NIPRO ENZYMES

PHOSPHOGLUCOSE ISOMERASE (PGI)

[EC 5. 3. 1. 9]

from *Bacillus stearothermophilus*

D-Glucose-6-phosphate ↔ D-Fructose-6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 400 U/mg protein	
Contaminants	:	(as PGI activity = 100 %)	
		Phosphofructokinase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		NADPH oxidase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 200,000	
Subunit molecular weight	:	ca. 54,000	
Optimum pH	:	9.0 - 10.0	(Fig. 1)
pH stability	:	6.0 - 10.5	(Fig. 2)
Isoelectric point	:	4.2	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(95 mM Tris-HCl buffer, pH 9.0, at 30 °C)	
		Fructose-6-phospate	0.27 mM

STORAGE

Stable at -20 °C for at least one year

ASSAY

Principle

The change in absorbance is measured at 340 nm according to the following reactions.

$$\text{Fructose-6-phosphate} \xrightarrow{\text{PGI}} \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{Gluconolactone-6-phosphate} + \text{NADPH} + \text{H}^+$$

Unit Definition

One unit of activity is defined as the amount of PGI that forms 1 μmol of glucose-6-phosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 9.0
Ⅱ Fructose-6-phosphate (F6P) solution ; 100 mM (0.310 g F6P disodium salt/10 mL distilled water)
Ⅲ $NADP^+$ solution ; 22.5 mM (0.188 g $NADP^+$ sodium salt·$4H_2O$/10 mL distilled water)
Ⅳ Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast, Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/2 mL) approx. 140 U/mg at 25 °C

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.

Solution Ⅰ	28.44 mL	Solution Ⅲ	0.60 mL
Solution Ⅱ	0.90 mL	Solution Ⅳ	0.06 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of the curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \text{ X } (3.00 + 0.01)}{6.22 \text{ X } 0.01} \text{ X d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADPH (cm^2/μmol)
*Protein concentration ; determined by Bradford's method

REFERENCE

1. Muramatsu, N., and Nosoh, T.; *Arch. Biochem. Biophys.*, **144**, 245 (1971)

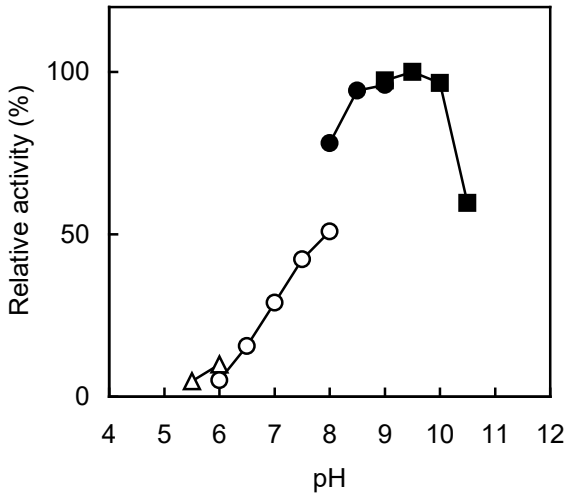

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

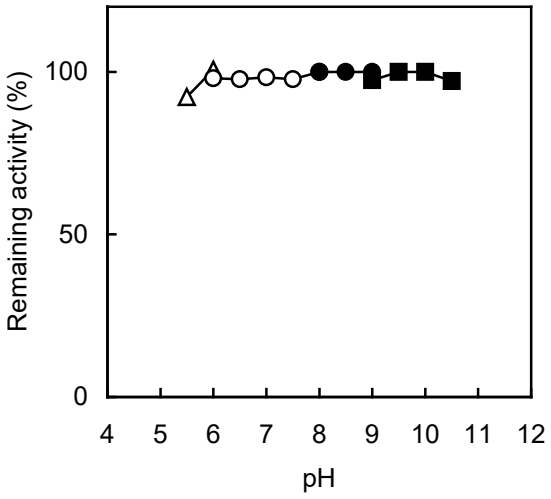

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

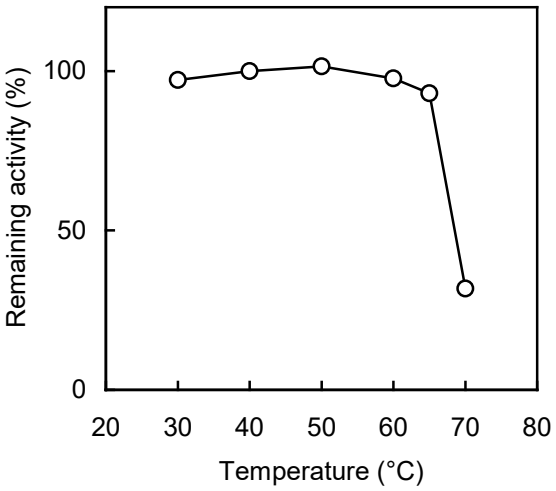

Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 8.5

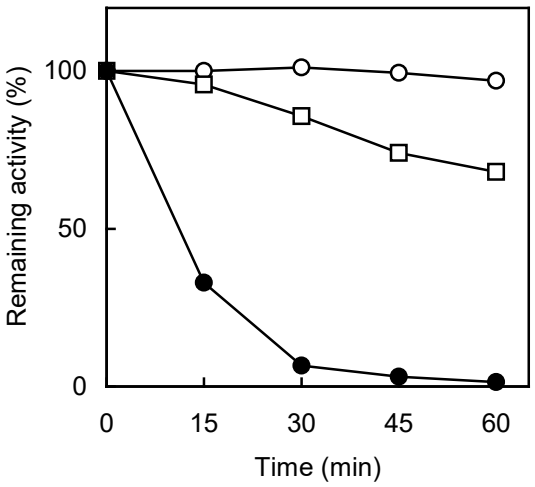

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.5
○ 60 °C, □ 65 °C, ● 70 °C