NIPRO ENZYMES

ALANINE DEHYDROGENASE (AlaDH)

[EC 1. 4. 1. 1]

from *Bacillus stearothermophilus*

L-Alanine + NAD^+ + H_2O ↔ Pyruvate + NH_4^+ + NADH

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 10 U/mg protein	
Contaminants	:	(as AlaDH activity = 100 %)	
		NADH oxidase	< 0.01 %
		Lactate dehydrogenase	< 0.10 %

PROPERTIES

Molecular weight	:	ca. 230,000	
Subunit molecular weight	:	ca. 38,000	
Optimum pH	:	10.4	(Fig. 1)
pH stability	:	7.0 - 11.5	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 70 °C.	(Fig. 3, 4)
Michaelis constants	:	(125 mM Glycine-NaOH buffer, pH 10.5, at 30 °C)	
		L-Alanine	10.0 mM
		NAD^+	0.26 mM
Substrate specificity	:	L-Alanine	100 %
		L-Leucine	0 %
		L-Isoleucine	0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of L-alanine.

ASSAY

Principle

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

$$\text{L-Alanine} + NAD^+ + H_2O \xrightarrow{\text{AlaDH}} \text{Pyruvate} + NH_4^+ + NADH$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 250 mM Glycine-NaOH, pH 10.5

Ⅱ L-Alanine solution ; 150 mM (1.336 g L-alanine/80 mL distilled water, adjusted to pH 10.5 with 1 N-NaOH and filled up to 100 mL with distilled water)

Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+/ 10 mL with distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM glycine - NaOH buffer, pH 9.5.

Procedure

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

Solution Ⅰ	15.00 mL	Solution Ⅲ	1.50 mL
Solution Ⅱ	10.00 mL	H_2O	3.50 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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Sakamoto, Y., Nagata, S., Esakl, N., Tanaka, H. and Soda, K.; *J. Ferment. Bioeng.*, **69**, 154 (1990)

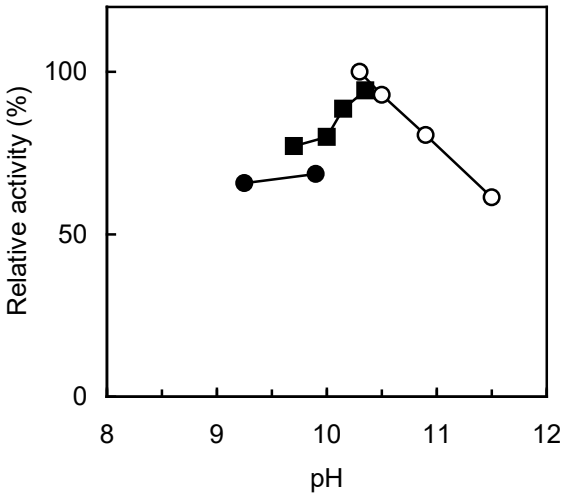

Fig. 1 pH profile

● Tris-HCl, ■ Gly-KOH,
○ phosphate

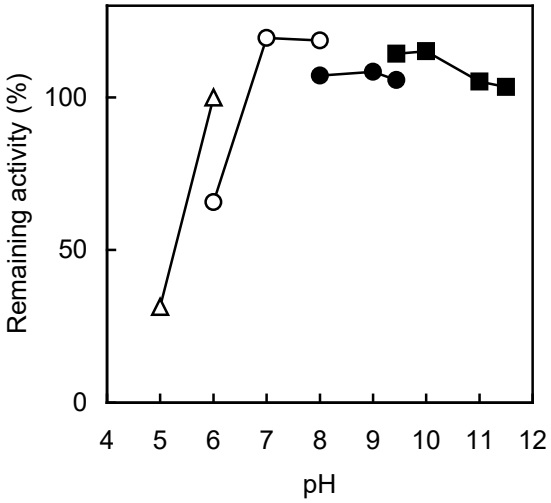

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

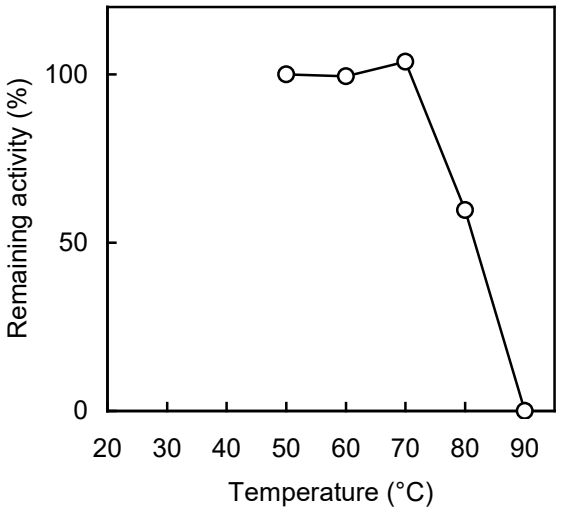

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Gly-KOH buffer, pH 9.0

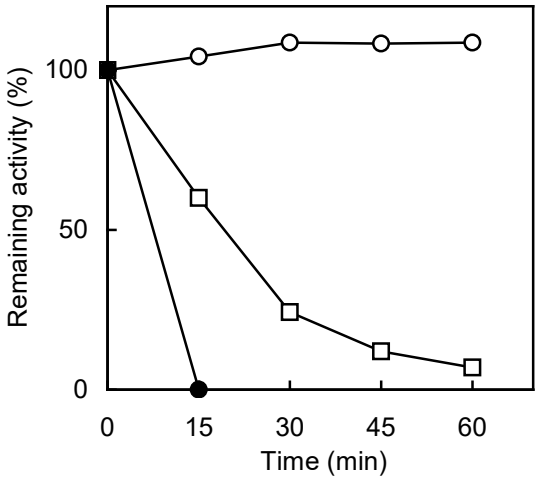

Fig. 4 Thermal stability

treated in 0.1 M Gly-KOH buffer, pH 9.0
○ 70 °C, □ 80 °C, ● 90 °C