NIPRO ENZYMES

PHENYLALANINE DEHYDROGENASE (PheDH)

[EC 1.4.1.20]

from *Thermoactinomyces intermedius*

L-Phenylalanine + NAD^+ + H_2O ↔ Phenylpyruvate + NH_4^+ + NADH

SPECIFICATION

State	:	Ammonium sulphate suspension	
Specific activity	:	more than 30 U/mg protein	
Contaminants	:	(as PheDH activity = 100 %)	
		NADH oxidase	< 0.01 %
		Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 380,000	
Subunit molecular weight	:	ca. 40,000	
Optimum pH	:	11.5	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	(200 mM Gly-KCl-KOH buffer, pH 11.0, at 30 °C)	
		L-Phenylalanine	0.66 mM
		NAD^+	0.05 mM
Substrate specificity	:	L-Phenylalanine	100 %
		L-Tyrosine	7.6 %
		L-Methionine	1.5 %

STORAGE

Stable at 4 °C for at least six months (Do not freeze)

ASSAY

Principle

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

$$\text{L-Phenylalanine} + NAD^+ + H_2O \xrightarrow{\text{PheDH}} \text{Phenylpyruvate} + NH_4^+ + NADH$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 400 mM Gly-KCl-KOH, pH 11.0
Ⅱ L-Phenylalanine solution ; 100 mM (0.165 g L-phenylalanine/10 mL distilled water)
Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)

Preparation of Enzyme Solution

Dilute the ammonium sulphate suspension of enzyme to 2 to 6 U/mL with 10 mM Tris-HCl buffer, pH 8.0, containing 50 mM NaCl.

Procedure

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

Solution Ⅰ	15.00 mL
Solution Ⅱ	3.00 mL
Solution Ⅲ	0.15 mL
H_2O	11.85 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 340nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

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

REFERENCE

1. Ohshima, T., Takada, H., Yoshimura, T., Esaki, N., and Soda, K.; *J. Bacteriol.*, **173**, 3943 (1991)

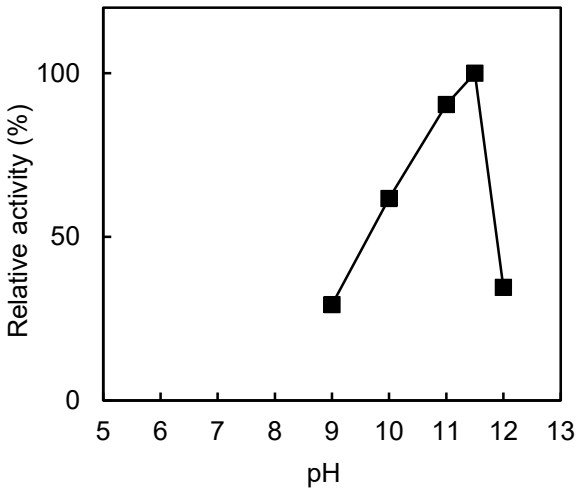

Fig. 1 pH profile

(■ Gly-KCl-KOH)

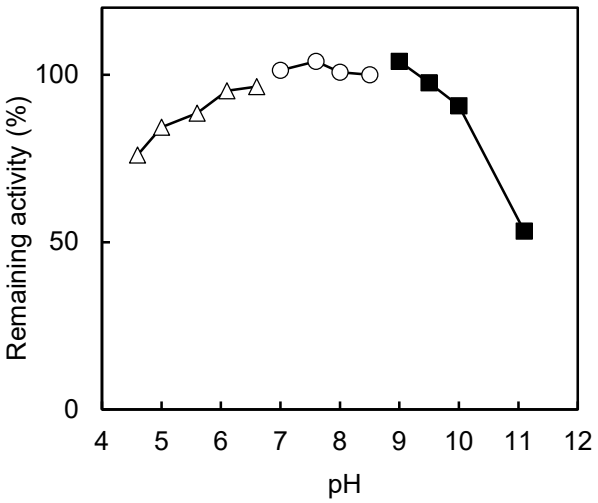

Fig. 2 pH stability

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

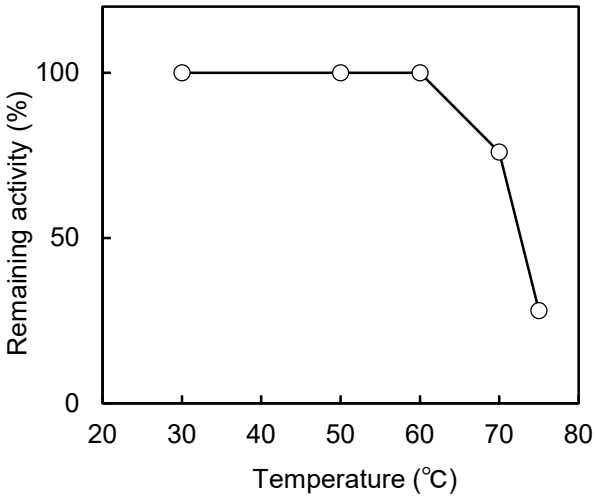

Fig. 3 Thermal stability

(treated for 15 min in 10 mM potassium phosphate buffer, pH 7.2)

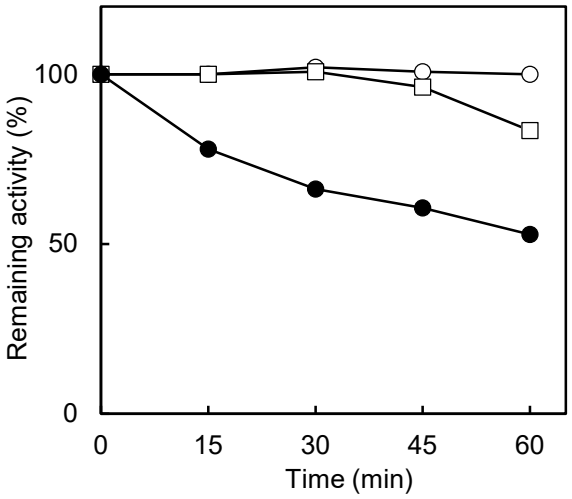

Fig. 4 Thermal stability

(treated in 10 mM potassium phosphate buffer, pH 7.2
○ 50 ℃, □ 60 ℃, ● 70 ℃)