NIPRO ENZYMES

α-GLUCOSIDASE (α-Glu)

[EC 3.2.1.20]

from *Bacillus stearothermophilus*

α-D-Glucoside + H_2O ↔ D-Glucose + Alcohol

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 40 U/mg protein	
Contaminants	:	(as α-Glu activity = 100 %)	
		Phosphoglucomutase	< 0.01 %
		NADH oxidase	< 0.01 %
		Alcohol dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 50,000	
Optimum pH	:	6.0 - 7.0	(Fig. 1)
pH stability	:	5.0 - 11.0	(Fig. 2)
Isoelectric point	:		
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(50 mM Potassium phosphate buffer, pH 6.3, at 30 °C)	
		p-Nitrophenyl-α-glucopyranoside (PNPG)	0.73 mM
		Maltose	1.3 mM
		Phenyl-α-glucopyranoside	2.4 mM
Substrate specificity	:	PNPG	100 %
		Maltose	177 %
		Phenyl-α-glucopyranoside	59 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, α-amylase determination.

ASSAY

Principle

The change in absorbance is measured at 400 nm according to the following reaction.

p-Nitrophenyl-α-glucopyranoside (PNPG) $\xrightarrow{\text{α-Glu}}$ p-Nitrophenol (PNP) + Glucose

Unit Definition

One unit of activity is defined as the amount of α-Glu that forms 1 μmol of PNP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Potassium phosphate buffer, pH 6.3

Ⅱ PNPG solution ; 20 mM (0.603 g PNPG/100 mL distilled water) (Stable for two weeks if stored at 0 - 5 °C)

Ⅲ Na_2CO_3 solution ; 0.2 M (2.12 g Na_2CO_3/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 0.006 to 0.022 U/mL with 10 mM Potassium phosphate buffer containing 1 mg/mL BSA, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 1.5 mL of reaction mixture into a test tube.
   Solution Ⅰ 10.0mL
   Solution Ⅱ 5.0mL
2. Incubate at 30 °C for 5 minutes.
3. Add 0.5 mL of the enzyme solution and mix.
4. Incubate at 30 °C for exactly 15 minutes.
5. After incubation, add 2.0 mL of Solution Ⅲ and mix.
6. Read absorbance at 400 nm (Abs•test).
   At the same time, prepare the blank with 1.5 mL of the reaction mixture, and add 2.0 mL of Solution Ⅲ after incubation at 30 °C for 15 minutes, followed by addition of the enzyme solution (Abs•blank).

Calculation

$$\text{Volume activity (U/mL)} = ((\text{Abs•test}) - (\text{Abs•blank})) \times \frac{4.0}{18.1 \times 15 \times 0.5} \times \text{d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)*}}$$

d.f. ; dilution factor

18.1 ; millimolar extinction coefficient of PNP (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

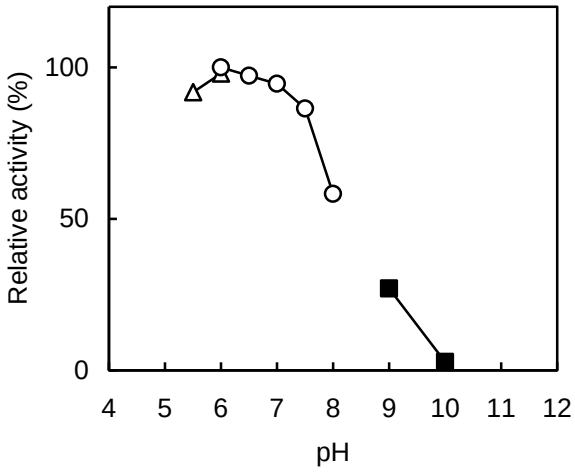

Fig. 1 pH profile

△ acetate, ○ phosphate,
■ Gly-NaOH

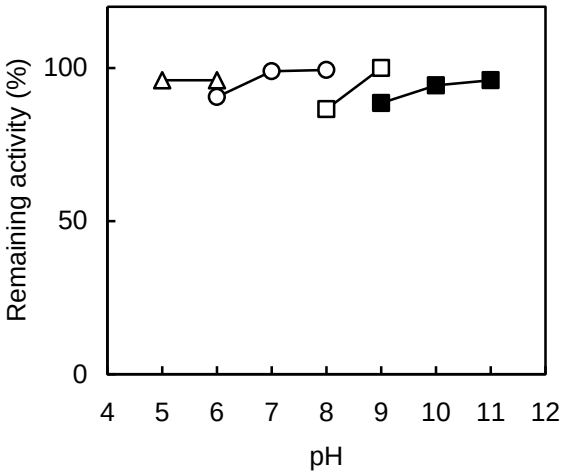

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
□ TEA-NaOH, ■ Gly-NaOH

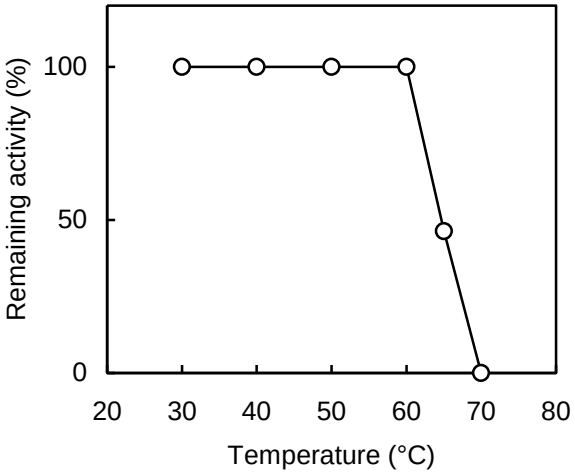

Fig. 3 Thermal stability

treated for 15 min in 0.1M potassium phosphate buffer, pH 8.0

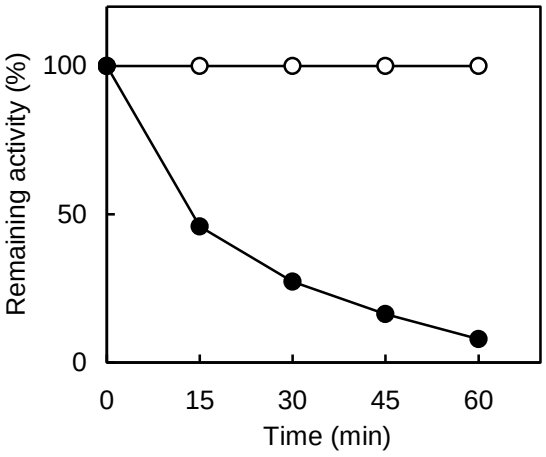

Fig. 4 Thermal stability

treated for in 0.1M potassium phosphate buffer, pH 8.0
○ 60 °C, ● 65°C