NIPRO ENZYMES

ACETATE KINASE (AK2)

[EC 2. 7. 2. 1]

from recombinant *E. coli*

ATP + Acetate ↔ ADP + Acetylphosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 1,100 U/mg protein	
Contaminants	:	(as AK2 activity = 100 %)	
		Lactate dehydrogenase	< 0.01 %
		Adenylate kinase	< 0.01 %
		NADH oxidase	< 0.01 %
		GOT	< 0.01 %
		GPT	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 172,000	
Subunit molecular weight	:	ca. 39,400	
Optimum pH	:	6.0	(Fig. 1)
pH stability	:	6.0 - 11.0	(Fig. 2)
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	130 mM
		Acetylphosphate	3.3 mM
		ATP	5.4 mM
		ADP	0.6 mM
Substrate specificity	:	Acetate	100 %
		Formate	0 %
		Propionate	4 %
		Butyrate	1 %
		Oxalate	0 %
		Malate	0 %
		Glycine	0 %
Activator	:	Fructose-1,6-bisphosphate	

STORAGE

Stable at -20 °C for at least one year

ASSAY

Principle

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

$$\text{ATP + Acetate} \xrightarrow{\text{AK2}} \text{ADP + Acetylphosphate}$$

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

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

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)

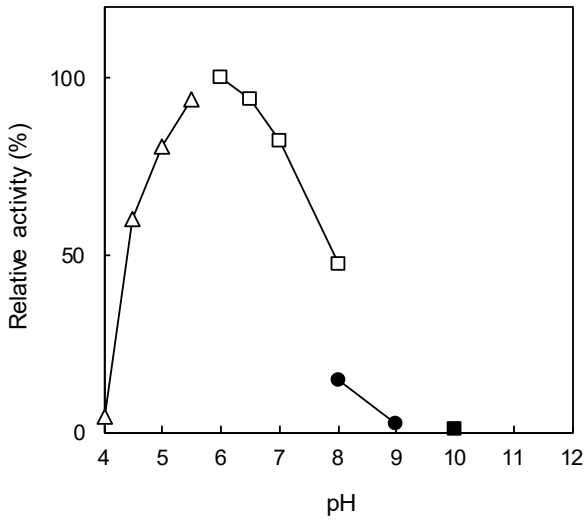

Fig. 1 pH profile

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

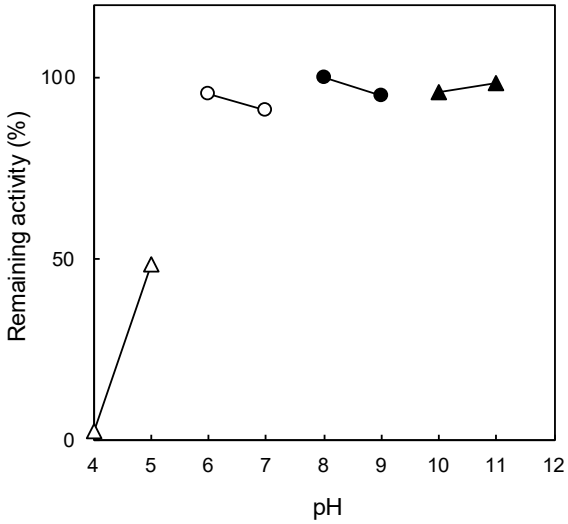

Fig. 2 pH stability

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

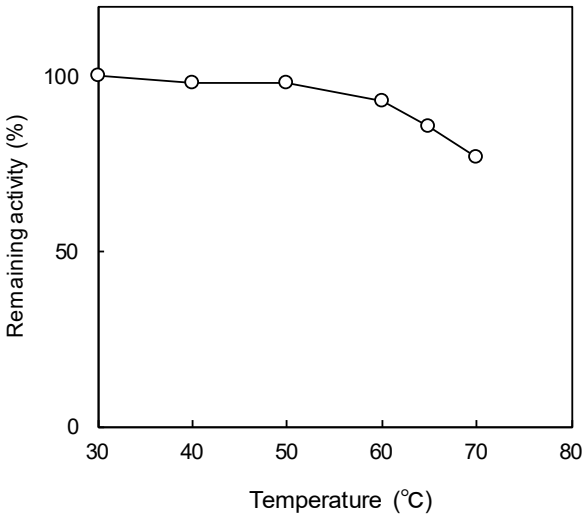

Fig. 3 Thermal stability

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

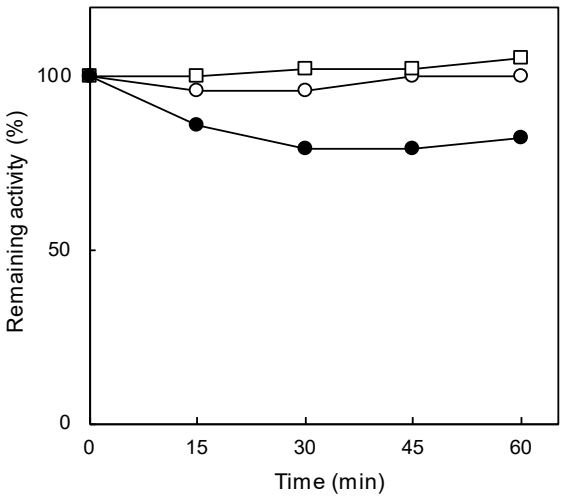

Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer, pH 7.5
○ 60 ℃, □ 65 ℃, ● 70 ℃