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Impact of inhibiting activity of indole inhibitors on phospholipid hydrolysis by phospholipase A_2

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Abstract

The influence of two indole inhibitors on the hydrolysis reaction of L-dipalmitoylphosphatidylcholine (L-DPPC) catalyzed by phospholipase A_2 (PLA_2) has been studied in the coexistence region using surface pressure as function of time (π–t) and Brewster angle microscopy (BAM) measurements. Similar experiments have been performed with L-DPPC vesicles made by ultrasonication method using transmission electron microscopy (TEM). Comparing the π–t curves and BAM images, which show defected domains after a certain reaction time, as well as TEM images of defected vesicles, one can conclude that the two indole inhibitors have different abilities in reducing the activity of PLA_2. The described experiments show that BAM and TEM can be successfully used to study the influence of newly designed inhibitors on the hydrolysis reaction catalyzed by PLA_2.

Keywords: Indole; PLA_2; Catalyzed

1. Introduction

Phospholipase A_2 (PLA_2) is a calcium dependent enzyme which extensively exists in the organism [1–3]. PLA_2 stereoselectively hydrolyses the sn-2 ester linkage of phospholipids, preferentially in the aggregated state [4], to release fatty acids and lysophospholipids [5]. The enzyme is widespread in bacteria, snake and bee venoms, mammalian cells and secretions [6,7]. In this report, we describe the reactions between PLA_2 and L-DPPC in the presence of indole inhibitors, 5-methoxy-2-methyl-1-(phenylmethyl)-1H-indole-3-acetamide (**I**) [8] and its derivative 1-(4-chlorbenzoyl)-5-methoxy-2-methyl-3-indolessigsaure (**II**) [9,10] at the air/subphase interface. Compound **I** is an inhibitor synthesized for human non-pancreatic secretory phospholipase A_2 (hnps-PLA_2). Its I_{c50} value is $0.84 \pm 0.17\,\mu M$ [11,12]. Inhibitors synthesized for inhibiting PLA_2 activity remain a potential target for the development of new drugs in the treatment of diseases. Compound **II** has a similar chemical structure, however, its inhibitory activity to PLA_2 is lower according to three-dimensional quantitative structure–activity relationship (3D-QSAR) calculations for R1 position (steric and electrostatic contributions) and R3 position (Ca^{2+} binding and hydrogen bonds network) [13]. In this report, two experimental methods were used to mix the inhibitor with the reaction system of PLA_2 and L-DPPC. The corresponding phenomena were recorded and observed by Brewster angle microscopy (BAM) and transmission electron microscopy (TEM), respectively.

2. Materials and methods

2.1. Materials

L-Dipalmitoylphosphatidylcholine (L-DPPC) and phospholipase A_2 (from Crotalus atrox Venom) were purchased

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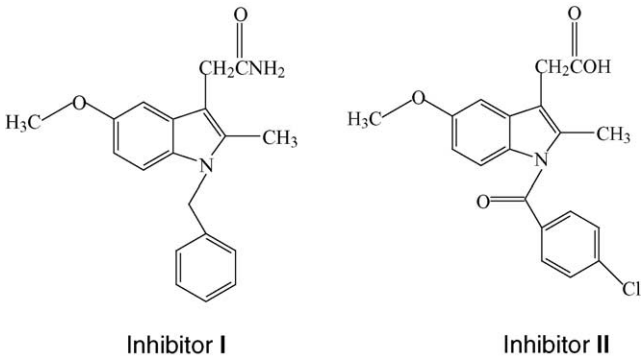

Fig. 1. Chemical structures of indole inhibitor **I** (5-methoxy-2-methyl-1-(phenylmethyl)-1H-indole-3-acetamide) and **II** (1-(4-chlorbenzoyl)-5-methoxy-2-methyl-3-indolessigsaure)).

from Sigma and used without further purification. The inhibitor **I**, 5-methoxy-2-methyl-1-(phenylmethyl)-1H-indole-3-acetamide was synthesized based on the procedure described in [8] and provided by Lai. It can be dissolved in chloroform and dimethylsulfoxide (DMSO). The inhibitor **II**, 1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid, was purchased from Fluka and used without further purification. The chemical structures of the two inhibitors are shown in Fig. 1. Chloroform of 99%+ purity was purchased from ACROS.

In the hydrolysis experiments, the L-DPPC monolayer was prepared from a 1 mM phospholipid/chloroform solution by a commercial LB trough. The monolayer was compressed to a suitable initial surface pressure and enzyme buffer solution was injected into the subphase for the cleavage reaction. The isotherm and morphology changes of the formed monolayer domains were recorded simultaneously. In all experiments, the compression rate was 2.5 $Å^2$/Molec./min. The subphase was an aqueous buffer solution at pH = 8.9, which contains 150 mM NaCl, 5 mM $CaCl_2$, and 10 mM Tris. The PLA_2 concentration in the subphase was 0.12 units/ml. The enzyme solution was injected into the subphase by a tiny syringe. The temperature was maintained at $20 \pm 0.1\,^{\circ}C$. The water in all experiments was purified by a Milli-Q system.

2.2. Methods

2.2.1. Brewster angle microscopy (BAM)

Brewster angle microscope (BAM) from NFT (Germany) mounted on the film balance was used for observation of the monolayer in the micrometer range. The microscope is sensitive to changes of the refractive index resulting from differences in thickness, density, and molecular orientation. The resolution is about 2 μm. Optical anisotropy caused by different molecular orientation in the monolayer is detected by an analyzer in the reflected beam path. The reflected light passes through a lens to a CCD (charge coupled device) camera, and the resulting video signal is fed into a video system. The BAM images are treated with an image processing software from Compic (Germany) to correct the distortion resulting from the observation at the Brewster angle. The surface tension was measured by the Wilhelmy method within 0.1 mN/m.

2.2.2. Transmission electron microscopy (TEM)

TEM measurements were performed on a Philips Tecnai 20 instrument. Samples for TEM were prepared by depositing an aqueous solution of the L-DPPC vesicles on a carbon-coated copper grid. The solution was then allowed to air-dry, and the extra solution was blotted off. The Philips Tecnai 20 is capable of magnifications of 100,000 times and extreme resolution.

3. Results and discussion

Inhibitor **I** can be easily dissolved in chloroform and DMSO but is only slightly soluble in water. In the first method, the inhibitor **I** and PLA_2 were mixed together and dissolved in the buffer solution using 5% (v/v) DMSO in order to increase the solubility of the inhibitor. L-DPPC was spread on the buffer surface and compressed to an initial surface pressure of 8.0 mN/m. The PLA_2 and inhibitor **I** mixed solution was injected into the subphase by a tiny syringe, the final concentration of PLA_2 was 0.12 units/ml. The pressure was measured as a function of time and the corresponding BAM images were recorded simultaneously during the reaction process. Fifty minutes after injection of the mixture of PLA_2 and inhibitor **I**, the L-DPPC domains have still a circular shape. Cleaved domains appeared after 55 min as shown in Fig. 2. New domains with high reflectivity can be seen after 60 min. This shows that the reaction products have formed their own domains on the surface. The observed stability of the shape of L-DPPC domains indicates that the inhibitor **I** is capable to inhibit the activity of PLA_2 for a period of approximately 50 min.

Because of the unknown effect of DMSO on the L-DPPC arrangement at the surface, a second method was applied to verify the observed results. The inhibitor **I** can be easily dissolved in chloroform. According to the I_{c50} value, inhibitor **I** is mixed with L-DPPC and dissolved in chloroform in a molar ratio of 30:1. The phase transition of this mixture is increasing from 6–7 mN/m (pure L-DPPC) to 11–13 mN/m. The plateau region indicating the first-order transition from liquid-expanded to a condensed state is more smeared-out due to the presence of inhibitor **I** (Fig. 3). Similar to pure L-DPPC domains can be observed in the mixed system at this surface pressure. However, the domain size is smaller compared to those in a pure L-DPPC monolayer. PLA_2 was injected by a tiny syringe at an initial surface pressure of 11.8 mN/m. The molar ratio of PLA_2 to inhibitor **I** is roughly 1/100. However, one has to keep in mind that not all inhibitor molecules will stay at the surface because it has a slight solubility in the buffer solution. After PLA_2 injection into the subphase, the surface pressure rises from 11.8 to 15.4 mN/m within 12 min. The pressure as a function of time is shown in Fig. 4. The corresponding BAM images are shown previously