α-Glucosidase (αGLS-SE)

from recombinant *E. coli*

α-D-Glucoside glucohydrolase, EC 3.2.1.20

α-D-Glucoside + H_2O ⟶ α-D-Glucose + ROH

SPECIFICATION

Appearance	white lyophilizate
Activity	≧10 U/mg lyophilizate
Contaminant	α-amylase ≦5.0×10^{-3}%
Stabilizer	bovine serum albumin
Storage	at −20°C

PROPERTIES

Molecular weight	*ca*. 61 kDa (gel filtration)	
Structure	monomer of 60 kDa (SDS-PAGE)	
Michaelis constant	5.5×10^{-5} M (*p*-nitrophenyl-α-D-glucoside)	
pH Optimum	6.0–9.0	(Fig. 1)
pH Stability	5.0–10.0	(Fig. 2)
Optimum temperature	52–55°C	(Fig. 3)
Thermal stability	below 50°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least two weeks	(Fig. 5)
Stability (powder form)	stable at 30°C for at least one month	(Fig. 6)
Inhibitors	Hg^{2+}, Ag^{+}	
Specificity		

Substrate*[a]	Relative activity (%)*[b]
PNPG	100.0
PNPG2	9.4
PNPG5	1.1
Maltose	10.2
Maltotriose	112.9
Maltotetraose	13.9
Maltopentaose	1.4

*[a] Substrate concentration, 2.2 mM
*[b] Glucose-forming activity, pH 7.0 at 37°C

ASSAY PROCEDURE

Principle

$$p\text{-Nitrophenyl-}\alpha\text{-D-glucoside (PNPG)} \xrightarrow{\alpha\text{-glucosidase}} p\text{-Nitrophenol (PNP)} + \text{D-Glucose}$$

The appearance of *p*-nitrophenol is measured spectrophotometrically at 400 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of PNP per min at 37°C and pH 7.0 under the conditions described below.

Reagents

A. Potassium phosphate buffer, 0.1 M; pH 7.0: mix 0.1 M KH_2PO_4 solution and 0.1 M K_2HPO_4 solution to make a pH 7.0 solution.
B. PNPG solution, 20 mM: 603 mg of PNPG/100 ml of distilled water.
C. Sodium carbonate solution, 0.2 M: 21.2 g of Na_2CO_3/1000 ml of distilled water.
D. Enzyme dilution buffer: 10 mM potassium phosphate buffer, pH 7.0, containing 0.2% bovine serum albumin (BSA).

Sample: dissolve the lyophilized enzyme to a volume activity of 0.05–0.10 U/ml with ice-cold enzyme dilution buffer (Reagent D) immediately before measurement.

Procedure

1. Pipette the following reagents into a test tube.

1.0 ml	Potassium phosphate buffer	(Reagent A)
0.5 ml	PNPG solution	(Reagent B)
0.4 ml	Enzyme dilution buffer	(Reagent D)

2. Equilibrate at 37°C for about 5 min.
3. Add 0.1 ml of sample and incubate for 15 min at 37°C.
4. Add 2.0 ml of sodium carbonate solution (Reagent C) to stop the reaction.
5. Read the absorbance at 400 nm in a cuvette (light path: 1 cm) (A_S).
   The blank solution is prepared by reversing the sequence of addition of sample and sodium carbonate solution (Reagent C) (A_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_S - A_0) \times 4.0(\text{ml}) \times df}{18.1 \times 0.1(\text{ml}) \times 15(\text{min})} = \Delta A \times 0.147 \times df$$

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

18.1 : Millimolar extinction coefficient of PNP under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor
C : Content of α-glucosidase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of α-amylase and serum chloride in clinical analysis.

(Determination of α-amylase)

$$\text{G5-CNP} \xrightarrow{\alpha\text{-amylase}} \text{G3} + \text{G2-CNP}$$

$$\text{G2-CNP} \xrightarrow{\alpha\text{-glucosidase}} \text{G} + \text{G1-CNP}$$

$$\text{G1-CNP} \xrightarrow{\beta\text{-glucosidase}} \text{G} + \text{CNP}$$

(G5-CNP: 2-chloro-4-nitrophenyl β-maltopentaoside)

(Determination of serum chloride)

$$\text{EDTA-Ca} + \alpha\text{-Amylase (inactive)} \xrightarrow{Cl^-} \text{EDTA} + \text{Ca-}\alpha\text{-Amylase (active)}$$

$$\text{G7-CNP} \xrightarrow{\text{Ca-}\alpha\text{-amylase (active)}} \text{G2-CNP} + \text{G3-CNP} + \text{G4-CNP}$$

$$\text{G2-CNP} + \text{G3-CNP} \xrightarrow{\alpha\text{-glucosidase}} \text{G1-CNP}$$

$$\text{G1-CNP} \xrightarrow{\beta\text{-glucosidase}} \text{CNP}$$

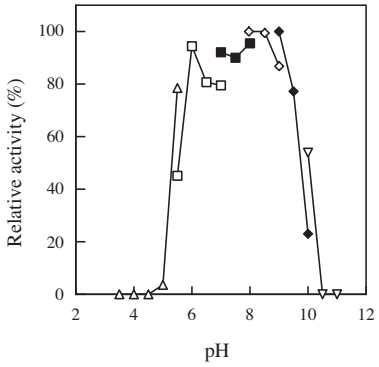
(G7-CNP: 2-chloro-4-nitrophenyl β-maltoheptaoside)

REFERENCES

Halvorson, H., "Methods in Enzymology," Vol. 8, Academic Press, New York, 1966, pp. 559–562.
Ono, T. *et al*., *Clin. Chem*., **34**, 552–553 (1988).

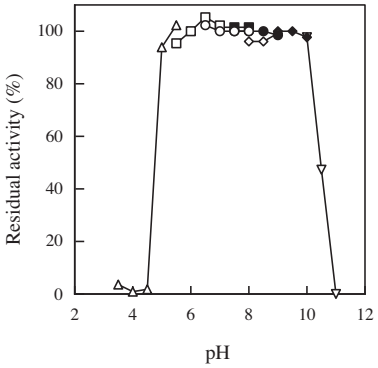
EXPERIMENTAL DATA

Fig. 1 pH Optimum



△: 70 mM acetate buffer
□: 70 mM MES–NaOH buffer
■: 70 mM HEPES–NaOH buffer
◇: 70 mM TAPS–NaOH buffer
◆: 70 mM CHES–NaOH buffer
▽: 70 mM CAPS–NaOH buffer

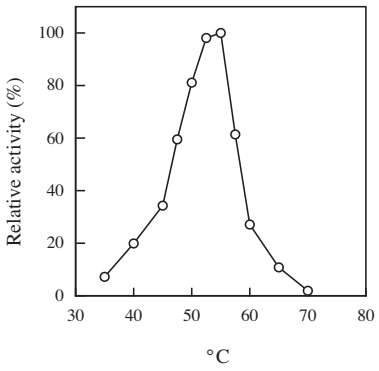
Fig. 2 pH Stability



Treatment: 25°C, 20 h

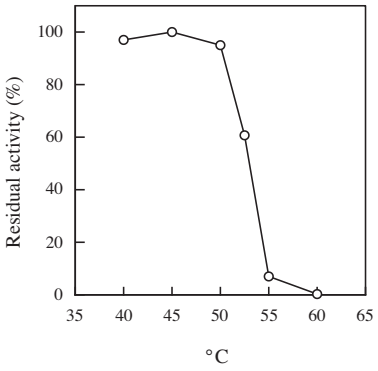
△: 0.1 M acetate buffer
□: 0.1 M MES-NaOH buffer
○: 0.1 M phosphate buffer
●: 0.1 M Tris-HCl buffer
■: 0.1 M HEPES-NaOH buffer
◇: 0.1 M TAPS-NaOH buffer
◆: 0.1 M CHES-NaOH buffer
▽: 0.1 M CAPS-NaOH buffer
(each buffer solution containing 0.2% BSA)

Fig. 3 Optimum temperature



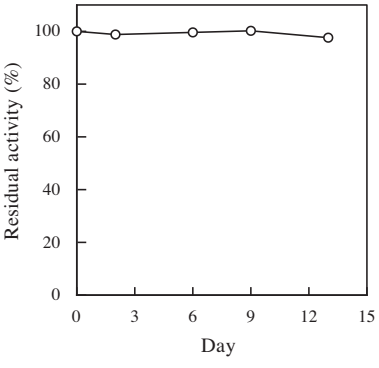
Buffer: 50 mM phosphate buffer, pH 7.0

Fig. 4 Thermal stability



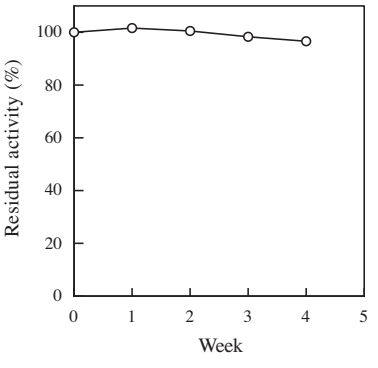
Treatment: 0.1 M phosphate buffer, pH 7.0, containing 0.2% BSA, 15 min

Fig. 5 Stability (liquid form) at 37°C



(Kept in 50 mM Tris-HCl buffer, pH 7.5)

Fig. 6 Stability (powder form) at 30°C



(Kept under dry conditions)