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CHOLINE OXIDASE [COD Ⅲ]

from Microorganism
(Choline: oxygen 1-oxidoreductase, EC 1.1.3.17)

$$\text{Choline} + O_2 \longrightarrow \text{Betaine aldehyde} + H_2O_2$$
$$\text{Betaine aldehyde} + O_2 + H_2O \longrightarrow \text{Betaine} + H_2O_2$$

Preparation and Specification

Appearance : Yellowish amorphous powder, lyophilized
Specific activity : More than 2 U/mg solid
Contaminants :

Alkaline phosphatase	Less than 2% (U/U)
Glycerophosphorylcholine phosphodiesterase	Less than 2% (U/U)

Properties

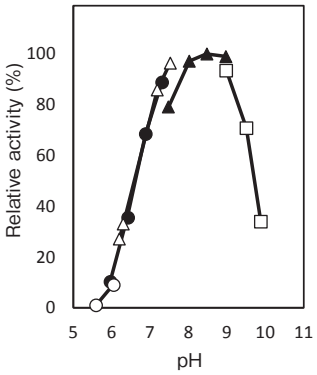
Molecular weight	: 60 kDa (SDS-PAGE)	
Isoelectric point	: 5.0 (estimated from amino acid sequence)	
Michaelis constant	: Choline 6.9 × 10^{-4}M	
Optimum pH	: 7.0–9.0	Figure 1
pH stability	: 5.5–10.0 (37℃, 60 min)	Figure 2
Thermal stability	: Stable at 55℃ and below (pH7.0, 30 min)	Figure 3
Optimum temperature	: 37℃	Figure 4

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **phospholipids** coupled with phospholipase D [PLD Ⅱ (T-222)].

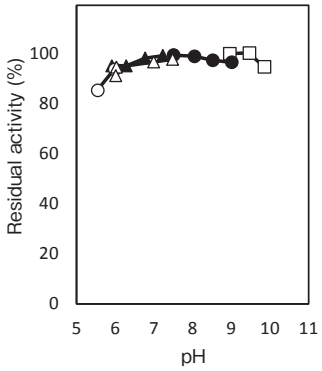
$$\text{Phosphatidylcholine} + H_2O \xrightarrow{\text{PLD}\,\text{Ⅱ}} \text{Choline} + \text{Phosphatidic acid}$$
$$\text{Choline} + 2\,O_2 + H_2O \xrightarrow{\text{COD}\,\text{Ⅲ}} \text{Betaine} + 2\,H_2O_2$$
$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

Fig. 1 Optimum pH



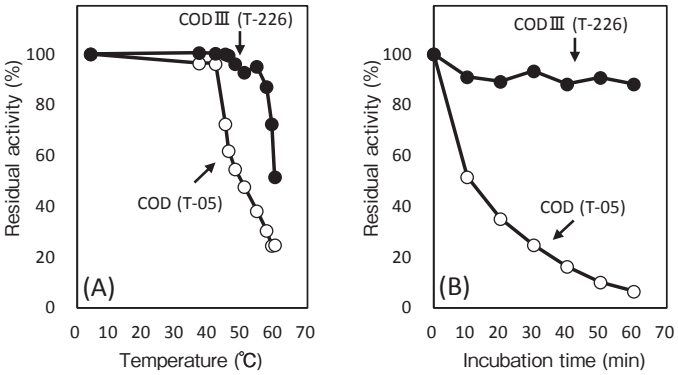
○: MES-NaOH buffer
●: PIPES-NaOH buffer
△: Phosphate buffer
▲: Tris-HCl buffer
□: CES-NaOH buffer

Fig. 2 pH stability



37℃, 60 min
○: MES-NaOH buffer
●: PIPES-NaOH buffer
△: Phosphate buffer
▲: Tris-HCl buffer
□: CES-NaOH buffer

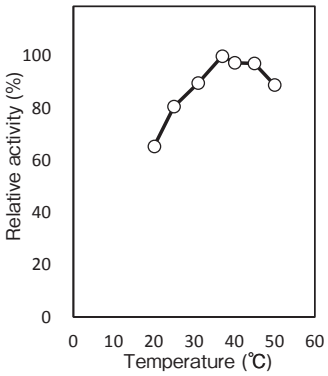
Fig. 3 Thermal stability



(A) pH 7.0, 30 min
50 mM Phosphate buffer
●: CODⅢ (T-226)
○: COD (T-05)

(B) 55℃, pH 7.0
50 mM Phosphate buffer
●: CODⅢ (T-226)
○: COD (T-05)

Fig. 4 Optimum temperature



pH 8.0
100 mM Tris-HCl buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 500 nm as the formation of quinoneimine dye proceeds in the following reactions:

$$\text{Choline} + O_2 \xrightarrow{\text{COD Ⅲ}} \text{Betaine aldehyde} + H_2O_2$$

$$\text{Betaine aldehyde} + O_2 + H_2O \xrightarrow{\text{COD Ⅲ}} \text{Betaine} + H_2O_2$$

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

■ Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of H_2O_2 per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture
   1.211 g of Tris (hydroxymethyl) amino methane, 2.1 g of choline chloride and 2 ml of 1 % (W/V) phenol are dissolved with 1 N HCl and adjusted to pH 8.0 (25℃). Then, 1 ml of 1 % (W/V) 4-AA and 3 ml of 100 PPU/ml POD are added to make a total of 100 ml.
2. Enzyme dilution buffer
   10 mM Tris-HCl buffer (pH 8.0) containing 2 mM EDTA and 1 % (W/V) KCl
   EDTA: Ethylenediaminetetraacetic acid
3. Reagents
   Choline chloride:
   FUJIFILM Wako Pure Chemical Corporation
   1st Grade #033-09812
   4-AA: NACALAI TESQUE, INC. Special grade #01907-52
   POD: Sigma Chemical Co. Type Ⅱ #P-8250
   EDTA (2 Na·2H_2O) : KISHIDA CHEMICAL Co., Ltd.
   #060-29133

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■ **Enzyme solution**

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ **Procedure**

1. Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate it at 37℃.
2. After 5 min, add 50 μl of enzyme solution and mix to start the reaction at 37℃.
3. After starting the reaction, measure the rate of increase per minutes in absorbance at 500 nm from 2 min to 7 min.

$$\triangle A/min \leqq 0.040\ Abs/min$$

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/min}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

12.0 : millimolar extinction coefficient of quinoneimine dye at 500 nm ($cm^2/\mu mole$)
1/2 : multiplier derived from the fact that 2 mole of H_2O_2 produce 1 mole of quinoneimine dye.
3.05 : final volume (ml)
0.05 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20° C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition.

References

1. Ikuta, S., Matsuura, K., Imamura, S., Misaki, H. and Horiuchi, Y. (1977) J. Biochem., **82**, 157–163.
2. Ikuta, S., Imamura, S., Misaki, H. and Horiuchi, Y. (1977) ibid, **82**, 1741–1749.
3. Ohta–Fukuyama, M., Miyake, Y., Emi, S. and Yamano, T. (1989) J. Biochem., **88**, 197–203.
4. Takayama, M. et al. (1977) Clin. Chim. Acta, **79**, 93.
5. Sugawara, K. and Kihara, A. (1978) Eisei Kensa, **27** (1), 106–111.
6. Okabe, H. et al. (1977) Clin. Chim. Acta, **80**, 87.

COD Ⅲ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液
   トリス（ヒドロキシメチル）アミノメタン 1.211g と塩化コリン 2.1g 及び 1%（W/V）フェノール液 2ml を精製水に溶解した後、1N HCl で pH8.0（25℃）に調整し、さらに 1%（W/V）4–AA 溶液 1ml と 100PPU/ml POD 溶液 3ml を加えて溶かし、全容 100ml とする。
2. 酵素溶解希釈用液
   2mM EDTA と 1%（W/V）KCl を含む 10mM トリス－ HCl 緩衝液 pH8.0 溶液
3. 試薬
   塩化コリン：富士フイルム和光純薬製　一級　#033–09812
   4-AA：ナカライテスク製　特級　#01907–52
   POD：シグマ製　Type Ⅱ　#P–8250
   EDTA（エチレンジアミン四酢酸・2Na・$2H_2O$）：キシダ化学製 #060–29133

Ⅱ．酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 3.0ml を正確に分注し 37℃で予備加温する。
2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和し、37℃で反応を開始する。
3. 反応開始後、500nm における 2 分目から 7 分目までの吸光度を測定し 1 分間当たりの吸光度変化を求める。

$$\Delta A/min \leqq 0.040\ Abs/min$$

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/min}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

12.0 ：キノンイミン色素の 500nm におけるミリモル分子吸光係数（$cm^2/\mu mole$）
1/2 ：H_2O_2 2 モルからキノンイミン色素 1 モルが生成することによる係数
3.05 ：反応総液量（ml）
0.05 ：反応に供した酵素試料液量（ml）
X ：酵素試料液の検品濃度（mg/ml）