Fructosyl-peptide Oxidase (FPOX-CE)

from recombinant *E. coli*

Fructosyl-peptide : oxygen oxidoreductase, EC 1.5.3

$$\text{Fructosyl-peptide} + H_2O + O_2 \longrightarrow \text{Peptide} + \text{Glucosone} + H_2O_2$$

SPECIFICATION

Appearance	yellow lyophilizate
Activity	≧4 U/mg lyophilizate
Stabilizers	EDTA, glutamate
Storage	at −20°C

PROPERTIES

Molecular weight	*ca.* 60 kDa (gel filtration)	
Structure	monomer of 52 kDa (SDS-PAGE)	
Michaelis constants	3.4×10^{-3} M (fructosyl-valyl-histidine)	
	4.4×10^{-3} M (fructosyl-glycine)	
	8.9×10^{-3} M (N^{ε}-fructosyl-lysine)	
pH Optimum	7.5–8.0	(Fig. 1)
pH Stability	6.0–9.5	(Fig. 2)
Optimum temperature	35–42°C	(Fig. 3)
Thermal stability	below 45°C	(Fig. 4)
Stability (powder form)	stable at 37°C for at least one month	(Fig. 5)
Specificity	fructosyl-valyl-histidine (100), fructosyl-glycine (53) N^{ε}-fructosyl-lysine (84)	

ASSAY PROCEDURE

Principle

$$\text{Fructosyl-L-amino acid} + H_2O + O_2 \xrightarrow{\text{fructosyl-peptide oxidase}} \text{L-Amino acid} + \text{Glucosone} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{TOOS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\ H_2O$$

The appearance of quinoneimine dye is measured spectrophotometrically at 555 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of hydrogen peroxide per min at 37°C and pH 8.0 under the conditions described below.

Reagents

A. Potassium phosphate buffer, 0.1 M; pH 8.0: mix 0.1 M KH_2PO_4 solution and 0.1 M K_2HPO_4 solution to make a pH 8.0 solution.
B. TOOS solution, 15 mM: 0.50 g of TOOS (*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine)/100 ml of distilled water.
C. Peroxidase (POD)-4-aminoantipyrine (4-AA) solution: dissolve 5 mg of POD preparation (200 guaiacol U/mg) in 800 ml of potassium phosphate buffer (Reagent A), then add 100 mg of 4-AA and dilute with potassium phosphate buffer (Reagent A) to 1000 ml.
D. Fructosyl-glycine solution, 600 mM: 1.43 g of fructosyl-glycine/10 ml of distilled water.
E. Enzyme dilution buffer: 10 mM potassium phosphate buffer, pH 8.0, containing 0.15% bovine serum albumin (BSA).

Sample: dissolve the lyophilized enzyme to a volume activity of 0.2–0.5 U/ml in ice-cold enzyme dilution buffer (Reagent E) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).
   0.1 ml TOOS solution (Reagent B)
   2.7 ml POD-4-AA solution (Reagent C)
   0.1 ml sample
2. Equilibrate at 37°C for about 5 min.
3. Add 0.1 ml of fructosyl-glycine solution (Reagent D) and mix.
4. Record the increase of absorbance at 555 nm in a spectrophotometer thermostated at 37°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding distilled water instead of fructosyl-glycine solution (Reagent D) (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.0(\text{ml}) \times df}{39.2 \times 1/2 \times 0.1(\text{ml})} = \Delta A \times 1.53 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

39.2 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions ($cm^2/\mu mol$)
1/2 : Factor based on the fact that 1 mol of hydrogen peroxide produces 1/2 mol of quinoneimine dye
df : Dilution factor
C : Content of fructosyl-peptide oxidase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of fructosyl-peptide and fructosyl-L-amino acid.

$$\underset{\text{(Fructosyl-L-amino acid)}}{\text{Fructosyl-peptide}} + H_2O + O_2 \xrightarrow{\text{fructosyl-peptide oxidase}} \underset{\text{(L-Amino acid)}}{\text{Peptide}} + \text{Glucosone} + H_2O_2$$

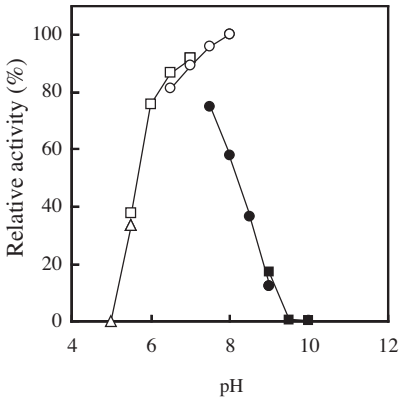
$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{TOOS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\ H_2O$$

REFERENCES

Horiuchi, T. *et al.*, *Agric. Biol. Chem.*, **53**, 103–110 (1989).
Hirokawa, K. *et al.*, *Arch. Microbiol.*, **180**, 227–231 (2003).
Hirokawa, K. *et al.*, *Biochem. Biophys. Res. Commun.*, **311**, 104–111 (2003).

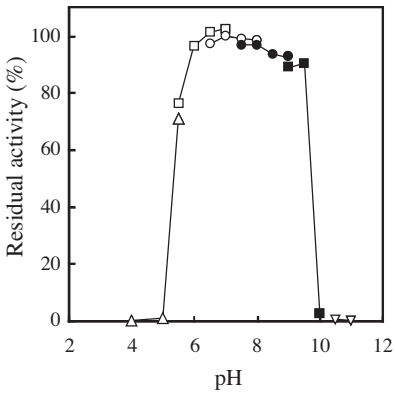
EXPERIMENTAL DATA

Fig. 1 pH Optimum



△ : 0.1 M acetate buffer
□ : 0.1 M MES-NaOH buffer
○ : 0.1 M phosphate buffer
● : 0.1 M Tris-HCl buffer
■ : 0.1 M CHES-NaOH buffer

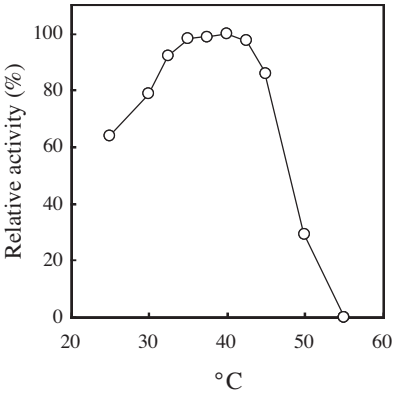
Fig. 2 pH Stability



Treatment: 30°C, 60 min

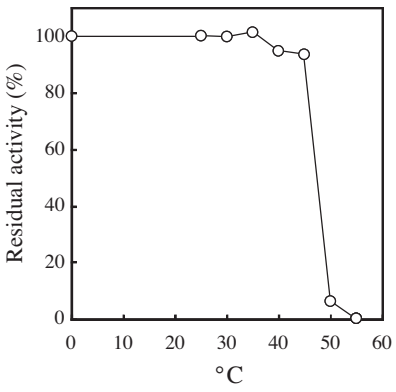
△ : 0.1 M acetate buffer
□ : 0.1 M MES-NaOH buffer
○ : 0.1 M phosphate buffer
● : 0.1 M Tris-HCl buffer
■ : 0.1 M CHES-NaOH buffer
▽ : 0.1 M CAPS-NaOH buffer

Fig. 3 Optimum temperature



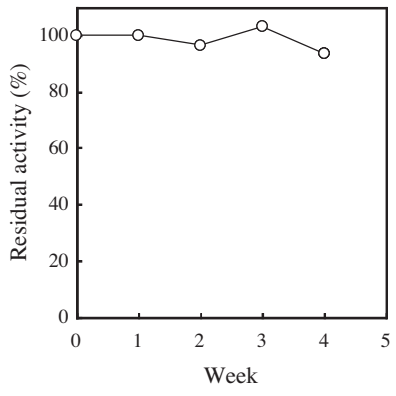
Buffer: 0.1 M phosphate buffer, pH 8.0

Fig. 4 Thermal stability



Treatment: 0.1 M phosphate buffer, pH 8.0, 10 min

Fig. 5 Stability (powder form) at 37°C



(Kept under dry conditions)