Cholesterol Esterase (CHE-XE)

from recombinant *E. coli*

Steryl-ester acylhydrolase, EC 3.1.1.13

Cholesterol ester + H_2O ⟶ Cholesterol + Fatty acid

SPECIFICATION

Appearance	light yellow lyophilizate
Activity	≧5 U/mg lyophilizate
Stabilizer	sucrose
Storage	at −20°C

PROPERTIES

Molecular weight	*ca*. 54 kDa (gel filtration)	
Structure	monomer of 54 kDa (SDS-PAGE)	
Michaelis constant	1.9×10^{-5} M (cholesterol linoleate)	
pH Optimum	5.5–7.0	(Fig. 1)
pH Stability	5.0–10.0	(Fig. 2)
Optimum temperature	40°C	(Fig. 3)
Thermal stability	below 50°C	(Fig. 4)
Specificity	cholesterol linoleate (100), cholesterol acetate (2) cholesterol oleate (98), cholesterol palmitate (74) cholesterol stearate (68), cholesterol arachidonate (46)	

ASSAY PROCEDURE

Principle

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

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

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 cholesterol per min at 37°C and pH 6.0 under the conditions described below.

Reagents

A. MES-NaOH buffer, 0.2 M; pH 6.0: dissolve 8.53 g of MES in 180 ml of distilled water, adjust to pH 6.0 with 2 N NaOH solution and dilute with distilled water to 200 ml.
B. Cholesterol linoleate solution, 0.6 mM: dissolve 39 mg of cholesterol linoleate in 2.0 ml of isopropyl alcohol with heating, mix with 80 ml of hot 1.0% Triton X-100 solution, keep on a water bath (74°C) for about 30 min until the solution turns clear, cool down to room temperature in running water, dissolve 600 mg of sodium cholate and dilute with 1.0% Triton X-100 solution to 100 ml.
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, 150 U/ml: 75 mg of POD (200 guaiacol U/mg)/100 ml of MES-NaOH buffer (Reagent A).
F. Cholesterol oxidase (CHO-CE) solution: 200 U/ml of MES-NaOH buffer (Reagent A).
G. Enzyme dilution buffer: 50 mM potassium phosphate buffer, pH 7.5, containing 0.2% bovine serum albumin and 2 mM $MgCl_2$.
H. Working solution: mix the following reagents. (Prepare immediately before measurement)

7.5 ml	MES-NaOH buffer	(Reagent A)
5.0 ml	Cholesterol linoleate solution	(Reagent B)
0.25 ml	4-AA solution	(Reagent C)
0.50 ml	Phenol solution	(Reagent D)
0.50 ml	POD solution	(Reagent E)

Sample: dissolve the enzyme preparation to a volume activity of 0.08–0.20 U/ml with ice-cold enzyme dilution buffer (Reagent G) immediately before measurement.

Procedure

1. Pipette 2.2 ml of working solution (Reagent H) into a cuvette (light path: 1 cm).
2. Equilibrate at 37°C for about 5 min.
3. Add 0.08 ml of CHO-CE solution (Reagent F) and equilibrate at 37°C for about 2 min.
4. Add 0.08 ml of sample and mix.
5. 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 G) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 2.36\ (\text{ml}) \times df}{13.8 \times 1/2 \times 0.08(\text{ml})} = \Delta A \times 4.28 \times df$$

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

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
C : Content of cholesterol esterase preparation in sample (mg/ml)

APPLICATIONS

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

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

REFERENCES

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Flegg, H. M., *Ann. Clin. Biochem*., **10**, 79 (1973).
Alain, C. C. *et al.*, *Clin. Chem*., **20**, 470 (1973).
Tarbutton, P. N. and Gunter, C. R., *Clin. Chem*., **20**, 724 (1974).
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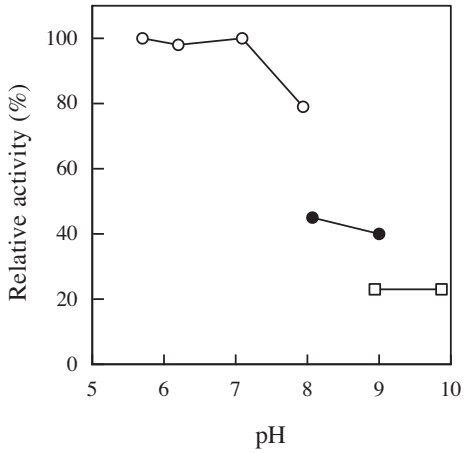
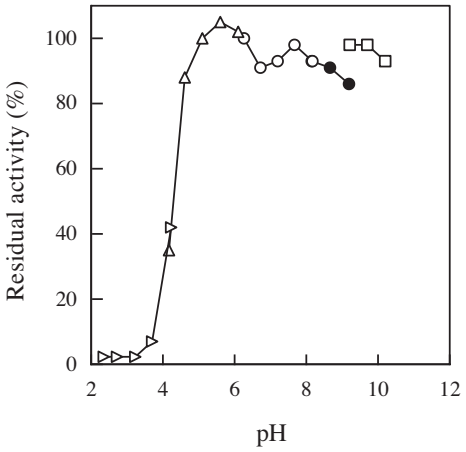
EXPERIMENTAL DATA

Fig. 1 pH Optimum



○: 0.1 M phosphate buffer
●: 0.1 M Tris-HCl buffer
□: 0.1 M glycine-NaOH buffer

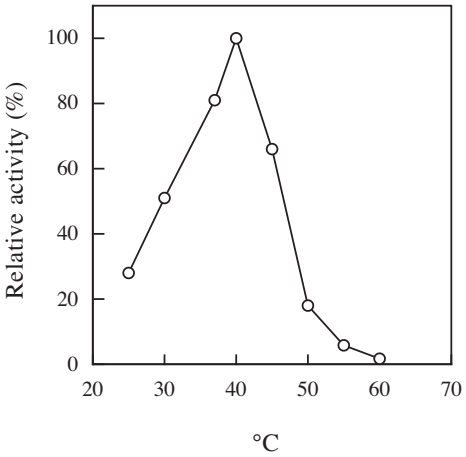
Fig. 2 pH Stability



Treatment: 25°C, 17 h

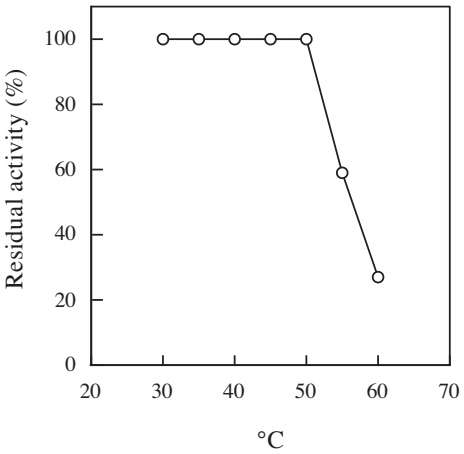
▷: 50 mM citrate buffer
△: 50 mM acetate buffer
○: 50 mM phosphate buffer
●: 50 mM Tris-HCl buffer
□: 50 mM glycine-NaOH buffer

Fig. 3 Optimum temperature



Buffer: 0.1 M phosphate buffer, pH 6.0

Fig. 4 Thermal stability



Treatment: 50 mM HEPES-NaOH buffer, pH 7.0, 15 min