NIPRO ENZYMES

PHOSPHOTRANSACETYLASE (PTA2)

[EC 2. 3. 1. 8]

from recombinant *E. coli*

Acetyl-CoA + Pi ↔ Acetylphosphate + CoA

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 5,000 U/mg protein	
Contaminants	:	(as PTA2 activity = 100 %)	
		Acetate kinase	< 0.01 %
		Adenylate kinase	< 0.01 %
		Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 69,700	
Subunit molecular weight	:	ca. 33,500	
Optimum pH	:	7.4	(Fig. 1)
pH stability	:	4.0 - 11.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(87mM Tris-HCl buffer, pH 7.5, at 30 °C)	
		Coenzyme A	0.1 mM
		Acetyl Phosphate	0.5 mM

STORAGE

Stable at -20 °C for at least one year

ASSAY

Principle

The change in absorbance is measured at 233 nm according to the following reaction.

$$\text{Acetylphosphate + CoA} \xrightarrow{\text{PTA2}} \text{Acetyl-CoA + Pi}$$

Unit Definition

One unit of activity is defined as the amount of PTA that forms 1 μmol of acetyl-CoA per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 7.5
Ⅱ CoA solution ; 6.4 mM (50 mg CoA trilithium salt/10 mL distilled water)
Ⅲ Acetylphosphate solution ; 217 mM (0.400 g acetylphosphate potassium lithium salt/10 mL distilled water)
Ⅳ Ammonium sulfate (AmS) solution ; 1 M (13.2 g AmS/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 20 U/mL with 50 mM Tris-HCl buffer, pH 8.0.

Procedure

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

Solution Ⅰ	26.0 mL	Solution Ⅲ	1.0 mL
Solution Ⅱ	2.0 mL	Solution Ⅳ	1.0 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 233 nm per minute (ΔAbs_{233}) in the linear portion of curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{233}) \text{ X } (3.00 + 0.01)}{4.44 \text{ X } 0.01} \text{ X d.f.}$$

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

d.f. ; dilution factor
4.44 ; differential millimolar extinction coefficient between acety-CoA and CoA ($cm^2/\mu mol$)
*Protein concentration ; determined by Bradford's method

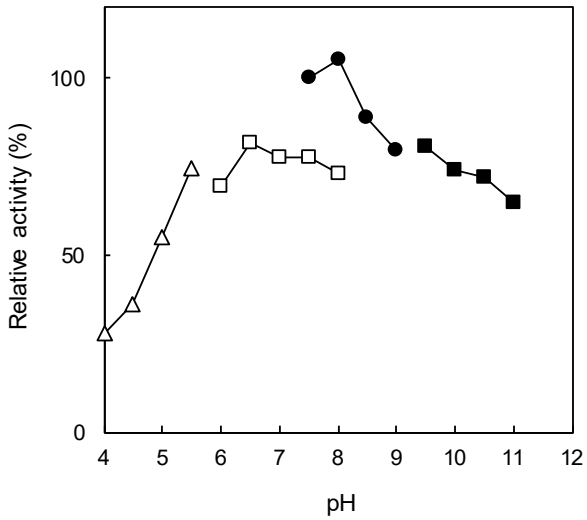

Fig. 1 pH profile

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

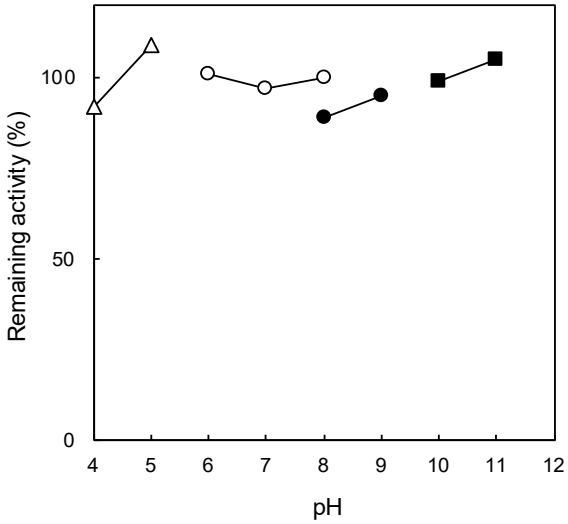

Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the following buffer solution (50 mM);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Glycine-KOH

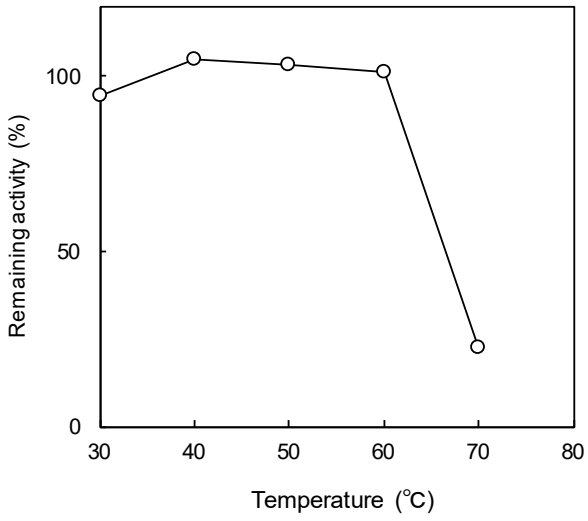

Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 8.5, 0.1 % BSA

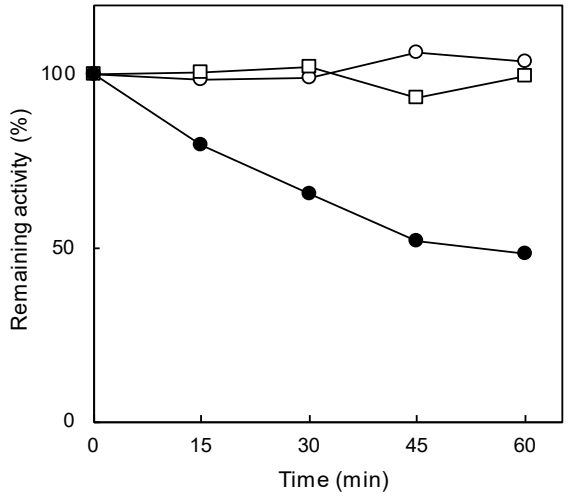

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.5, 0.1 % BSA
○ 55 ℃, □ 60 ℃, ● 65 ℃