.0	(Fig. 1)
pH Stability	5.0–10.0	(Fig. 2)
Optimum temperature	60°C	(Fig. 3)
Thermal stability	below 50°C	(Fig. 4)
Inhibitors	heavy metal ions, Na^+, K^+, NH_4^+, suramin, thiourea	
Specificity	specific for urea	

ASSAY PROCEDURE

Principle

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} CO_2 + 2\ NH_3$$

$$2\ NH_3 + 2\ \alpha\text{-Ketoglutarate} + 2\ NADH + 2\ H^+ \xrightarrow{\text{glutamate dehydrogenase}} 2\ \text{L-Glutamate} + 2\ NAD^+$$

The disappearance of NADH is measured spectrophotometrically at 340 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 2 μmol of ammonia per min at 25°C and pH 8.0 under the conditions described below.
One unit under these conditions is equivalent to 2.4 Nessler units.

Reagents

A. Potassium phosphate buffer, 50 mM; pH 7.4: mix 50 mM KH_2PO_4 solution and 50 mM K_2HPO_4 solution to make a pH 7.4 solution.
B. Buffered water (potassium phosphate buffer, 5.0 mM; pH 7.4): dilute potassium phosphate buffer (Reagent A) to 10-fold volume with distilled water.
C. NADH solution, 8.5 mM: 30 mg of NADH/5 ml of buffered water (Reagent B).
D. α-Ketoglutarate solution, 25 mM: dissolve 91.3 mg of α-ketoglutarate in 10 ml of distilled water, adjust to pH 5.0 with 2 N NaOH solution and dilute with distilled water to 25 ml.
E. Urea solution, 0.3 M: 90 mg of urea/5 ml of distilled water. (Prepare freshly)
F. Glutamate dehydrogenase (GLDH) solution: 250 U/ml of potassium phosphate buffer (Reagent A).
G. Enzyme dilution buffer: dissolve 6.06 g of Tris(hydroxymethyl)aminomethane and 0.37 g of EDTA·Na_2·$2H_2O$ in 800 ml of distilled water, adjust to pH 8.0 with 6.0 N HCl and dilute with distilled water to 1000 ml.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.10–0.25 U/ml with ice-cold enzyme dilution buffer (Reagent G) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).

0.1 ml	NADH solution	(Reagent C)
0.1 ml	α-Ketoglutarate solution	(Reagent D)
0.1 ml	GLDH solution	(Reagent F)
2.5 ml	Enzyme dilution buffer	(Reagent G)

2. Equilibrate at 25°C for about 5 min.
3. Add 0.1 ml of sample and mix.
4. Add 0.1 ml of urea solution (Reagent E) and mix.
5. Record the decrease of absorbance at 340 nm in a spectrophotometer thermostated at 25°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding enzyme dilution buffer (Reagent G) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_0 - \Delta A_S) \times 3.0\,(\text{ml}) \times df}{6.2 \times 0.1\,(\text{ml}) \times 2} = \Delta A \times 2.42 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

6.2 : Millimolar extinction coefficient of NADH at 340 nm ($cm^2/\mu mol$)
2 : Factor to allow for 2 mol of ammonia produced from 1 μmol of urea
df : Dilution factor
C : Content of urease preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of urea in clinical analysis.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} CO_2 + 2\ NH_3$$

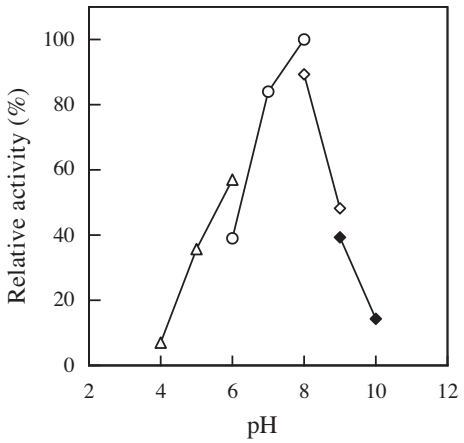
$$2\ NH_3 + 2\ \alpha\text{-Ketoglutarate} + 2\ \text{NADH} + 2\ H^+ \xrightarrow{\text{glutamate dehydrogenase}} 2\ \text{L-Glutamate} + 2\ NAD^+$$

REFERENCES

Sumner, J. B. and Hand, D. B., *J. Am. Chem. Soc*., **51**, 1255–1260 (1929).
Gorin, G. and Chin, C., *Biochim. Biophys. Acta*, **99**, 418–426 (1965).
Fishbein, W. N. et al., *J. Biol. Chem*., **245**, 5985–5992 (1970).
Fishbein, W. N. and Nagarajan, K., *Arch. Biochem. Biophys*., **144**, 700–714 (1971).
Contaxis, C. and Reithel, F. J., *Canadian J. Biochem*., **50**, 461–473 (1972).
Schlegel, H. and Kaltwasser, H., "Methods of Enzymatic Analysis," Vol. 2 (2nd ed.), Verlag Chemie, Weinheim, Germany, 1974, pp. 1081–1085.
Bergmeyer, H. U., "Methods of Enzymatic Analysis," Vol. 2 (3rd ed.), Verlag Chemie, Weinheim, Germany, 1983, pp. 320–321.

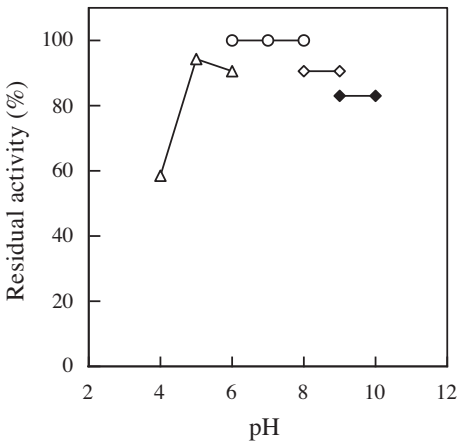
EXPERIMENTAL DATA

Fig. 1 pH Optimum



△: 50 mM acetate buffer
○: 50 mM phosphate buffer
◇: 50 mM TAPS-NaOH buffer
◆: 50 mM CHES-NaOH buffer

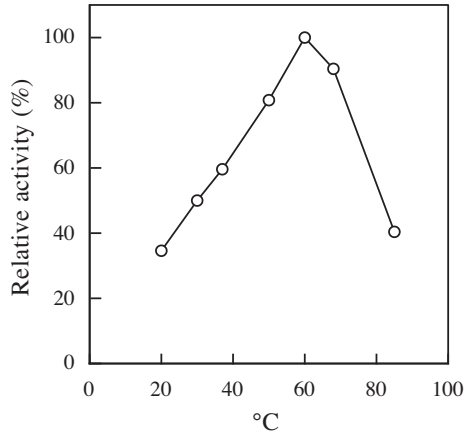
Fig. 2 pH Stability



Treatment: 5°C, 2 h

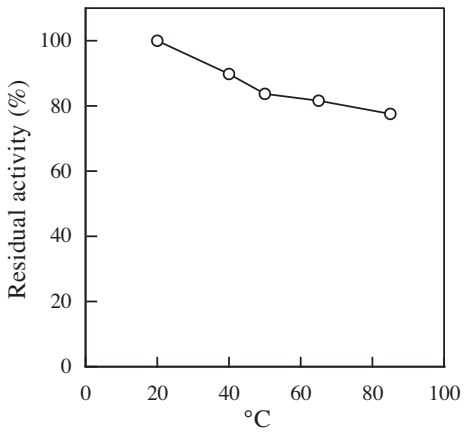
△: 50 mM acetate buffer
○: 50 mM phosphate buffer
◇: 50 mM TAPS-NaOH buffer
◆: 50 mM CHES-NaOH buffer

Fig. 3 Optimum temperature



Buffer: 50 mM Tris-HCl buffer, pH 8.0,
containing 1 mM EDTA

Fig. 4 Thermal stability



Treatment: 20 mM phosphate buffer, pH 7.0,
containing 1 mM EDTA, 60 min