NIPRO ENZYMES

POLYNUCLEOTIDE PHOSPHORYLASE (PNPase)

[EC 2. 7. 7. 8]

from *Bacillus stearothermophilus*

RNA_{n+1} + Pi ↔ RNA_n + Nucleoside diphosphate

FOR DEPOLYMERIZATION REACTION

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 2,000 U/mg protein	

PROPERTIES

Molecular weight	: 300,000 - 340,000	
Subunit molecular weight	: ca. 85,000	
Optimum pH	: 9.0 - 9.5	(Fig. 1)
pH stability	: 9.0 - 11.0	(Fig. 2)
Isoelectric point	: 4.0	
Thermal stability	: No detectable decrease in activity up to 55 °C.	(Fig. 3, 4)
Michaelis constants	: (38 mM Tris-HCI buffer, pH 9.5, at 60 °C)	
	Poly A	0.27 mM**
	KH_2PO_4	3.0 mM
	**concentration of poly A was calculated as AMP concentration	
Effectors	: cations and anions	(Fig. 5, 6)

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for the preparation of polyribonucleotide.

ASSAY

Principle

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

$$\text{Poly } A_n + Pi \xrightarrow{\text{PNPase}} \text{Poly } A_{n-1} + ADP \qquad (I)$$

$$ADP + PEP \xrightarrow{PK} ATP + Pyruvate$$

$$Pyruvate + NADH + H^+ \xrightarrow{LDH} Lactate + NAD^+ \qquad (II)$$

Unit Definition

One unit of activity is defined as the amount of PNPase that forms 1 µmol of ADP per hour at 60 °C by depolymerizing of Poly A.

Solutions

(Reaction I)

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 9.5 ((1.212 g Tris + 0.074 g EDTA + 0.014 mL 2-mercaptoethanol + 0.610 g $MgCl_2 \cdot 6H_2O$ + 0.746 g KCl)/80 mL distilled water, adjusted to pH 9.5 with 1 N-HCl and filled up to 100 mL with distilled water)

Ⅱ KH_2PO_4 solution ; 65 mM (0.088 g KH_2PO_4/10 mL distilled water)

Ⅲ polyadenylate (Poly A) solution ; (25 mg Poly A potassium salt/1 mL distilled water; ca. 35 mM based on AMP concentration)

(Reaction II)

Ⅳ Buffer solution ; 100 mM Triethanolamine buffer, pH 7.6 ((9.300 g triethanolamine-HCl + 0.407 g $MgCl_2 \cdot 6H_2O$ + 0.373 g KCl)/400 mL distilled water, adjusted to pH 7.6 with 1 N-NaOH and filled up to 500 mL with distilled water)

Ⅴ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

Ⅵ Phosphoenolpyruvate (PEP) solution ; 56mM (0.150 g PEP MCA salt/10 mL distilled water)

Ⅶ Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C

Ⅷ Lactate dehydrogenase (LDH) ; (from pig heart, Oriental Yeast Co. Ltd., Product Code: LDH-02) ammonium sulfate suspension, approx. 5,000 U/mL at 25 °C

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 1 to 5 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

Procedure

(Reaction I)

1. Prepare the following reaction mixture and pipette 0.55 mL of reaction mixture into a test tube.

Solution Ⅰ	2.50mL	Solution Ⅲ	1.00mL
Solution Ⅱ	1.00mL	H_2O	1.00mL

2. Add 0.10 mL of enzyme solution and mix.
3. Incubate at 60 °C for exactly 10 minutes.
4. After incubation, add 0.01 mL conc. HCl and mix.
5. Centrifuge at 10,000 rpm for 30 seconds.
   At the same time, repeat the Procedure 1 to 5 using distilled water in place of enzyme solution in Procedure 2 (as blank).

(Reaction II)

6. Prepare the following reaction mixture and pipette 2.50 mL of the reaction mixture into a cuvette.

Solution Ⅳ	24.18mL	Solution Ⅶ	0.12mL

Solution Ⅴ	0.40mL	Solution Ⅷ	0.05mL
Solution Ⅵ	0.25mL		

7. Incubate at 30 °C for about 3 minutes.
8. Add 0.10 mL of supernatant of Procedure 5 and mix.
9. Read absorbance at 340 nm (Abs•test).
   Repeat the Procedure using blank (Abs•blank).

Calculation

$$\text{Volume activity (U/mL)} = ((\text{Abs•blank}) - (\text{Abs•test})) \times \frac{2.60 \times 0.65}{6.22 \times 0.10 \times 0.10} \times \frac{60}{10} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

REFERENCES

1. Smith, J.C., and Eaton, M.A.W.; *Nucleic Acids Research*, **1**, 1763 (1974)
2. Wood, J.N., and Hutchinson, D.W.; *ibid*., **3**, 219 (1976)

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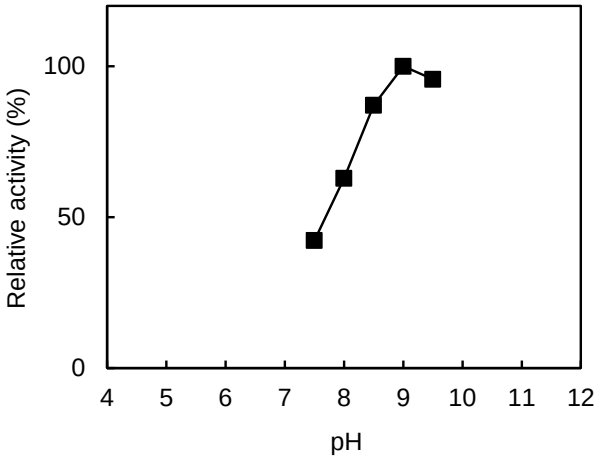

Fig. 1 pH profile

■ Tris-HCl

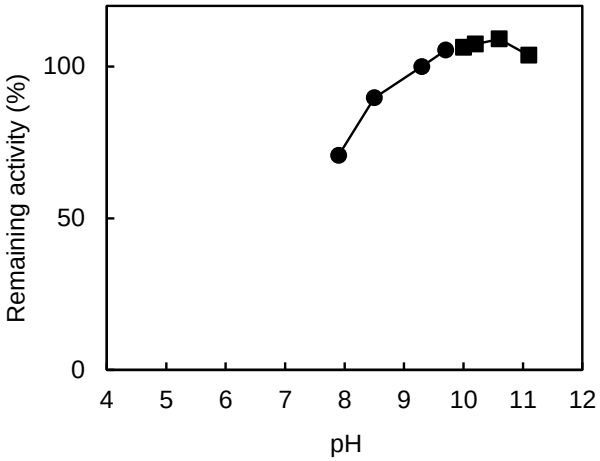

Fig. 2 pH stability

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

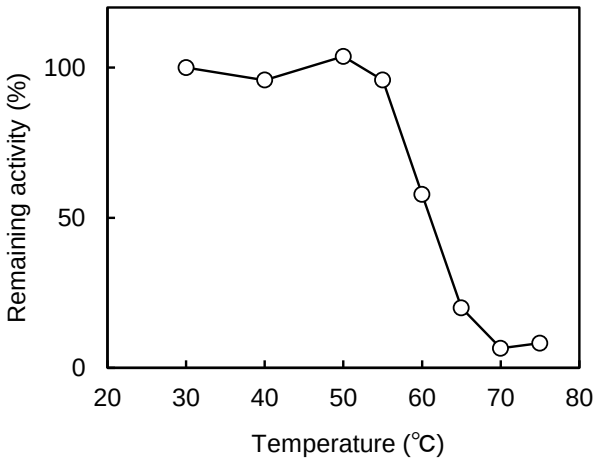

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 8.5

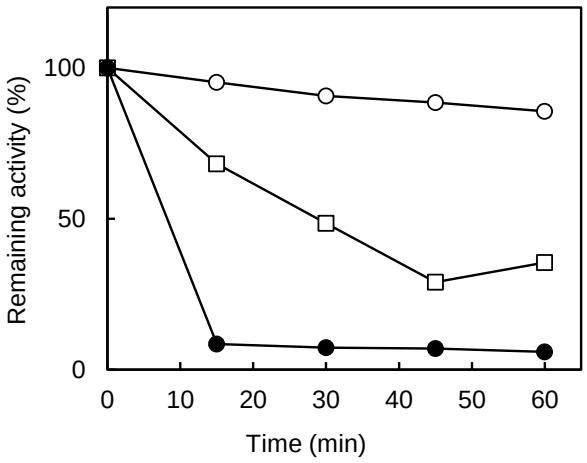

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 8.5
○ 55 ° C, □ 60 ° C, ● 65 ° C

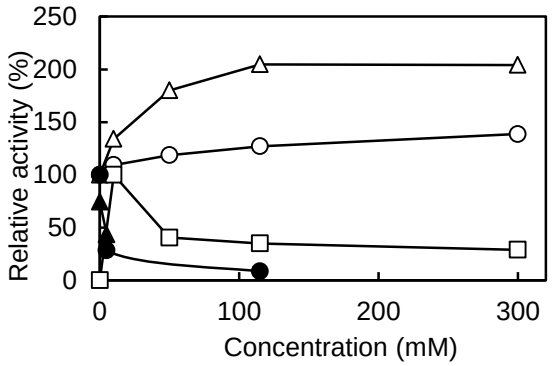

Fig. 5 Effect of various cations on the activity of Polynucleotide phosphorylase in the following *Assay Method*

Measurement : 0.015 mL of each cation solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 ° C. After 10 minutes, the quantity of ADP was determined.
○ NaCl, △ KCl, □ $MgCl_2$, ● $CaCl_2$, ▲$ZnCl_2$

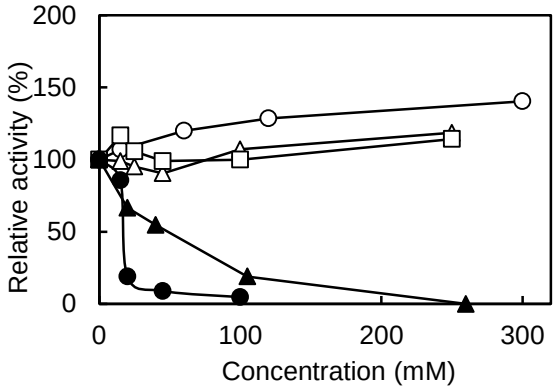

Fig. 6 Effect of various anions on the activity of Polynucleotide phosphorylase in the following *Assay Method*

Measurement : 0.015 mL of each anion solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 ° C. After 10 minutes, the quantity of ADP was determined.
○ NaCl, △CH_3COONa, □ Na_2SO_4, ●$NaHCO_3$, ▲ NaH_2PO_4