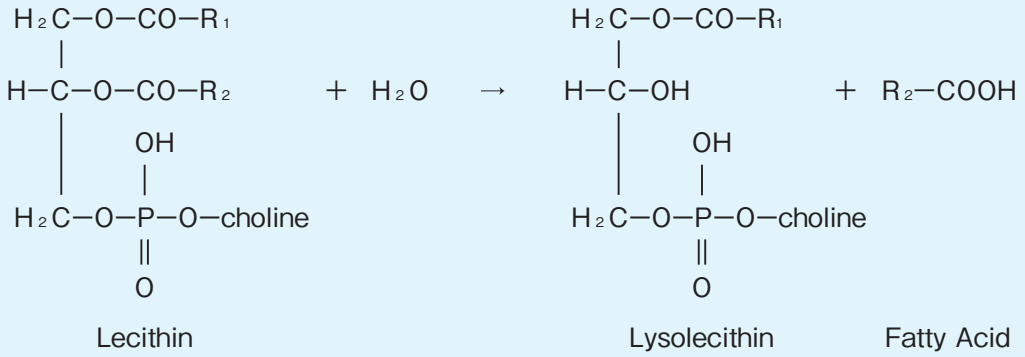
PHOSPHOLIPASE A_2 [PLA_2ⅢL]

from *Streptomyces violaceoruber*
(Phosphatidylcholine 2-acylhydrolase, EC 3.1.1.4)



Preparation and Specification

Appearance : Colorless to brownish solution
Specific activity : More than 200 U/ml

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 15 kDa (Sephadex G-100)	
Isoelectric point	: pH 7.51	
Michaelis constants	: Lecithin 5.0×10^{-3}M	
Optimum pH	: 7.3-8.3	Figure 1
pH stability	: 6.0-9.5 (55℃, 10 min)	Figure 2
Thermal stability	: Stable at 50℃ and below (pH8.0, 10 min, + $CaCl_2$)	Figure 3
Stabilizer	: Ca^{2+}	
Activators	: Ca^{2+}, Non-ionic detergents	
Inhibitor	: EDTA	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **lecithin** when coupled with lysophospholipase (T-32), glycerophosphorylcholine phosphodiesterase (T-33) and choline oxidase (T-05).

$$\text{Lecithin} + H_2O \xrightarrow{PLA_2\text{ⅢL}} \text{Lysolecithin} + \text{Fatty acid}$$

$$\text{Lysolecithin} + H_2O \xrightarrow{LYPL} \text{Glycerophosphorylcholine} + \text{Fatty acid}$$

$$\text{Glycerophosphorylcholine} + H_2O \xrightarrow{GPCP} \text{Glycerophosphate} + \text{Choline}$$

$$\text{Choline} + 2\,O_2 + H_2O \xrightarrow{COD} \text{Betaine} + 2\,H_2O_2$$

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

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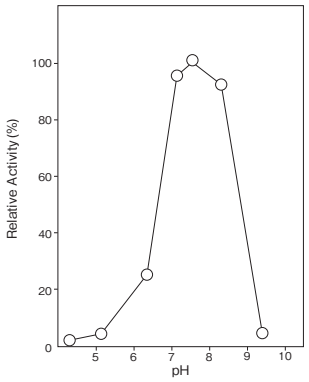
Table 1. Substrate specificity

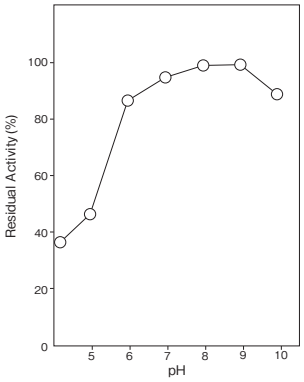
Substrate	Relative activity (%)
Phosphatidylcholine	100
Phosphatidylethanolamine	30
Phosphatidic Acid	10
Lysophosphatidylcholine	0
Triolein	0
Trilaurin	0

Fig.1 pH Optimum



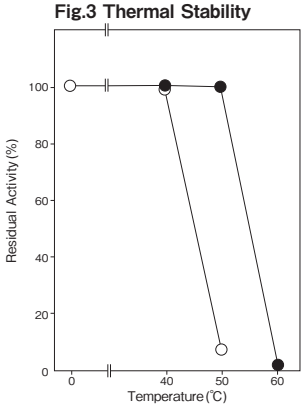
20 mM Britton-Robinson buffer

Fig.2 pH Stability



55℃, 10 min.
20 mM Britton-Robinson buffer containing 50 mM $CaCl_2$

Fig.3 Thermal Stability



20 mM Britton-Robinson buffer pH 8.0 for 10 min.
○ : None
● : +50 mM $CaCl_2$

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:

$$\text{Lecithin} + H_2O \xrightarrow{PLA_2\text{II L}} \text{Lysolecithin} + \text{Fatty acid}$$

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

$$\text{Acyl-CoA} + O_2 \xrightarrow{\text{ACOD}} \text{2, 3-Enoyl acyl-CoA} + H_2O_2$$

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

ACS: Acyl-CoA synthetase
ACOD: Acyl-CoA oxidase
ATP: Adenosine triphosphate

■ Unit definition

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

■ Reagents

1. Reaction mixture for the first reaction

0.2 M Tris-HCl buffer pH 8.0	0.20 ml
20 mM DPPC solution [1)]	0.05 ml
1 M $CaCl_2$ solution	0.025 ml
0.2 M ATP solution pH 7.0	0.01 ml
0.1 M CoA solution pH 6.0 [2)]	0.02 ml
10.6 U/ml ACS solution [3)]	0.05 ml
Distilled water	0.145 ml

1): 20 mM DPPC solution
Dissolve 147 mg of DPPC with 10 ml of 2% (W/V) Triton X-100 solution.

2): 0.1 M CoA solution pH 6.0
Dissolve 7.85 g (purity calculation) of CoA with 80 ml of distilled water and adjust pH to 6.0 (25℃) with 4 N NaOH and add distilled water to make a total of 100 ml.

3): 10.6 U/ml ACS solution
Dissolve 106 U of ACS with 10 ml of Tris-HCl buffer pH 8.0.

DDPC: Dipalmytoylphosphatidylcholine

2. Reaction mixture for the second reaction

0.5 M PIPES-NaOH buffer pH 7.5	0.04 ml
0.3% (W/V) 4-AA solution	0.10 ml
0.2% Phenol solution	0.10 ml
50 U/ml POD solution [4)]	0.045 ml
300 U/ml ACOD solution [5)]	0.05 ml
10% (W/V) Triton X-100 solution	0.01 ml
0.2 M ATP solution pH 7.0	0.05 ml
10 mM FAD solution	0.005 ml
Distilled water	0.10 ml

4): 50 U/ml POD solution
Dissolve 500 U (PPU) of POD with 10 ml of distilled water.

5): 300 U/ml ACOD solution
Dissolve 3,000 U of ACOD with 10 ml of PIPES-NaOH buffer pH 7.5.

FAD: Flavine adenine dinucleotide

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3. 20 mM NEM
   NEM: N-ethylmaleimide
4. Reaction stopper
   0.1 M EDTA solution containing 0.5% (W/V) SDS pH8.0
   SDS: Sodium dodecyl sulfate
   EDTA: Ethylenediamine tetraacetic acid
5. Enzyme dilution buffer
   10 mM Tris-HCl buffer pH 8.0 containing 0.05% (W/V) BSA.
6. Reagents:
   DPPC: Nippon Fine Chemical Co., Ltd.
   Coenzyme A (CoA・Li_3) : Kohjin Co.
   ATP (2Na・$3H_2O$) : Kyowa Hakko Co., Ltd.
   PIPES [Piparazine-1,4-bis (2-ethanesulphonic acid)] : Dojindo Laboratories #345-02225
   Triton X-100: The Dow Chemical Company
   NEM: FUJIFILM Wako Pure Chemical Corporation #058-02061
   ACS: Asahi Kasei Pharma Corporation #T-16
   ACOD: Asahi Kasei Pharma Corporation #T-17
   SDS: NACALAI TESQUE, INC. #316-06
   4-AA: NACALAI TESQUE, INC. Special grade #01907-52
   POD: Sigma Chemical Co. Type Ⅱ #P-8250
   EDTA (2Na・$2H_2O$): KISHIDA CHEMICAL Co., Ltd. #060-29133

■ **Enzyme solution**

Dilute accurately 0.5 ml of the sample with enzyme dilution buffer to make a 50-fold Solution. Dilute it with enzyme dilution buffer to adjust the concentration as required

■ **Procedure**

1. Pipette accurately 0.50 ml of reaction mixture for the first reaction into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 50 μl of enzyme sample 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 at this point.
3. After 10 min, add 0.5 ml of 20 mM NEM and add 0.5 ml of reaction mixture for the second reaction after 15 seconds later and mix to start the second reaction at 37℃.
4. At 5 min after starting the reaction, add 1.5 ml of the reaction stopper to stop the reaction.
5. Measure the absorbance at 500 nm.

   Absorbance sample : As
   blank : Ab
   △A = (As − Ab) ≦ 0.20 Abs

■ **Calculation**

$$\text{Activity (U/ml)} = \frac{\triangle A/10}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times D$$

12.0 : millimolar extinction coefficient of quinoneimine dye at 500 nm ($cm^2/\mu mol$)
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoeimine dye
10 : reaction time (min.)
3.05 : final volume (ml)
0.05 : volume of enzyme solution (ml)
D : times of dilution in enzyme solution

Storage

Storage at 2～8℃ in the presence of a desiccant is recommended.

References

1. Scandella, C. J. and Kornberg, A. C. (1971) Biochemistry, **10**, 4447-4456.
2. Nishijima, M., Akamatsu, Y. and Nojima, S. (1974) J. Biol. Chem., **249**, 5658-5667.
3. Waite, M. and Sisson, P. (1971) Biochemistry, **10**, 2377-2383.
4. Kannagi, R. and Koizumi, K. (1979) Biochim. Biophys. Acta, **556**, 423-433.
5. Van Dam-Mieras, M. C. E., Slotboom, A. J., Pieterson, W. A. and de Haas, G. H. (1975) Biochemistry, **14**, 5387-5393.

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PLA_2ⅡL活性測定法 (Japanese)

Ⅰ. 試薬液

1. 第一反応試薬混合液

0.2M トリス-HCl 緩衝液 pH8.0	0.20 ml
20mM DPPC 溶液 [1]	0.05 ml
1M 塩化カルシウム溶液	0.025 ml
0.2M ATP 溶液 pH7.0	0.01 ml
0.1M CoA 溶液 pH6.0 [2]	0.02 ml
10.6U/ml ACS 溶液 [3]	0.05 ml
精製水	0.145 ml

1) : 20mM DPPC 溶液
DPPC 147mg を 2% (W/V) トリトン X-100 溶液 10ml で溶解する。

2) : 0.1M CoA 溶液 pH6.0
CoA 7.85g (純度換算) を精製水 80ml に溶解した後、4N NaOH で pH6.0 (25℃) に調整し、精製水で全容 100ml とする。

3) : 10.6U/ml ACS 溶液
ACS 106 単位 (U) を 10mM トリス－HCl 緩衝液 pH8.0 10ml で溶解する。

2. 第二反応試薬混合液

0.5M PIPES-NaOH 緩衝液 pH7.5	0.04 ml
0.3% (W/V)4-AA 溶液	0.10 ml
0.2% (W/V) フェノール液	0.10 ml
50U/ml POD 溶液 [4]	0.045 ml
300U/ml ACOD 溶液 [5]	0.05 ml
10% (W/V) トリトン X-100 溶液	0.01 ml

0.2M ATP 溶液 pH7.0　0.05 ml
10mM FAD 溶液　0.005 ml
精製水　0.10 ml

4)：50U/ml POD 溶液
POD 500 単位（PPU）を精製水 10ml で溶解する。

5)：300U/ml ACOD 溶液
ACOD 3,000 単位（U）を 10mM PIPES-NaOH 緩衝液 pH7.5 10ml で溶解する。

3. 20mM NEM 溶液
4. 反応停止液
0.5%（W/V）SDS を含む 0.1M EDTA 溶液 pH8.0
5. 酵素溶解希釈用液
0.05%（W/V）BSA を含む 10mM トリス－HCl 緩衝液 pH8.0
6. 試薬
DPPC（ジパルミトイルフォスファチジルコリン）：日本精化製
CoA（コエンザイム・Li_3）：興人製
ATP（アデノシン三リン酸・2Na・$3H_2O$）：協和発酵製
PIPES[ピペラジン -1,4- ビス(2- エタンスルホン酸)]：同仁化学製　#345-02225
トリトン X-100：Dow chemical 製
FAD（フラビンアデニンジヌクレオチド・2Na）：協和発酵製
NEM（N- エチルマレイミド）：富士フイルム和光純薬製　特級　#058-02061
ACS：旭化成ファーマ製　#T-16
ACOD：旭化成ファーマ製　#T-17
SDS（ドデシル硫酸ナトリウム）：ナカライテスク製　#316-06
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 0.5ml を酵素溶解希釈用液で 50 倍に希釈する。
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. 小試験管に第一反応試薬混合液 0.50ml 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℃で第一反応を開始する。
※盲検は酵素試料液の代わりに酵素溶解希釈緩衝液 50 μl を加える。
3. 10 分経過後、20mM NEM 溶液 0.50ml を加えて混和し、15 秒後に第二反応試薬混合液 0.50ml を加えて混和し、37℃で第二反応を開始する。
4. 5 分経過後、反応停止液 1.50ml を加えて混和し、反応を停止する。
5. 500nm 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。

$$\Delta A = (As - Ab) \leqq 0.20\ Abs.$$

Ⅳ．計算

$$活性\ (U/ml) = \frac{\Delta A/10}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times D$$

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（$cm^2/\ \mu mol$）
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
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）
D　：酵素試料液の調製希釈倍数