NIPRO ENZYMES

GLUCOKINASE (GlcK)

[EC 2. 7. 1. 2]

from *Bacillus stearothermophilus*

ATP + D-Glucose ↔ ADP + D-Glucose 6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 350 U/mg protein	
Contaminants	:	(as GlcK activity = 100 %)	
		Glucose-6-phosphate dehydrogenase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 68,000	
Subunit molecular weight	:	ca. 32,000	
Optimum pH	:	8.5	(Fig. 1)
pH stability	:	8.0 - 11.0	(Fig. 2)
Isoelectric point	:	5	
Optimum temperature	:	65 °C	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(60mM Tris-HCI buffer, pH 8.5, at 30 °C)	
		Glucose	0.1 mM
		ATP	0.05 mM
Substrate specificity	:	D-Glucose	100 %
		D-Mannose	25 %
		D-Fructose	0 %

STORAGE

Stable at -20 to 5 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.

ASSAY

Principle

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

$$\text{ATP + Glucose} \xrightarrow{\text{GlcK}} \text{ADP + Glucose 6-phosphate}$$

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

Unit Definition

One unit of activity is defined as the amount of GlcK 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 ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅳ $NADP^+$ solution ; 22.5 mM mM [(0.172 g $NADP^+$ monosodium salt or 0.177 g $NADP^+$ disodium salt)/10 mL distilled water]
Ⅴ Glucose solution ; 40 mM (0.072 g glucose (anhyd.)/10 mL distilled water)
Ⅵ 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	17.97mL	SolutionⅣ	1.20mL
Solution Ⅱ	1.20 mL	Solution Ⅴ	9.00mL
SolutionⅢ	0.60 mL	SolutionⅥ	0.03mL

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 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. Hengartner, H., and Zuber, H.; FEBS *Lett.*, **37**, 212 (1973)
2. Kamei, S., Tomita, K., Nagata, K., Okuno, H., Shiraishi, T., Motoyama, A., Ohkubo, A., and Yamanaka, H.; *J. Clin. Biochem. Nutr.*, **3**,1 (1987)
3. Tomita, K., Kamei, S., Nagata, K., Okuno, H., Shiraishi, T., Motoyama, A., Ohkubo, A., and

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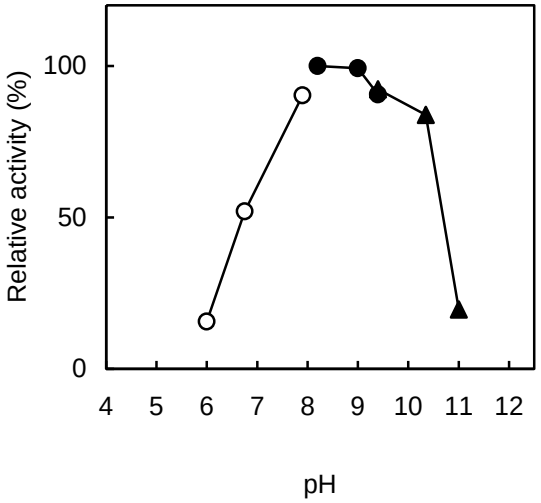

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
▲ carbonate

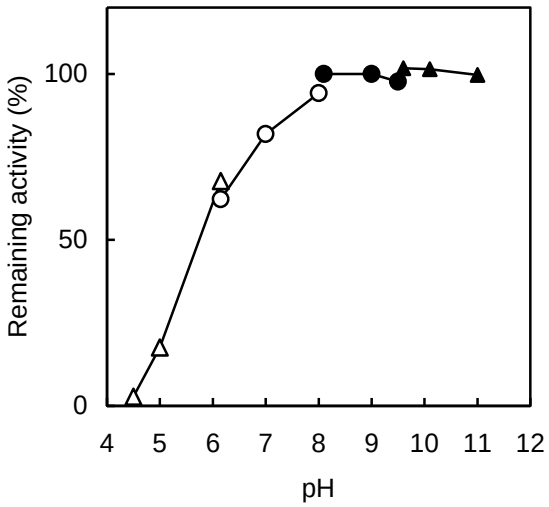

Fig. 2 pH stability

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

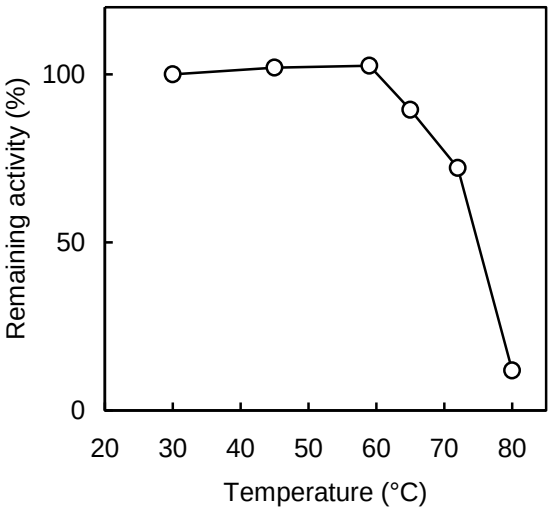

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 8.9

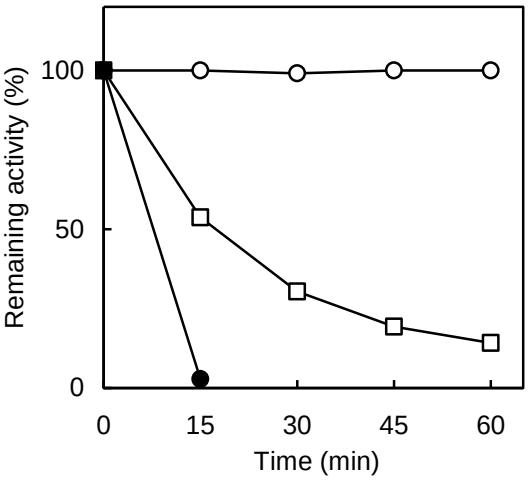

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 8.9
○ 60 °C, □ 70 °C, ● 80 °C