ACYL-CoA SYNTHETASE [ACS]

from *Pseudomonas fragi*
(Acid: CoA ligase (AMP forming), EC 6.2.1.3)

$$RCOOH + ATP + CoASH \longrightarrow RCO\text{-}SCoA + PPi + AMP$$

Preparation and Specification

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

Properties

Substrate specificity	: See Table 1		
Molecular weight	: 60 kDa (Sephadex G-150) SDS-PAGE 62 kDa		
Isoelectric point	: pH 5.2		
Michaelis constants	: Palmitic acid	1.1×10^{-5}M	
	ATP	1.7×10^{-4}M	
	CoA	3.2×10^{-4}M	
Optimum pH	: Palmitic acid	7.7	Figure 1
	: Serum fatty acids	7.7	Figure 2
pH stability	: 6.0–8.0 (37℃, 2 hr)		Figure 3
Thermal stability	: Stable at 50℃ and below		
	: (pH 7.5, 10 min)		Figure 4
Storage stability	: At least one year at −20℃		Figure 5
Effect of various chemicals	: See Table 2 and Table 3		
Stabilizer	: ATP		
Activator	: Triton X-100		

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **fatty acid** when coupled with Acyl-CoA oxidase (T-17).

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

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

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

FFA: Free fatty acid

Table 1. Substrate specificity (Fatty acids)

Substrate		Relative activity (%)	Km value (10^{-5}M)
Caproic acid	(6:0)	38	
Caprylic acid	(8:0)	64	3.7
Capric acid	(10:0)	24	0.5
Lauric acid	(12:0)	21	0.95
Myristic acid	(14:0)	40	0.71
Palmitic acid	(16:0)	66	1.10
Stearic acid	(18:0)	78	3.0
Arachidic acid	(20:0)	100	
Myristoleic acid	(14:1)	35	
Palmitoleic acid	(16:1)	40	
Palmitelaidic acid	(16:1)	48	
Oleic acid	(18:1)	78	0.91
Elaidic acid	(18:1)	60	
Linoleic acid	(18:2)	57	0.34
Linolenic acid	(18:3)	62	1.10
Arachidonic acid	(20:4)	63	
Erucic acid	(22:1)	92	
Nervonic acid	(24:1)	9	

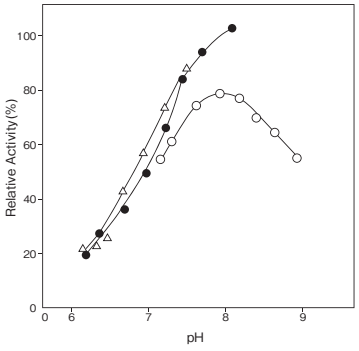
Table 2. Effect of detergents on ACS activity

Detergent (%)		Relative activity (%)
None		100
Deoxycholate	0.1	69.6
	0.25	41.1
SDS	0.1	0
Cetyltrimethyl-ammoniumchloride	0.1	96.4
	0.25	0
Cetylpyridinium chloride	0.1	96.4
	0.25	0
Sarcosinate PN	0.1	64.3
	0.25	0

Table 3. Effect of metal ions on ACS activity

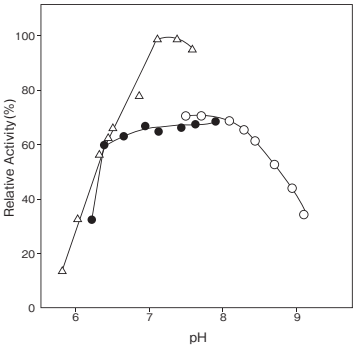
Metal ion	Concentration	Relative activity (%)
None	–	38
KCl	0.1M	29
NaCl	0.1	35
LiCl	0.1	26
NH_4Cl	0.1	29
$MgCl_2$	1mM	100
$CaCl_2$	1	94
$ZnCl_2$	1	31
$BaCl_2$	1	38
$MnCl_2$	1	117
$CuCl_2$	1	0
$NiCl_2$	1	87
EDTA	1	0
1mM $MgCl_2$ + $CaCl_2$	1	103
1mM $MgCl_2$ + $ZnCl_2$	1	55
1mM $MgCl_2$ + $CuCl_2$	1	0
1mM $MgCl_2$ + $NiCl_2$	1	99
1mM $MgCl_2$ + $BaCl_2$	1	100
1mM $MgCl_2$ + $MnCl_2$	1	117

Fig.1 pH Optimum (Palmitic acid)



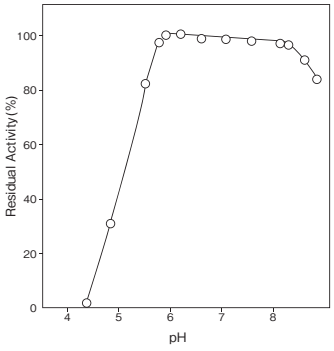
△ : 3,3-Dimethylglutarate-NaOH buffer
● : Phosphate buffer
○ : Tris-HCl buffer

Fig.2 pH Optimum (Serum NEFA)



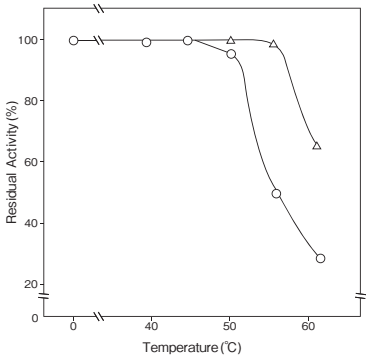
△ : 3,3-Dimethylglutarate-NaOH buffer
● : Phosphate buffer
○ : Tris-HCl buffer

Fig.3 pH Stability



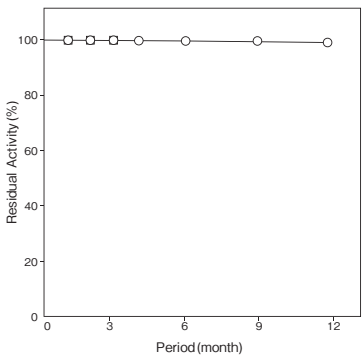
37℃, 2 hr
pH 4.0—6.5 3,3-Dimethylglutarate-NaOH buffer
pH 6.5—7.5 Phosphate buffer
pH 7.5—9.0 Tris-HCl buffer

Fig.4 Thermal Stability



pH 7.5, 10 min.
Phosphate buffer
△ : +2 mM ATP
○ : Non addition

Fig.5 Storage (lyophilized powder)



○ : −20℃
□ : 5℃

Assay

■ Principle

The assay is based on the increase in absorbance at 550 nm as the formation of quinonediimine dye proceeds in the following reactions:

$$\text{Palmitic Acid + CoA + ATP} \xrightarrow{\text{ACS}} \text{Palmitoyl CoA + AMP + PPi}$$

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

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

ACOD: Acyl-CoA oxidase
MEHA: 3-Methyl-N-ethyl-N- (2-hydroxymethyl) aniline

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μmole of fatty acid to acyl-CoA per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture for the first reaction

0.2 M KH_2PO_4-K_2HPO_4 buffer pH 7.5	0.20 ml
10 mM ATP solution pH 7.5	0.10 ml
10 mM $MgCl_2$ solution	0.10 ml
1 mM Palmitic acid-5 % (W/V) Triton X-100 solution pH 7.5 [1]	0.20 ml
Distilled water	0.35 ml
10 mM CoA solution pH 6.5 [2]	0.05 ml

1): 1 mM Palmitic acid-5 % (W/V) Triton X-100 solution pH 7.5
Dissolve 26 mg of palmitic acid with 90 ml of 5% (W/V) Triton X-100, adjust pH to 7.5 at 25℃ with 4 N NaOH, add 5% (W/V) Triton X-100 to make a total of 100 ml.

2): 10mM CoA solution pH 6.5
Dissolve 154 mg (purity calculation) of CoA with 15 ml of distilled water, adjust pH to 6.5 at 25℃ with 4 N NaOH, and add distilled water to make a total of 20 ml.

2. Reaction mixture for the second reaction

0.2 M KH_2PO_4-K_2HPO_4 buffer pH 7.5	0.50 ml
20mM NEM	0.10 ml
15mM 4-AA solution	0.30 ml
0.3% (W/V) MEHA solution pH 5.8	0.25 ml
100 u/ml POD solution [3]	0.10 ml
0.5% (W/V) NaN_3 solution	0.10 ml
Distilled water	0.55 ml
120 U/ml ACOD solution [4]	0.10 ml

NEM: N-Ethylmaleimide

3): 100 U/ml POD solution
Dissolve 1,000 U (PPU) of POD with 10 ml of distilled water.

4): 120 U/ml ACOD solution
Dissolve 1,200 U of ACOD with 10 ml of ACOD dilution buffer [※].

※): ACOD dilution buffer
Dissolve 1.36 g of KH_2PO_4 and 1.82 g of ATP with distilled water, adjust pH to 7.0 with 4 N NaOH, add 10 ml of 1 mM FAD, and finally add distilled water to make a total of 1 L.

3. Enzyme dilution buffer
10 mM phosphate buffer (pH 7.5) containing 2 mM ATP, 0.5% (W/V) BSA and 0.1 % (W/V) Triton X-100.

4. Reagents:
ATP (2Na・$3H_2O$): Kyowa Hakko Co., Ltd.
Palmitic acid : Wako Pure Chemical Industries, Ltd. #169-00105
Triton X-100: The Dow Chemical company
NEM: Wako Pure Chemical Industries, Ltd. #058-02061
4-AA: NACALAI TESQUE, INC. Special grade #01907-52
MEHA: Tokyo Kasai Kogyo Co., Ltd. #E0220
POD: Sigma Chemical Co. Type Ⅱ #P-8250
ACOD: Asahi Kasei Pharma Corporation #T-17
FAD (2Na): Kyowa Hakko Co., Ltd.
BSA: Millipore Fraction V pH 5.2 #81-053

ATP: Adenosine triphosphate
FAD: Flavine adenine dinucleotide

■ **Enzyme solution**

Weigh about 20 mg of test sample exactly and add enzyme dilution buffer to make a total of 20 ml. Dissolve it with enzyme dilution buffer to adjust the concentration to within 0.04–0.06 U/ml.

■ **Procedure**

1. Pipette accurately 1.0 ml of reagent mixture for the first reaction into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 50 μl of enzyme solution and mix to start the first reaction at 37℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
3. After 10 min, add 2.0 ml of reagent mixture for the second reaction to stop the first reaction and mix to start the second reaction at 37℃.
4. After 5 min, measure the absorbance at 550 nm.

Absorbance sample : As
blank : Ab
△A = (As − Ab) ≦ 0.30 Abs

■ **Calculation**

$$\text{Activity (U/mg)} = \frac{\triangle A/10}{32.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

32.0 : millimolar extinction coefficient of quinonediimine dye at 550 nm (cm^2/ μmole)
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinonediimine dye
10 : reaction time (min)
3.05 : final volume (ml)
0.05 : volume of enzyme solution (ml)
X : concentration of ACS 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.

References

1. Yamada, H., Shimizu, S. and Tani, Y. (1980) Vitamin (Japanese), **54**, 489.
2. Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1980) Anal. Biochem., **107**, 193.
3. Okabe, H., Uji, Y., Nagashima, K. and Noma, A. (1980) Clin. Chem., **26**, 1540.
4. Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1979) Anal. Biochem., **98**, 341.

ACS 活性測定法 (Japanese)

Ⅰ. 試薬液

1. 第一反応試薬混合液

0.2M KH_2PO_4–K_2HPO_4 緩衝液 pH7.5	0.20 ml
10mM ATP 溶液 pH7.5	0.10 ml
10mM 塩化マグネシウム溶液	0.10 ml
1mM パルミチン酸 –5% (W/V) トリトン X–100 溶液 pH7.5 [1]	0.20 ml
精製水	0.35 ml
10mM CoA 溶液 pH6.5 [2]	0.05 ml

1): 1mM パルミチン酸 –5% (W/V) トリトン X–100 溶液 pH7.5
パルミチン酸 26mg を 5% (W/V) トリトン X-100 溶液 90ml で加温溶解した後、4N NaOH で pH7.5 (25℃) に調整し、5% (W/V) トリトン X–100 溶液で全容 100ml とする。

2): 10mM CoA 溶液 pH6.5
CoA 154mg (純度換算) を精製水 15ml に溶解した後、4N NaOH で pH6.5 (25℃) に調整し、精製水で全容 20ml とする。

2. 第二反応試薬混合液

0.2M KH_2PO_4–K_2HPO_4 緩衝液 pH7.5	0.50 ml
20mM NEM 溶液	0.10 ml
15mM 4–AA 溶液	0.30 ml
0.3% (W/V) MEHA 溶液 pH5.8	0.25 ml
100U/ml POD 溶液 [3]	0.10 ml
0.5% (W/V) NaN_3 溶液	0.10 ml
精製水	0.55 ml
120U/ml ACOD 溶液 [4]	0.10 ml

3): 100U/ml POD 溶液
POD1,000 単位 (PPU) を精製水 10ml で溶解する。

4): 120U/ml ACOD 溶液
ACOD1,200 単位 (U) を ACOD 希釈緩衝液※) 10ml で溶解する。
※): ACOD 希釈緩衝液
KH_2PO_4 1.36g と ATP 1.82g を精製水に溶解した後、4N NaOH で pH7.0 (25℃) に調製し、さらに 1mM FAD 溶液 10ml を加えて精製水で全容 1L とする。

3. 酵素溶解希釈用液
2mM ATP と 0.5% (W/V) BSA 及び 0.1% (W/V) トリトン X–100 を含む 10mM リン酸緩衝液 pH7.5

4. 試薬
ATP (アデノシン三リン酸・2Na・$3H_2O$) : 協和発酵製
CoA (コエンザイム A) : 興人製
パルミチン酸 : 和光純薬工業製　特級 #169–00105
トリトン (トリトン X–100) : Dow Chemical 社製
NEM (N– エチルマレイミド) : 和光純薬工業製 #058–02061
4–AA : ナカライテスク社製　特級　#01907–52

MEHA［N-エチル-N-2-ヒドロキシエチル -m- トルイジン］:
東京化成製　#E0220
POD:シグマ社製　Type Ⅱ　#P-8250
ACOD（アシル-CoA 酸化酵素）:旭化成ファーマ製
#T-17
FAD（フラビンアデニンジヌクレオチド･2Na）:
協和発酵製
BSA: Millipore 社製　Fraction V pH5.2 #81-053

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 0.04～0.06U/ml 濃度となるように適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に第一反応試薬混合液 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 50 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 50 μl を加える。
3. 10 分経過後、第二反応試薬混合液 2.0ml を正確に加えて混和し、第一反応を停止させ、37℃で第二反応を開始する。
4. 5 分経過後、550nm における吸光度を測定する。求められた吸光度を試料液は As、盲検液は Ab とする。

$$\Delta A = (As - Ab) \leqq 0.300\ Abs$$

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/10}{32.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

32.0：キノンジイミン色素の 550nm におけるミリモル分子吸光数（$cm^2/\ \mu mole$）
1/2：H_2O_2 2 モルからキノンジイミン色素 1 モルが生成することによる係数
10　：反応時間（min）
3.05：反応総液量（ml）
0.05：反応に供した酵素試料液量（ml）
X　：酵素試料液中の検品濃度（mg/ml）