NIPRO ENZYMES

POLYNUCLEOTIDE PHOSPHORYLASE (PNPase3)

[EC 2. 7. 7. 8]

from recombinant *E. coli*

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

FOR DEPOLYMERIZATION REACTION

SPECIFICATION

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

PROPERTIES

Subunit molecular weight	: ca. 79,000	
Optimum pH	: 9.0 - 9.5	(Fig. 1)
pH stability	: 8.0 - 11.0	(Fig. 2)
Thermal stability	: No detectable decrease in activity up to 55 °C.	(Fig. 3, 4)
Effectors	: cations and anions	(Fig. 5, 6)

STORAGE

at -20 °C

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.

$$\text{Poly } A_n + Pi \xrightarrow{\text{PNPase3}} \text{Poly } A_{n-1} + ADP \qquad (I)$$

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

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

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 ; 56 mM (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 2 to 150 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	2.50 mL	Solution Ⅲ	1.00 mL
Solution Ⅱ	1.00 mL	H_2O	1.00 mL

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	24.18 mL	Solution Ⅶ	0.12 mL

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

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 280 nm (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)

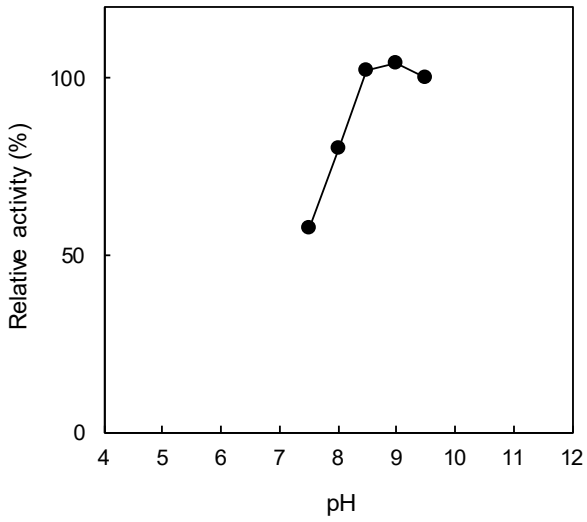

Fig. 1 pH profile

● Tris-HCl

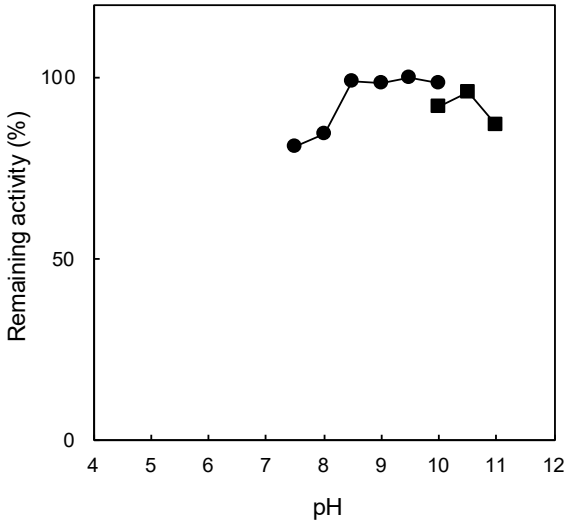

Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the following buffer solution (0.1 M);
● Tris-HCl, ■ Glycine-KOH

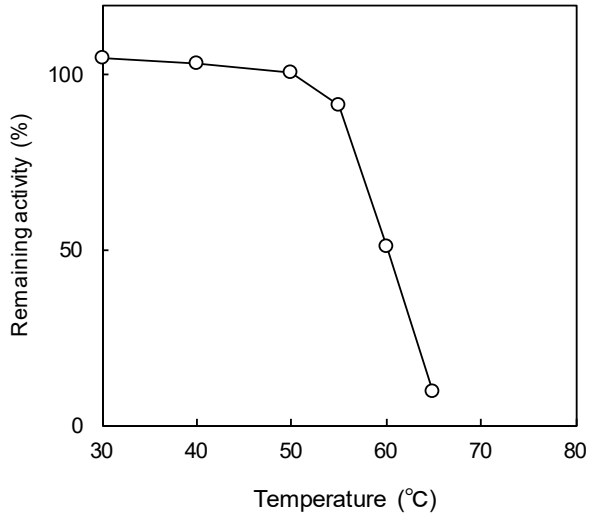

Fig. 3 Thermal stability

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

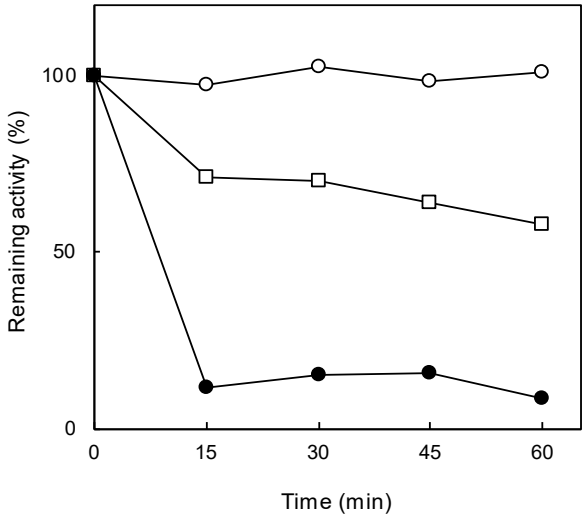

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.5
○ 55 ℃, □ 60 ℃, ● 65 ℃

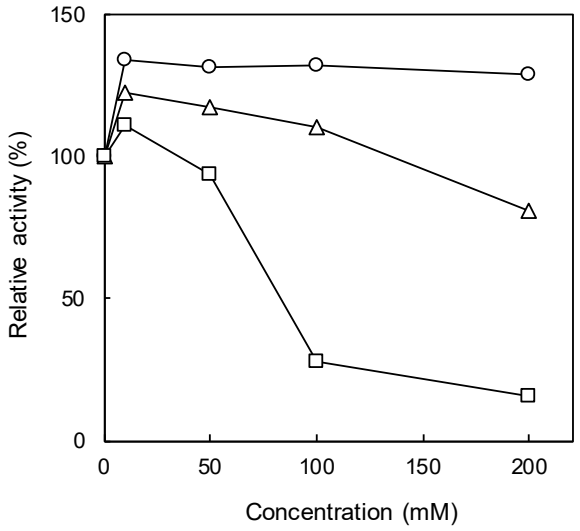

Fig. 5 Effect of various cations on the activity of PNPase3 in the following Assay

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 ℃. After 10 minutes, the quantity of ADP was determined.
○ NaCl, △ KCl, □ $MgCl_2$

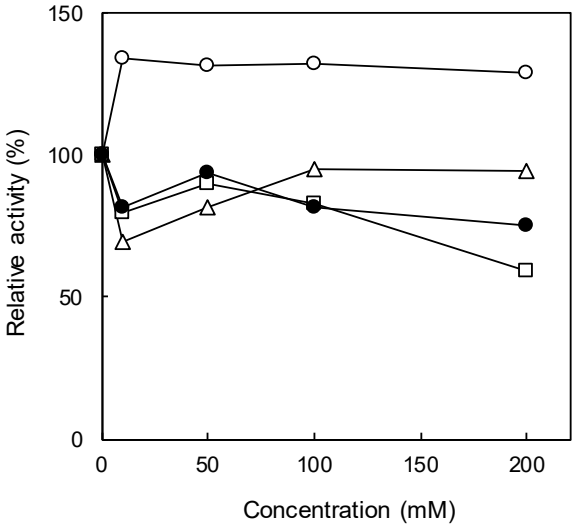

Fig. 6 Effect of various anions on the activity of PNPase3 in the following Assay

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 ℃. After 10 minutes, the quantity of ADP was determined.
○ NaCl, △ CH_3COONa, □ Na_2SO_4, ● NaH_2PO_4