ACYL-CoA OXIDASE [ACOD]

from *Arthrobacter* sp.
(Acyl-CoA: oxygen 2-oxidoreductase, EC 1.3.3.6)

Acyl-CoA + O_2 ⟶ *trans*-2,3-dehydroacyl-CoA + H_2O_2

Preparation and Specification

Appearance : Yellowish amorphous powder, lyophilized
Specific activity : More than 20 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 210 kDa (Sephadex G-150)	
Isoelectric point	: pH 4.7	
Michaelis constant	: Palmitoyl-CoA 2.0 × 10^{-5}M	
Optimum pH	: Serum Acyl-CoA 7.5	Figure 1
	: Palmitoyl-CoA 8.5	Figure 1
pH stability	: 6.0-7.5 (37℃, 60 min)	Figure 2
Thermal stability	: Stable at 40℃ and below (pH 7.0, 10 min)	Figure 3
Storage stability	: At least one year at −20℃	Figure 4
Effects of various chemicals	: See Table 2	
Stabilizer	: FAD	
Activator	: Triton X-100	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **fatty acid** when coupled with Acyl-CoA synthetase (T-16).

$$\text{FFA} + \text{CoA} + \text{ATP} \xrightarrow{\text{ACS}} \text{Acyl} - \text{CoA} + \text{PPi} + \text{AMP}$$

$$\text{Acyl} - \text{CoA} + O_2 \xrightarrow{\textbf{ACOD}} \text{Enoyl} - \text{CoA} + H_2O_2$$

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

FFA: Free fatty acid

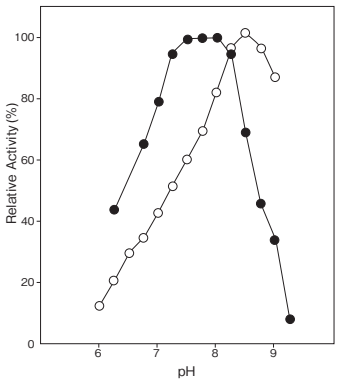
Table 1. Substrate specificity of ACOD

Substrate		Relative activity (%)	Km value (10^{-5}M)
Hexanoyl-CoA	(6:0)	11	
Octanoyl-CoA	(8:0)	60	
Decanoyl-CoA	(10:0)	73	
Dodecanoyl-CoA	(12:0)	87	
Tetradecanoyl-CoA	(14:0)	99	
Hexadecanoyl-CoA	(16:0)	53	2.0
Octadecanoyl-CoA	(18:0)	16	3.8
Icosanoyl-CoA	(20:0)	7	
9-Tetradecenoyl-CoA	(14:1)	100	
9-Hexadecenoyl-CoA	(16:1)	65	
9,12-Hexadecadienoyl-CoA	(16:2)	55	
cis-9-Octadecenoyl-CoA	(18:1)	45	4.0
trans-9-Octadecenoyl-CoA	(18:1)	31	
d-12-Hydroxy-*trans*-9-octadecenoyl-CoA	(18:1)	7	
cis-9, *cis*-12-Octadecenoyl-CoA	(18:2)	31	3.8
cis-6, *cis*-9, *cis*-12-Octadecenoyl-CoA	(18:3)	95	1.67
15-Tetracosenoyl-CoA	(24:1)	7	

Table 2. Effect of various chemicals on ACOD activity

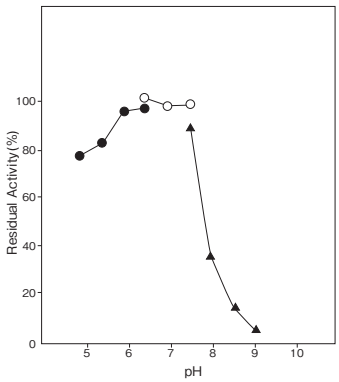
Additive	Consentration	Relative activity (%)
None	–	100
NaCl	1mM	102
KCl	1mM	104
LiCl	1mM	101
NH_4Cl	1mM	101
$MgCl_2$	1mM	138
$BaCl_2$	1mM	147
$CaCl_2$	1mM	137
$MnCl_2$	1mM	155
$ZnCl_2$	1mM	99
$CoCl_2$	1mM	121
$FeCl_3$	1mM	101
EDTA	1mM	74
Triton X-100	0.1%	140
Adekatol SO-120	0.1%	147
Sodium laurylbenzene sulfonate	0.1%	54
Sodium laurylsulfate	0.1%	43
Deoxycholate	0.1%	106

Fig.1 pH Optimum



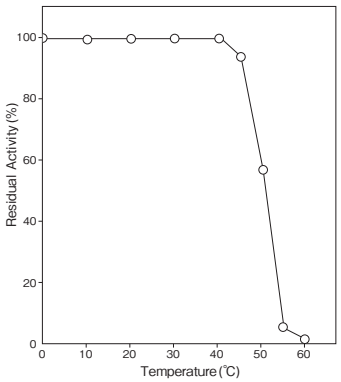
pH 6－7 Phosphate buffer
pH 7－9 Tris-HCl buffer
○ : Palmitoyl-CoA
● : Serum Acyl-CoA

Fig.2 pH Stability



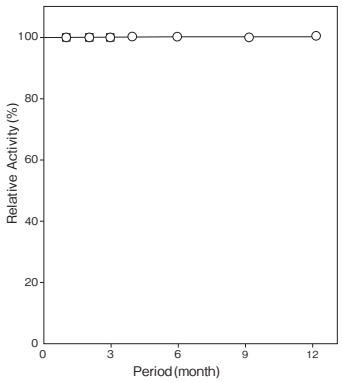
● : 3,3-Dimethylglutarate -NaOH buffer
○ : Phosphate buffer
▲ : Tris-HCl buffer

Fig.3 Thermal Stability



pH 7.0, 10 min.
Phosphate buffer

Fig.4 Storage (lyophilized powder)



○ : −20℃
□ : 5℃

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:

$$\text{Palmitoyl-CoA} + O_2 \xrightarrow{\text{ACOD}} \text{2-Hexadecenoyl-CoA} + H_2O_2$$

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

■ 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

0.2 M Tris-HCl buffer pH 8.0	0.20 ml
15 mM 4-AA solution	0.10 ml
0.2% (W/V) Phenol solution	0.10 ml
50 U/ml POD solution [1)]	0.10 ml
1% (W/V) Triton X-100 solution	0.10 ml
5 mM Palmitoyl-CoA solution [2)]	0.10 ml
Distilled water	0.30 ml

1): 50 U/ml POD solution
Dissolve 500 U (PPU) of POD with 10 ml of distilled water.

2): 5 mM Palmitoyl-CoA solution
Dissolve 50.3 mg (purity calculation) of palmitoyl-CoA with 10 ml of 10 mM KH_2PO_4-NaOH buffer pH 7.0.

2. Enzyme dilution buffer
10 mM KH_2PO_4-NaOH buffer (pH 7.0) containing 3 mM ATP and 10 μM FAD

3. Reagents
4-AA: NACALAI TESQUE, INC. Special grade #01907-52
POD: Sigma Chemical Co. Type Ⅱ # P-8250
Triton X-100: The Dow Chemical Company
Palmitoyl-CoA: Asahi Kasei Pharma Corporation
ATP (2Na・3H_2O): Kyowa Hakko Co., Ltd.
FAD (2Na): Kyowa Hakko Co., Ltd.
ATP: Adenosine triphosphate
FAD: Flavine adenine dinucleotide

■ **Enzyme solution**

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20ml. Dilute it with enzyme dilution buffer to adjust the concentration to within 0.2–0.5 U/ml.

■ **Procedure**

1. Pipette accurately 1.0 ml of reaction mixture into a reaction cuvette (1 ml volume black cuvette) and preincubate at 37℃.
2. After 5 min, add 20 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 20 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase in absorbance at 500 nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample : As/min
blank : Ab/min
△A/min = (As/min − Ab/min) ≦ 0.060 Abs/min

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\triangle\ \text{A/min}}{12.0 \times 1/2} \times \frac{1.02}{0.02} \times \frac{1}{X}$$

12.0 : millimolar extinction coefficient of quinoneimine dye at 500 nm (cm^2/ μmole)
1/2 : a multiplier derived from the fact that 2 mol of H_2O_2 produce 1 mol of quinoneimine dye
1.02 : final volume (ml)
0.02 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4).

References

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3. Hosaka, K., Kikuchi, T, and Mitsuhida, N. (1979) Proceedings of the Symposium on Chemical Physiology, **19**, 180.
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5. Hosaka, K., Kikuchi, T., Mitsuhida, N. and Kawaguchi, A. (1981) J. Biochem., **89**, 1799–1803.
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ACOD 活性測定法 (Japanese)

Ⅰ. 試薬液

1. 反応試薬混合液

0.2M トリス –HCl 緩衝液 pH8.0	0.20 ml
15mM 4–AA 溶液	0.10 ml
0.2% (W/V) フェノール溶液	0.10 ml
50U/ml POD 溶液 [1)]	0.10 ml
1% (W/V) トリトン X–100 溶液	0.10 ml
5mM パルミトイル –CoA 溶液 [2)]	0.10 ml
精製水	0.30 ml

1): 50U/ml POD 溶液
POD500 単位(PPU)を精製水 10ml で溶解する。
2): 5mM パルミトイル –CoA 溶液
パルミトイル –CoA 50.3mg (純度換算) を 10mM KH_2PO_4–NaOH 緩衝液 pH7.0 10ml で溶解する。

2. 酵素溶解希釈用液
3mM ATP と 10 μM FAD を含む 10mM KH_2PO_4–K_2HPO_4 緩衝液 pH7.0

3. 試薬
4–AA：ナカライテスク製 特級 #01907–52
POD：シグマ製 Type Ⅱ #P–8250
トリトン X–100：Dow Chemical 製
パルミトイル –CoA：旭化成ファーマ製
ATP (アデノシン三リン酸・2Na・$3H_2O$)：協和発酵製
FAD (フラビンアデニンジヌクレオチド・2Na)：協和発酵製

Ⅱ. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。
その液を酵素溶解希釈用液で 0.2〜0.5U/ml 濃度となるように適宜希釈する。

Ⅲ. 測定操作法

1. 反応セル (1ml 用ブラックセル) に反応試薬混合液 1.0ml を正確に分注して 37℃で予備加温する。
2. 5 分経過後、酵素試料液 20 μl を加えて混和し、37℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 20 μl を加える。
3. 反応開始後、500nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。
   求められた吸光度変化を試料液は As/min、盲検液は Ab/min とする。
   Δ A/min = (As/min − Ab/min) ≦ 0.060 Abs/min

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta\ \text{A/min}}{12.0 \times 1/2} \times \frac{1.02}{0.02} \times \frac{1}{X}$$

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/ μmole)
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
1.02 : 反応総液量 (ml)
0.02 : 反応に供した酵素試料液量 (ml)
X : 酵素試料液中の検品濃度 (mg/ml)