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Phospholipase A_2-like catalytic antibody

Shigeki Isomura, Kazuo Ito, and Mitsumasa Haruna*

Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku, Nagoya 468, Japan

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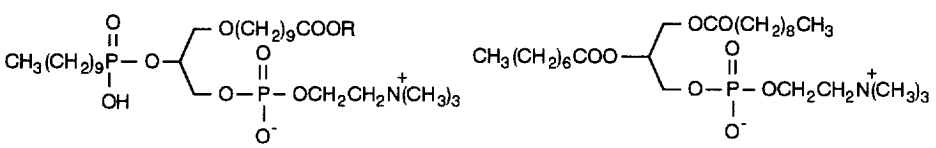
Abstract: The phospholipase A_2-like catalytic antibody 13C2-1F6 was elicited against the hapten **1** as the transition state analog for the hydrolysis of the C_2 ester in the phospholipid. The Michaelis-Menten kinetics for the hydrolysis of the phospholipid **2** by 13C2-1F6 afforded a k_{cat} of 1.0×10^{-2} min^{-1} and a K_m of 71 μM. This antibody hydrolyzes the C_2 ester in (*R*)-**2**, regio- and enantioselectively.

Since catalytic antibodies were developed by Lerner[1] and Schultz[2] in 1986, a large number of reactions catalyzed by antibodies have been reported in the past decade.[3] The development of antibodies with the function of disfavored chemical transformation[4] and C-C bond formation such as the Diels-Alder reaction[5] and aldol-condensation[6] are particularly interesting. Recently, antibodies with a prodrug activation[7], cocaine[8] and paraoxon[9] inactivation, and an RNase activity[10] were derived from haptens with phosphonate as the transition state analogs and bait and switch strategy. Furthermore, although many hydrolytic antibodies have been reported,[11] the production of the antibody for the hydrolysis of a phospholipid is still unknown.

Phospholipase A_2 [PLA_2, EC.3.1.1.4] catalyzes the hydrolysis of the *sn*-2 ester of phospholipids in a membrane to release lysophospholipids and functional fatty acids such as arachidonic acid that are metabolized to prostaglandins and leukotrienes.[12] Lysophospholipids, which form spherical micelles, have the remarkable property of being an amphoteric surfactant and are known to cause instability in the cell membrane such as fusion and disruption depending on their concentrations.[13] Thus, an antibody with PLA_2-like activity is expected to be a potentially useful functional molecule. We report herein the production of a PLA_2-like



1 : R = H
3 : R = CH_3

2

Figure 1.

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catalytic antibody and its reactivity.

Hapten **1**,[14] the racemic transition state analog chosen by the considering the biomembrane stability,[15] was directly conjugated to the carrier protein keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) with EDC [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide][16], as shown in Figure 1. Lymphocytes from the spleen of BALB/c mice immunized with the KLH-hapten **1** conjugates were fused using mouse myeloma cells (sp-2/0-Ag 14), and hybridomas were prepared and subcloned by standard protocols.[17] Forty-one monoclonal antibodies were selected by enzyme-linked immunosorbent assay (ELISA) to the BSA-hapten **1** conjugates. These clones were individually injected into mice for the production of ascites fluid. This fluid was purified by salt precipitation followed by anti-mouse antibody[18] and Protein G[19] affinity chromatography. Antibodies were judged to be homogeneous by SDS polyacrylamide gel electrophoresis.[20]

The initial screening for catalytic activity of an antibody (5 μM) against (±)-1-decanoyl-2-octanoyl-phosphatidylcholine **2**[21] (120 μM) was performed by fast atom bombardment mass spectrometry (FABMS)[22] at 25 °C in 50 mM Tris-HCl buffer, pH 7.6. Nineteen out of the forty-one monoclonal antibodies were found to accelerate the hydrolysis of (±)-**2** to the free fatty acid and the lysophospholipid at a rate significantly above the uncatalyzed reaction. The hydrolysis of (±)-**2** by BSA (5 μM) was not observed under the same conditions. Antibody 13C2-1F6 showed the highest rate enhancement and was characterized in more detail.

The initial rate for the antibody-catalyzed hydrolysis was analyzed by FABMS under the same assay condition. Quantitative analysis of the lysophospholipid was determined by the relative peak intensities of *m/z* 412 (lyso-*H*) and *m/z* 413 that is the molecular ion peak of the labeled lysophospholipid (lyso-d_1) used as the internal standard.[23] Michaelis-Menten kinetics for the hydrolysis of (±)-**2** by 13C2-1F6 afforded a k_{cat} of 1.0×10^{-2} min^{-1} and a K_m of 71 μM. Furthermore, the uncatalyzed reaction of (±)-**2** could not be observed under the same conditions for 10 hrs.[24] This hydrolytic reaction was competitively inhibited (K_i=10.0 μM) by the hapten methyl ester **3**, namely hydrolyzing at the active site on the antibody, as shown in Figure 2.

The enantioselectivity of 13C2-1F6 was identified from its hydrolytic activity to (+)-**2**[25], the (*R*)-configuration, because the amount of liberated lysophospholipid from (+)-**2** by 13C2-1F6 during the hydrolytic reaction was approximately two-fold more than that from (±)-**2**. Thus, antibody 13C2-1F6 recognized the (*R*)-configuration at the glycerol C_2 position of the phospholipid.

The regioselectivity of antibody 13C2-1F6 during the hydrolytic reaction can be clearly demonstrated by FABMS using (±)-**2** that has different side chain lengths at the glycerol C_1 and C_2 positions. During the analysis of the lysophospholipid, (±)-**2** afforded a molecular ion peak at *m/z* 412, 1-decanoyl-2-hydroxylphosphatidylcholine **4** ($C_{18}H_{38}NO_7P$, Mw 411 and *m/z* 412, MH^+), that indicated the hydrolysis of

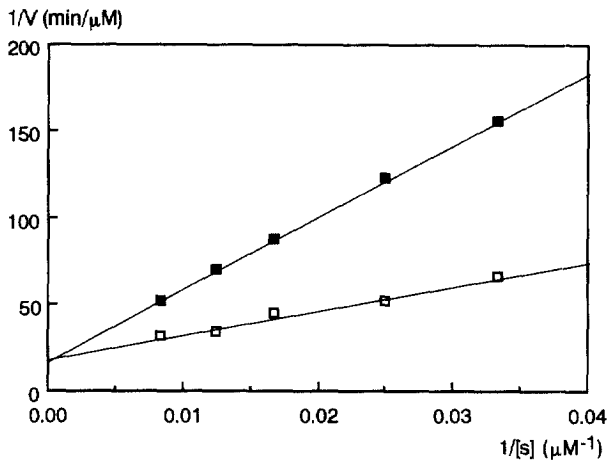

Figure 2. Lineweaver-Burk plots of antibody 13C2-1F6 (5 μM) against substrate (±) **2** (120-30 μM) under 50 mM Tris-HCl, pH 7.6 at 25 °C; open square: in the absence and closed square: in the presence of the phosphonate **3** (20 μM).

the C_2 ester, whereas no peak at *m/z* 384, 1-hydroxyl-2-octanoylphosphatidylcholine **5** ($C_{16}H_{34}NO_7P$, Mw 383 and *m/z* 384, MH^+), was observed. Furthermore, the alteration of the peak intensity at *m/z* 258 that is the molecular ion peak of glycerophosphorylcholine **6** ($C_8H_{20}NO_6P$, Mw 257 and *m/z* 258, MH^+) derived from the lysophospholipid was not observed under the given assay conditions. These findings indicated that the antibody 13C2-1F6 strictly hydrolyzed the ester side chain at C_2 in the phospholipid.

Thus, the antibody 13C2-1F6 elicited against the haptenic phosphonate **1** as the transition state analog was proved to be a catalytic antibody with the same regio- and enantioselectively, as those of phospholipase A_2.

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24. The uncatalyzed rate ($k_{un\text{-}2}$) of the hydrolysis at the C_2 ester in (±)-**2** without antibody could not determined by FABMS. In the uncatalyzed reaction, the C_1 ester is more rapidly hydrolyzed than the C_2 ester of (±)-**2**, $k_{un\text{-}2} < k_{un\text{-}1}$, by increasing the concentration of the hydroxide ion and reaction times, as shown in Figure 3. In the colorimetric assay,[26] the liberated fatty acids by all uncatalyzed reaction corresponding to the $k_{un\text{-}x}$ can not observed by the reaction for 88 hrs. Since the k_{uncat} values for hydrolysis of ester with secondary alcohols reported[7,11] to $5.0 \pm 2.11 \times 10^{-5}$ min^{-1}, the rate acceleration (k_{cat}/k_{uncat}) by the catalytic antibody ($k_{cat} = 1.0 \times 10^{-2}$ min^{-1}) was estimated as over 200 times.

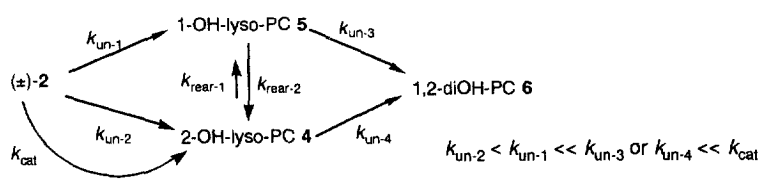

Figure. 3

25. Substrate (+)-**2** was prepared from L-α-didecanoylphosphatidylcholine, the (*R*)-configuration, with PLA_2 followed by acylation of octadecanoyl chloride.
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