NIPRO ENZYMES

ACETATE KINASE (AK)

[EC 2. 7. 2. 1]

from *Bacillus stearothermophilus*

ATP + Acetate ↔ ADP + Acetylphosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 1,100 U/mg protein	
Contaminants	:	(as AK activity = 100 %)	
		Lactate dehydrogenase	< 0.01 %
		Adenylate kinase	< 0.01 %
		NADH oxidase	< 0.01 %
		GOT	< 0.01 %
		GPT	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 160,000	
Subunit molecular weight	:	ca. 40,000	
Optimum pH	:	7.2	(Fig. 1)
pH stability	:	7.0 - 8.0	(Fig. 2)
Isoelectric point	:	4.8	
Thermal stability	:	No detectable decrease in activity up to 65 °C.	(Fig. 3, 4)
Michaelis constants	:	(57 mM Imidazole- HCl buffer, pH 7.2, at 30 °C)	
		Acetate	120 mM
		Acetylphosphate	2.3 mM
		ATP	1.2 mM
		ADP	0.8 mM
Substrate specificity	:	Acetate	100 %
		Formate	0 %
		Propionate	5 %
		Butyrate	0 %
		Oxalate	0 %
		Citrate	0 %
		Malate	0 %
		Glycine	0 %
Activator	:	Fructose-1,6-bisphosphate	

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of acetate or for ATP regeneration system.

ASSAY

Principle

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

$$\text{ATP + Acetate} \xrightarrow{\text{AK}} \text{ADP + Acetylphosphate}$$

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

$$\text{Pyruvate + NADH + H}^{+} \xrightarrow{\text{LDH}} \text{Lactate + NAD}^{+}$$

Unit Definition

One unit of activity is defined as the amount of AK that forms 1 μmol of ADP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Imidazole-HCl, pH 7.2
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 Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
Ⅳ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)
Ⅴ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$ /100 mL distilled water)
Ⅵ KCl solution ; 2.5 M (18.64 g KCl/100 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
Ⅸ Sodium acetate solution ; 2 M (27.22 g sodium acetate·$3H_2O$/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.4 mL of reaction mixture into a cuvette.

Solution Ⅰ	16.92 mL	Solution Ⅴ	0.60 mL
Solution Ⅱ	3.00 mL	Solution Ⅵ	0.90 mL
Solution Ⅲ	1.80 mL	Solution Ⅶ	0.12 mL
Solution Ⅳ	0.60 mL	Solution Ⅷ	0.06 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.60 mL of Solution Ⅸ and 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

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

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

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

REFERENCE

1. Nakajima, H., Suzuki, K., and Imahori, K. ; *J. Biochem*., **84**, 193 (1978)
2. Nakajima, H., Suzuki, K., and Imahori, K. ; *ibid*., **84**, 1139 (1978)
3. Nakajima, H., Suzuki, K., and Imahori, K. ; *ibid*., **86**, 1169 (1979)

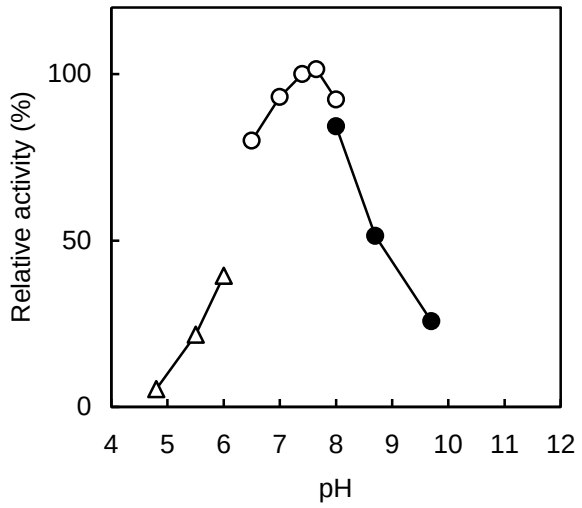

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl

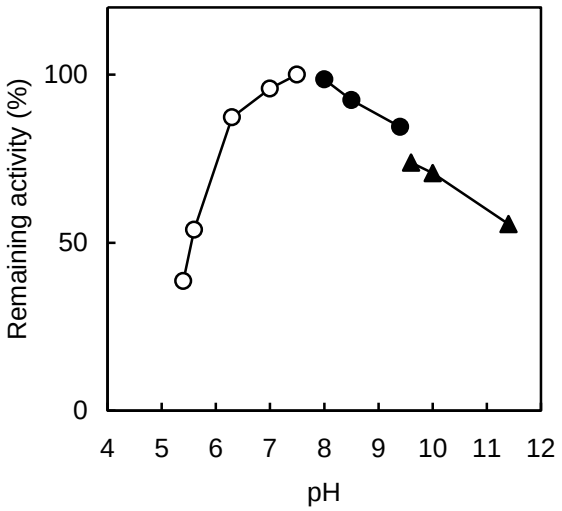

Fig. 2 pH stability

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

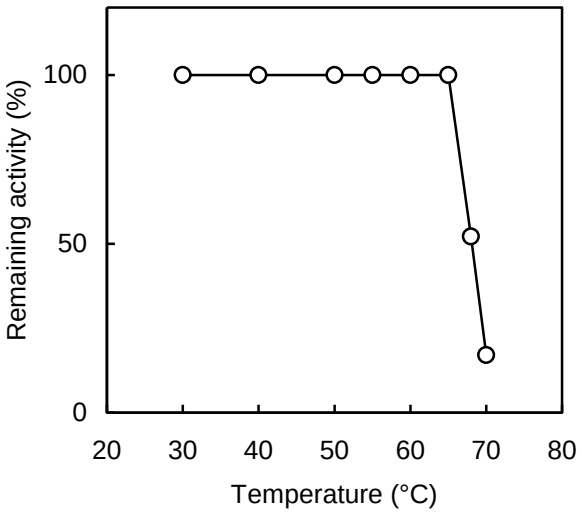

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

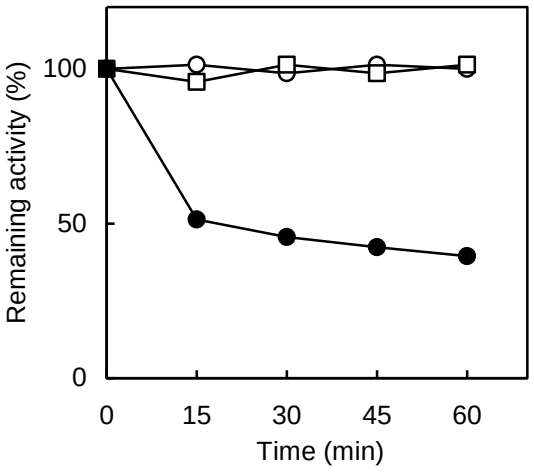

Fig. 4 Thermal stability

treated in 0.1M potassium phosphate buffer, pH 7.5
○ 60 °C, □ 65 °C, ● 70 °C