NIPRO ENZYMES

ADENYLATE KINASE (AdK)

[EC 2. 7. 4. 3]

from *Bacillus stearothermophilus*

ATP + AMP ↔ 2 ADP

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 200 U/mg protein	
Contaminants	:	(as AdK activity = 100 %)	
		ATPase	< 0.01 %
		Phosphoglycerate kinase	< 0.10 %

PROPERTIES

Molecular weight	:	ca. 20,000	
Optimum pH	:	6.5	(Fig. 1)
pH stability	:	8.0 - 10.5	(Fig. 2)
Isoelectric point	:	5.0	
Thermal stability	:	No detectable decrease in activity up to 65 °C.	(Fig. 3, 4)
Michaelis constants	:	(89 mM Imidazole-HCl buffer, pH 6.5, at 30 °C)	
		ATP	0.04 mM
		ADP	0.05 mM
		AMP	0.02 mM

STORAGE

Stable at -20 °C for at least one year

APPLICATION

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

ASSAY

Principle

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

$$ATP + AMP \xrightarrow{AdK} 2\ ADP$$

$$2\ ADP + 2PEP \xrightarrow{PK} 2\ ATP + 2\ Pyruvate$$

$$2\ Pyruvate + 2\ NADH + 2\ H^+ \xrightarrow{LDH} 2\ Lactate + 2\ NAD^+$$

Unit Definition

One unit of activity is defined as the amount of AdK that forms 2 μmol of ADP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Imidazole-HCl, pH 6.5
Ⅱ AMP solution ; 50 mM (0.250 g AMP disodium salt·$6H_2O$/10 mL distilled water)
Ⅲ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅳ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$ /10 mL distilled water)
Ⅴ Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
Ⅵ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅶ KCl solution ; 2.5 M (18.64 g KCl/100mL distilled water)
Ⅷ 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
Ⅸ 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 2.5 to 5 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

Procedure

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

Solution Ⅰ	26.70 mL	Solution Ⅵ	0.60 mL
Solution Ⅱ	0.24 mL	Solution Ⅶ	1.20 mL
Solution Ⅲ	0.30 mL	Solution Ⅷ	0.09 mL
Solution Ⅳ	0.60 mL	Solution Ⅸ	0.09 mL
Solution Ⅴ	0.18 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 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \times (3.00 + 0.01)}{2 \times 6.22 \times 0.01} \times \text{d.f.}$$

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

d.f. ; dilution factor
2 ; according to the reaction that forms 2 μmol of ADP, one unit of activity of Adk is defined to form 2 μmol of ADP.

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

REFERENCE

1. Imahori, K., Nakajima, H., Nagata, K., and Iwasaki, T.; *Seikagaku*, **53**, 829 (1981)

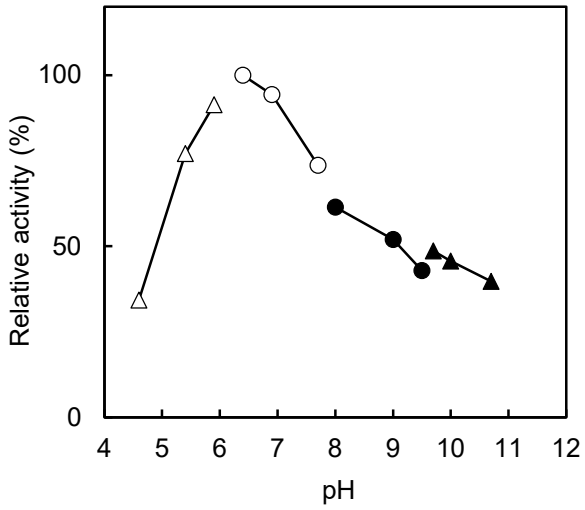

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲ carbonate

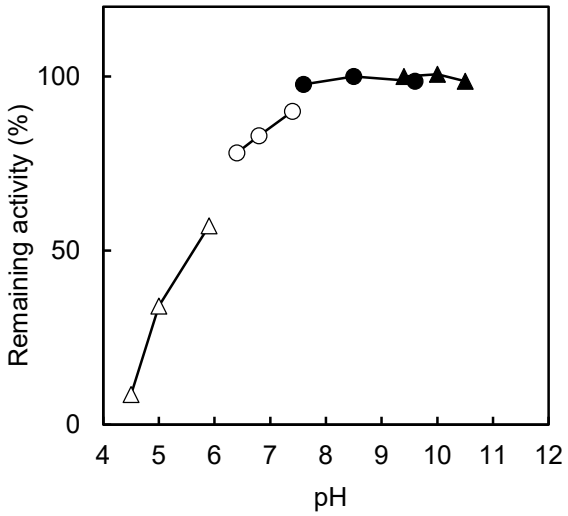

Fig. 2 pH stability

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

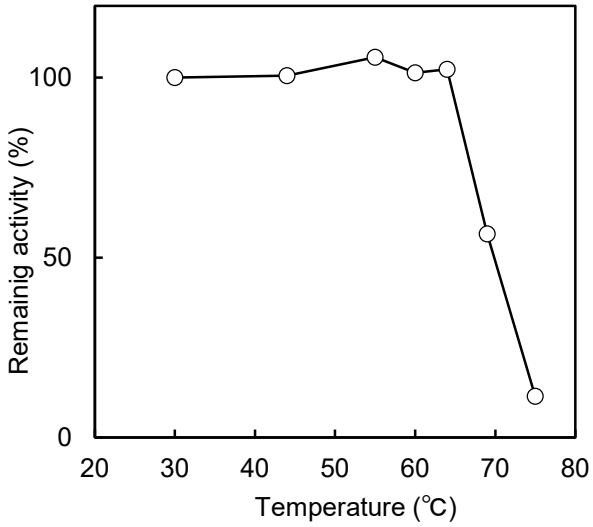

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 9.0

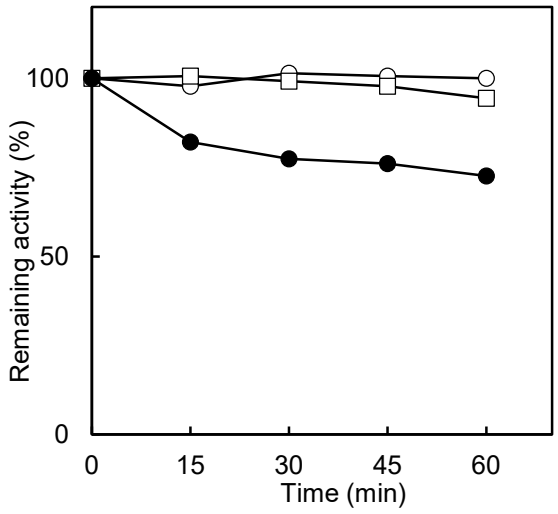

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 9.0
○ 60 ℃, □ 65 ℃, ● 70 ℃