Cholesterol Oxidase (CHO-PEWL)

from recombinant *E. coli*

Cholesterol : oxygen oxidoreductase, EC 1.1.3

Cholesterol + 2 O_2 ⟶ 6β-hydroperoxycholest-4-en-3-on + H_2O_2

SPECIFICATION

Appearance	liquid form (30% sucrose solution)
Activity	≧200 U/ml
Storage	at −20°C

PROPERTIES

Molecular weight	*ca.* 59 kDa (gel filtration)	
Structure	monomer of 60 kDa (SDS-PAGE)	
Michaelis constants	1.9×10^{-5} M (cholesterol)	
pH Optimum	6.5–8.0	(Fig. 1)
pH Stability	3.5–8.5	(Fig. 2)
Optimum temperature	55°C–65°C	(Fig. 3)
Thermal stability	below 70°C	(Fig. 4)
Stability (liquid form)	stable at 25°C for at least one month (Fig. 5)	
Inhibitors	Ag^+, Hg^{2+}	

ASSAY PROCEDURE

Principle

$$\text{Cholesterol} + 2\ O_2 \xrightarrow{\text{cholesterol oxidase}} 6\beta\text{-hydroperoxycholest-4-en-3-one} + H_2O_2$$

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

The appearance of quinoneimine dye is measured spectrophotometrically at 500 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 7.0 under the conditions described below.

Reagents

A. Potassium phosphate buffer, 0.1 M; pH7.0: mix 0.1 M KH_2PO_4 solution and 0.1 M K_2HPO_4 solution to make a pH 7.0 solution.
B. Cholesterol solution, 0.5%: dissolve 500 mg of cholesterol in 5.0 ml of Triton X-100 with heating. Add 90 ml of distilled water to the hot cholesterol-Triton X-100 solution by slowly pouring. Stir and boil for 30–60s. Cool under running water with gentle agitation. Dissolve 4.0 g of sodium cholate in the cholesterol-Triton X-100 solution and dilute with distilled water to 100 ml. This solution is stable for about one week at room temperature. If it becomes cloudy, warm slightly with stirring until becomes clear.
C. 4-Aminoantipyrine (4-AA) solution, 1.76%: 1.76 g of 4-AA/100 ml of distilled water.
D. Phenol solution, 6.0%: 6.0 g of phenol/100 ml of distilled water.
E. Peroxidase (POD) solution, 1150 U/ml: 75 mg of POD (200 guaiacol U/mg)/100 ml of potassium phosphate buffer (Reagent A).
F. Enzyme dilution buffer: 20 mM potassium phosphate buffer, pH 7.0, containing 0.2% bovine serum albumin (BSA).

Sample: dissolve the enzyme preparation to a volume activity of 0.1–0.5 U/ml with ice-cold enzyme dilution buffer (Reagent F).

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm)

2.55 ml	Potassium phosphate buffer	(Reagent A)
0.20 ml	Cholesterol solution	(Reagent B)
0.05 ml	4-AA solution	(Reagent C)
0.10 ml	Phenol solution	(Reagent D)
0.10 ml	POD solution	(Reagent E)

2. Equilibrate at 37°C for about 5 min.
3. Add 0.05ml of sample and mix.
4. Record the increase of absorbance at 500 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 enzyme dilution buffer (Reagent F) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.05\,(\text{ml}) \times df}{13.8^* \times 1/2 \times 0.05\,(\text{ml})} = \Delta A \times 8.84 \times df$$

13.8 : 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

APPLICATIONS

The enzyme is useful for the determination of cholesterol in clinical analysis.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + 2\,O_2 \xrightarrow{\text{cholesterol oxidase}} 6\beta\text{-hydroperoxycholest-4-en-3-one} + H_2O_2$$

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

REFERENCES

Aono, R. *et al.*, *Appl. Environ. Microbiol.*, **60**, 2518–2523 (1994).
Doukyu, N. *et al.*, *Appl. Environ. Microbiol.*, **64**, 1929–1932 (1998).
Doukyu, N. and Aono, R., *Biochem. J.*, **341**, 621–627 (1999).
Doukyu, N. and Aono, R., *Appl. Microbiol. Biotechnol.*, **57**, 146–152 (2001).
Aunpad, R. *et al.*, *Acta Cryst.*, **D58**, 2182–2183 (2002).

EXPERIMENTAL DATA

Fig. 1 pH Optimum

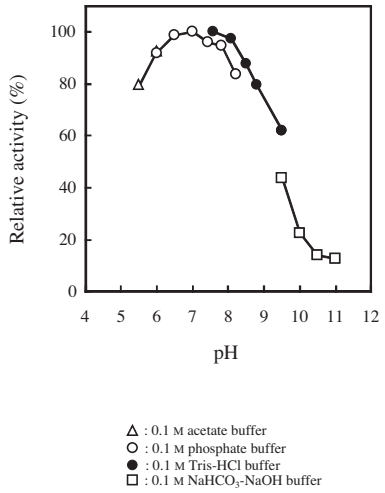

Fig. 2 pH Stability

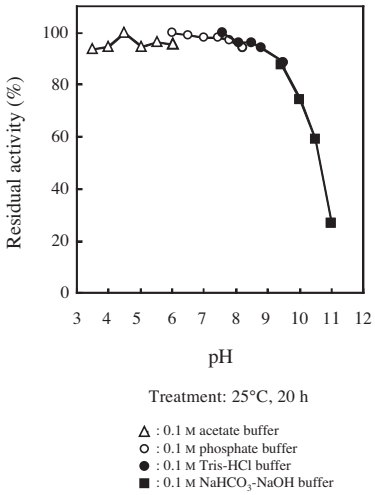

Fig. 3 Optimum temperature

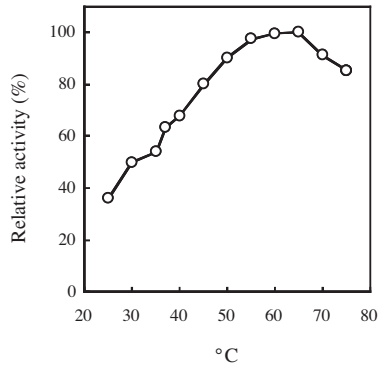

Fig. 4 Thermal stability

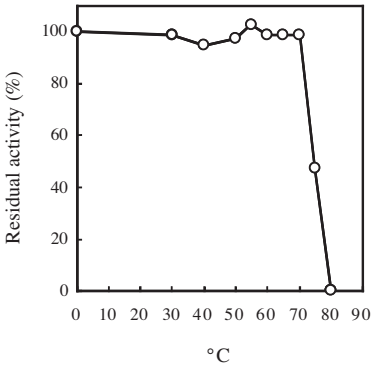

Fig. 5 Stability (liquid form)