

Research Article

α 1-Antitrypsin and Phospholipase A₂ in Respiratory Secretions of Patients with Cystic Fibrosis

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Citation:

Abstract

A 14 kDa secretory phospholipase A₂ (sPLA₂) in the lower respiratory tract may play a significant role in surfactant phospholipid degradation and tissue damage in the lungs of patients with cystic fibrosis (CF). We examined bronchoalveolar lavage fluid (BALF) from patients with CF to investigate the regulation of sPLA₂ activity on membrane phospholipid degradation. Substantial amounts of 50 kDa N-terminal truncated α 1-antitrypsin (Nt-A1AT) were found to be present in BALF from patients with CF but were not present in BALF from healthy subjects. Nt-A1AT could markedly stimulate sPLA₂ to degrade membrane phospholipids. A1AT, which functions as a neutrophil elastase inhibitor, may promote sPLA₂-mediated surfactant degradation and tissue damage if it is degraded to Nt-A1AT. We suggest that adequate prevention of protease-mediated degradation of A1AT may be required to prevent membrane phospholipid degradation in the inflamed CF lung, especially if exogenously administered A1AT is used as an augmentation therapy to prevent lung disease progression in CF.

Keywords: α 1-antitrypsin, bronchoalveolar lavage fluid, cystic fibrosis, lung, phospholipase A₂

Introduction

Cystic fibrosis (CF) is a lethal hereditary disease caused by mutations of the gene encoding the CF transmembrane conductance regulator protein (CFTR) that regulates the flux of chloride, bicarbonate, and other ions across the apical surfaces of epithelial cell membranes [1]. Dysfunction of CFTR results in accumulation of adherent mucus plaques in the lungs, and thick mucus on airway surfaces not only obstructs airways also allows infection and intense inflammation to become established and cause progressive airway damage [2]. Airway inflammation and bronchiectasis eventually lead to progressive loss of lung function that ultimately results in respiratory failure and death. The only effective palliative therapy for end-stage CF lung disease is lung transplantation [3].

Airspace infection in patients with CF characteristically leads to massive and persistent recruitment of neutrophils into the airspaces [4,5]. These neutrophils may undergo primary or secondary necrosis and release large amounts of neutrophil elastase (NE) that overwhelm antiprotease defences, and neutrophils and NE play a major role in the damage to airway cells and supporting tissues that leads to extensive phlegm accumulation, bronchiectasis and airflow obstruction [6,7].

A number of substances that include bacterial products and inflammation-induced release of leukotriene B4 (LTB4) and interleukin 8 (IL-8) induce the massive influx of neutrophils into infected CF airways [8], and phospholipase A₂ (PLA₂) plays an important role in the production of LTB4. Lipopolysaccharide (LPS), the cell wall component of Gram-negative bacteria, markedly increases the expression and secretion of a PLA₂ isoform, secretory PLA₂ (sPLA₂), in macrophages during acute lung injury [9] and in human respiratory epithelial cell lines with the CFTR mutation [10]. Additionally, sPLA₂ also activates alveolar macrophages to release pro-inflammatory cytokines [11,12]. Consequently, the dysfunctional cycle of infection with generation of cytokines and lipid mediators that exists in the CF lung may be tightly linked to dysregulated sPLA₂ activity in the airways. Because sPLA₂ activity is highly affected by the surrounding environment and substrate properties, the purpose of this study was to determine whether components in the BALF of CF patients could affect sPLA₂ activity.

Materials and Methods

Porcine pancreatic sPLA₂, phospholipase C (PLC) (*Clostridium perfringens*), pancreatic lipase, dioleoyl phosphatidylcholine (DOPC), and phosphatidylglycerol (PG) were purchased from Sigma Chemical (St. Louis, MO). Fluorescence labeled 1,2-bis-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-undecanoyl)-sn-glycero-3-phosphocholine (BODIPY-PC) was obtained from Molecular Probes (Invitrogen Corp., Carlsbad, CA). L- α -[1-¹⁴C]dioleoyl PC was purchased from PerkinElmer (Norwalk, CT). Sequencing grade-modified trypsin was obtained from Promega (Madison WI). Mouse monoclonal anti-human secretory PLA₂-IIA antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Goat anti-mouse IgG conjugated with horseradish peroxidase were from Sigma Chemical Co. Chemiluminescence ECL Plus reagent and were products of Amersham Pharmacia Biotech (Poscataway, NJ).

Isolation of human bronchoalveolar lavage fluid (BALF) components

This study was approved by the Institutional Review Board of the University of Wisconsin School of Medicine and Public Health (Protocol Number 2000-471), and informed consent was obtained from all subjects for the collection of samples. All patients with CF (N=10) were young adults (age range 18-34 years), were chronically infected with *Pseudomonas aeruginosa*, had mild to moderate impairment of forced expiratory volume in one second (FEV₁) values, and were clinically stable outpatients without acute exacerbation when outpatient bronchoscopy with bronchoalveolar lavage was performed. Normal volunteers were young adults (age range 20-32 years, N=15) who were healthy and had normal spirometry testing, had never smoked, and had no respiratory symptoms or evidence of lung disorders. BALF specimens were centrifuged to obtain cell-free fluids as previously described [13]. BALF from all subjects with CF had neutrophil-dominated inflammation, and cell-free BALF supernatant had unopposed neutrophil elastase activity. The cell-free BALF was concentrated 10-fold using an Amicon Ultra 5,000 MWCO concentrator (Millipore) and stored at -70°C before use. The Lowry method [14] modified for 96-well plate analysis was used to determine the protein content of BALF.

Determination of sPLA₂ activity by the radiometric and fluorescent methods

The radiometric assay of sPLA₂ was performed as previously described [13]. The sPLA₂ substrate liposomes composed of 50% DOPC, 50% PG and traces of [1-¹⁴C]DOPC. The reaction mixture contained 10 mM CaCl₂, 20 μ g liposomes with 2×10^4 cpm [1-¹⁴C]DOPC, and 0.5 μ g of sPLA₂ in a final volume of 0.1 ml of 0.01 M Tris-HCl buffer (pH 7.4). The reaction was carried out at room temperature for 30 seconds. PC and lysoPC in the mixture were extracted, separated by silica gel thin-layer chromatography (TLC), and their radioactivity determined. The sPLA₂ activity was determined as percentage of PC hydrolysis [13].

Fluorescently labeled liposomes were prepared by mixing 1.5 mg DOPC, 1.5 mg PG and 0.021 mg BODIPY-PC [15]. The assay mixture contained 10 mM CaCl₂, 20 μ g fluorescently labeled liposomes, and 10 ng sPLA₂ (or otherwise

specified) in a final volume of 3 ml of 0.01M Tris-HCl, pH 7.4 in a quartz cuvette. The assay was conducted at room temperature for 2 min and the sPLA₂ activity was expressed as fluorescence intensity (FI) vs. time (sec) after the initial reading was subtracted [15].

Isolation and characterization of the sPLA₂-stimulating factor (PLA₂-sf) in CF BALF

Pooled BALF from two CF subjects (160 ml) was heated in hot water (90°C) for 5 min; the denatured proteins were discarded by centrifugation, and the supernatant was concentrated 10-fold as described above. Approximately 5 ml of the concentrate was used for protein isolation by the methods of column chromatography using the columns of Sephadex G100, a high-performance liquid chromatography (HPLC) of anionic exchange Mono Q, and the reverse phase HPLC Vydac C4 as detailed previously [16]. The sPLA₂-sf activity in these fractions was determined by the fluorescent method. Protein purity was analyzed using 10% sodium dodecyl sulfate (SDS) polyacrylamide Ready gel (Bio-Rad, Hercules, CA) electrophoreses (PAGE) under denaturing conditions, and proteins on the SDS gel were visualized by Coomassie brilliant blue staining.

Structure determination and identification of sPLA₂-stimulating factor

An amount of 5 μ g of purified sPLA₂-sf was used for peptide sequence analysis using the methods of “matrix-assisted laser desorption ionization” (MALDI) and tandem mass spectrometry (MS/MS) with time-of-flight (TOF) instruments conducted on campus by the University of Wisconsin Biotechnology Center (UWBC) [16]. To determine the N-terminal amino acid sequence of the purified sPLA₂-sf moiety, an amount of 6 μ g of protein was run by SDS-PAGE followed by electro-transblotting to a PVDF membrane (Millipore, 0.45 μ m, 9 \times 12 cm). The PVDF membrane was then stained in 0.1% Ponceau Red (Xylidine Ponceau 2R, Sigma) in 10% acetic acid solution for several min until a pink-colored band became visible; this was followed by destaining in 10% acetic acid and distilled/deionized water. The protein band on the PVDF was used for N-terminal amino acid sequence analysis conducted at the Harvard Microchemistry Facility.

Western blot analysis and fluorescent assay of BALF endogenous sPLA₂ activity

An amount of 100 μ g protein of the heat-treated and 10-fold concentrated BALF was employed for protein separation by SDS-PAGE as described above. Proteins separated on the gel were transferred onto a nitrocellulose membrane and then immunoblotted with primary antibody of mouse monoclonal anti-human secretory PLA₂-IIA antibody (40 μ g/10 ml blotting buffer) and a secondary antibody of goat anti-mouse conjugated with horseradish peroxidase (1:3000 dilution in 10 ml TBST) by the procedures described elsewhere [13]. The immunoblotted membrane was treated with chemiluminescence reagents and exposed to a sheet of Hyperfilm. Some samples of 10-fold concentrated BALF without heat treatment were also analyzed by the Western blot method.

To determine the endogenous sPLA₂ activity in CF BALF, we first applied 5 ml BALF to a 15 ml Amicon Ultra Centrifugal Filter with 30k Ultracel (Millipore) and centrifuged at 5,000 rpm in a Sorvall ST 16R centrifuge (Thermo Scientific) at 4°C for 40 min. The concentrate was marked as >30k fraction and stored at -70°C. The filtrate was transferred to a 5 ml Amicon Ultra Centrifugal Filter with 10k. Ultracel and centrifuged at 5,000 for 40 min as described above. The concentrate was marked as the <30k>10k fraction and used for determination of the 14 kDa sPLA₂ activity. The sPLA₂ assay was conducted in a 96-well microplate in 0.3 ml reaction mixture that contained 1/10th of the components used in the cuvette assay described above; except the fluorescent liposome, substrate was replaced with 100% PG to enhance the assay sensitivity as described previously [17]. Routinely, two samples were assayed per run and each sample was assayed in duplicates (four wells). The plate was placed in a fluorescent microplate reader (Gen5 Synergy HT, BioTek Instruments, Inc., Winooski, VM, USA), and the assay was conducted at 37°C. The fluorescence intensity (FI) in each well was recorded every 10 s for up to 20 min at 485 nm excitation and 528 nm emission. An initial reading was recorded as zero time and the activity was expressed as FI vs. time (min) after the initial reading was

subtracted from each subsequent reading (Δ FI) [15]. The initial rate of the reaction (FI/min) was determined from the reaction curve fitted with a second-order polynomial equation and the first-degree coefficient [15]. The baseline was determined without the presence of any proteins in the assay mixture.

Fluorescent assay of PLC and lipase

The reaction mixture for the fluorescent assay of PLC contained the same 50%DOPC-50%PG-BODIPY-PC liposome substrates and Ca^{2+} -Tris buffer as the sPLA₂ fluorescent assay, except sPLA₂ was replaced with a specified amount of PLC (0.01 to 0.5 unit). The reaction was carried out at room temperature, and FI was recorded every 10 s after addition of PLC for up to 2 min; then an amount of 0.14 unit of lipase was added to the reaction mixture. Fluorescence intensity was further recorded for another 2 min.

Results

Western blot analysis of BALF sPLA₂ and effect of BALF on sPLA₂ activity

The Western blot shows that the monoclonal antibody against sPLA₂-IIA detected the heat-stable sPLA₂-IIA protein in the heat-treated and concentrated CF BALF but not in the heat-treated and concentrated HS BALF samples (Figure 1A). The amount of sPLA₂-IIA in un-heated and un-concentrated BALF was too low to be detected (CF BALF1 and CF BALF2 in Figure 1A). Because of very low concentrations of sPLA₂ protein in the CF BALF, we used exogenous sPLA₂ as the enzyme source to investigate BALF effects on the sPLA₂ activity. The radiometric assay showed that presence of an amount of 100 μ g of CF BALF protein in the reaction mixture containing 0.5 μ g sPLA₂ enhanced sPLA₂ activity by nearly 2-fold (Figure 1B). CF BALF alone had no measurable sPLA₂ activity and the presence of 100 μ g of HS BALF protein in the sPLA₂ reaction mixture did not stimulate sPLA₂ activity, suggesting that some unknown factor(s) in the CF BALF could stimulate the sPLA₂ activity. Pre-incubation of CF BALF at 37°C for 1 h did not have any effect on sPLA₂ activity stimulation. Similarly, the CF BALF also induced the sPLA₂ activity in a CF BALF dose-dependent manner measured by the fluorescent assay, and CF BALF alone in the assay had no effect on FI (Figure 1C).

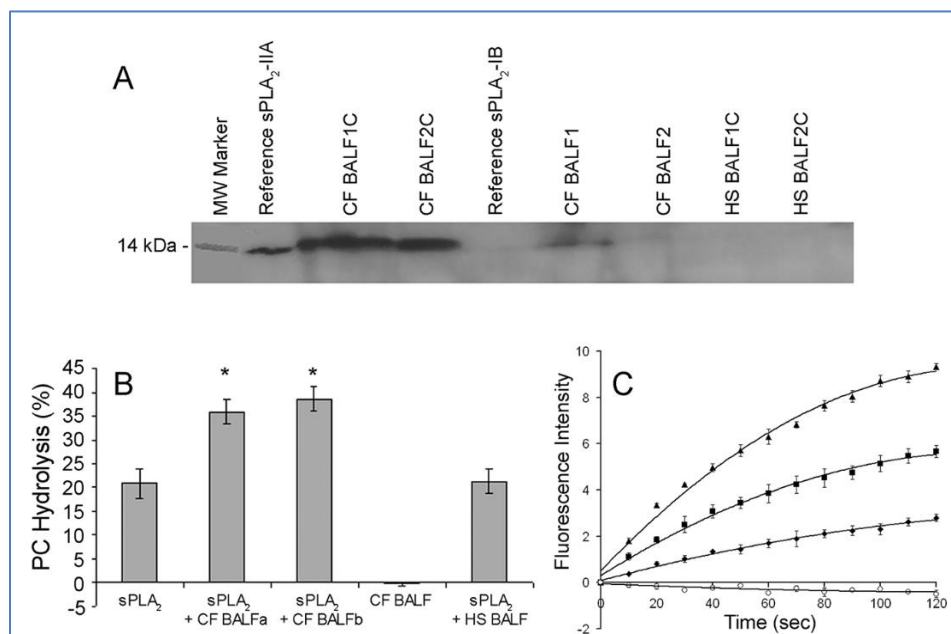


Figure 1: (A) Western blot analysis of sPLA₂ in BALF. Mouse monoclonal antibody against human sPLA₂-IIA was used to immunoblot the proteins in the BALF from CF and healthy subjects (HS). Color stained standard proteins were used as molecular weight (MW) markers. An amount of 0.1 mg of human synovial fluid proteins containing sPLA₂-IIA and 0.1 mg of exogenous sPLA₂ (sPLA₂-IB) were used as references. CF BALF1C, CF BALF2C, HS BALF1C, and HS BALF2C were each heat-treated and concentrated 10-fold from its original fluid volume. CF BALF1 and CF BALF2 were not heat treated but 10-fold concentrated samples. An amount of 0.1 mg of concentrated BALF proteins was employed for Western blot analysis. The monoclonal antibody against sPLA₂-IIA did not cross react with sPLA₂-IB protein. **(B)** In the radiometric assay, Column (sPLA₂) represents the reaction mixture contained 20 μ g of ¹⁴C-PC labeled liposomes and 0.5 μ g exogenous sPLA₂. Columns

(sPLA₂ + CF BALFa) and (sPLA₂ + CF BALFb) represent the reaction mixture of Column (sPLA₂) plus 0.1 μ g of CF BALF proteins without or with pre-incubation at 37°C for 1 h, respectively. Column (CF BALF) represents the reaction mixture containing 20 μ g of ¹⁴C-PC liposomes and 0.1 μ g CF BALF proteins without exogenous sPLA₂. Column (sPLA₂ + HS BALF) represents the reaction mixture of Column (sPLA₂) plus 0.5 μ g sPLA₂ and 0.1 μ g of HS BALF proteins. All BALF samples were 10-fold concentrated. The results are mean \pm SD (n = 4) and the symbol (*) represents p < 0.05 compared to the Column (sPAL₂). (C) In the fluorescent assay, the 3 ml reaction mixture in a cuvette contained 20 μ g 50% PC-50% PG liposomes labeled with BODIPY-PC, 10 mM calcium chloride, and 10 ng exogenous sPLA₂ (\blacklozenge). To determine the effect of CF BALF, an amount of 50 μ g (\blacksquare) or 100 μ g (\blacktriangle) of CF BALF protein was added to the reaction mixture prior to the addition of sPLA₂. The symbol of (O) represents the reaction contained 100 μ g CF BALF proteins in the absence of exogenous sPLA₂. The results are an average of duplicate assays and bars represent SD.

Isolation, characterization, and identification of sPLA₂-stimulating factor(s) in CF BALF

We used the methods of Sephadex G100 gel filtration, MonoQ, and reverse phase HPLC column chromatography as previously described [16] to isolate the sPLA₂-sf. The activity of sPLA₂-sf was determined by the fluorescent assay in each column chromatography (data not shown). sPLA₂-sf was finally isolated as a homogenous single protein by the reverse phase HPLC (Figure 2A). The SDS-PAGE analysis showed that the fractions eluted between 52-53 fractions contained a single protein with an apparent molecular weight approximately 50 kDa (Figure 2B). The purified sPLA₂-sf also markedly stimulated sPLA₂ activity in the fluorescent assay (Figure 2C). Presence of purified sPLA₂-sf protein in the reaction mixture without sPLA₂ did not induce any significant FI change. The peptide mass spectrometry and amino acid sequence results showed that the sPLA₂-sf peptide sequences matched human serum α 1-antitrypsin (A1AT) (Table 1). Because serum A1AT had a molecular weight of 52 kDa and CF BALF sPLA₂-sf had an apparent mass of 50 kDa, this suggests that CF BALF sPLA₂-sf was a truncated A1AT. The N-terminal sequence of the truncated A1AT was determined to be HDQDHPTFNKIT, indicating that A1AT in the CF respiratory secretions was cleaved between the His15 and His16 bond. Thus, sPLA₂-sf was an N-terminal truncated A1AT (Nt-A1AT).

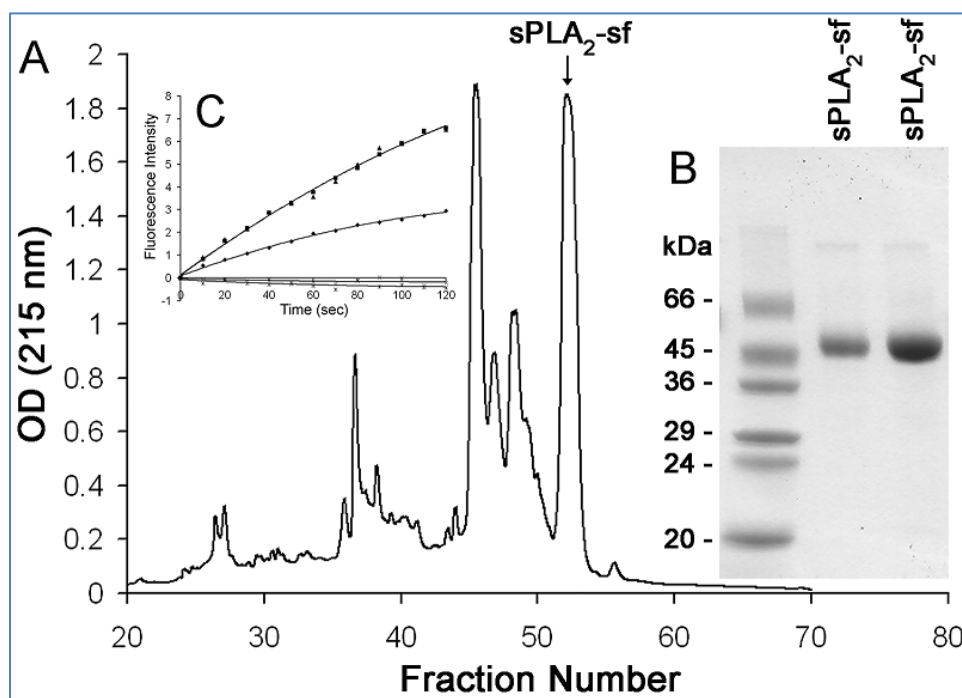


Figure 2: (A) Isolation of sPLA₂-stimulation factor (sPLA₂-sf) from CF BALF by reverse phase HPLC chromatography. sPLA₂-sf was eluted from the column at 52-53 fractions. (B) SDS gel electrophoresis of sPLA₂-sf showed a single band with MW 50 kDa. Left column protein bands are standard reference proteins with known molecular weights. (C) Fluorescent assay of sPLA₂-sf isolated from CF BALF. The reaction mixture contained 20 μ g 50% PC-50% PG UL, 10 mM CaCl₂, and 10 ng exogenous sPLA₂ (\blacklozenge), or in the presence of 10 ng exogenous sPLA₂ and 6 μ g sPLA₂-sf protein from two separate isolations (\blacktriangle , \blacksquare). The lines with symbols of (*, X) represent the reactions containing 6 μ g sPLA₂-sf protein from two separate isolations in the absence of sPLA₂.

Table 1: Peptide mass and sequence of PLA₂ stimulator isolated from CF BALF

Peptides	Observed mass/Z	Expected mass/Z	Calculated mass/Z	Amino acid sequences matching α 1-AT	Amino acid residue position at α 1-AT*
1	686.44	685.43	685.44	IVDLVK	169-174
2	538.8	1075.58	1075.61	LSSWVLLMK	235-243
3	1110.62	1109.61	1109.6	LSITGTYDLK	291-300
4	754.85	1507.69	1507.71	GTEAAGAMFLEAIP	344-357
5	821.42	1640.83	1640.86	ITPNLAEFASFSLYR	26-39
6	917.46	1832.9	1832.92	VFSNGADLSGVTEE	311-324
7	858.77	2573.28	2573.33	TLNQPDSQLQLTTG	102-115

To determine the effect of BALF Nt-A1AT on the endogenous sPLA₂-IIA activity, we separated BALF into two fractions; one fraction of MW >30 kDa which contained the 50 kDa Nt-A1AT, and the second fraction of MW <30 kDa >5 kDa which had the 14 kDa sPLA₂-IIA. We then used the 100% PG-BODIPY liposome as the substrate and assayed activity in a fluorescent microplate reader that was more sensitive than the 50% DOPC-50% PG-BODIPY liposome substrate [17]. Because of quenching of the substrate of BODIPY-PG-liposomes, the initial reading of the reaction was taken from 10 min and the reaction lasted for 20 min. The CF BALF fraction of MW <30 kDa >5 kDa exhibited a time-dependent increase in FI, a characteristic of the sPLA₂ activity, whereas the fraction of MW >30 kDa had a FI pattern similar to the substrate background (Figure 3A). A combination of the fraction of MW <30 kDa >5 kDa and the fraction of MW >30 kDa yielded 2-fold increase in the activity, suggesting a marked stimulation of BALF endogenous sPLA₂-IIA activity by the Nt-A1AT. Additional assays of the MW <30 kDa >5 kDa and MW >30 kDa fractions from a total of 6 CF BALF samples were shown in Figure 3B, whereas these fractions isolated from 4 healthy subjects had no detectable sPLA₂ and Nt-A1AT activities (Figure 3B).

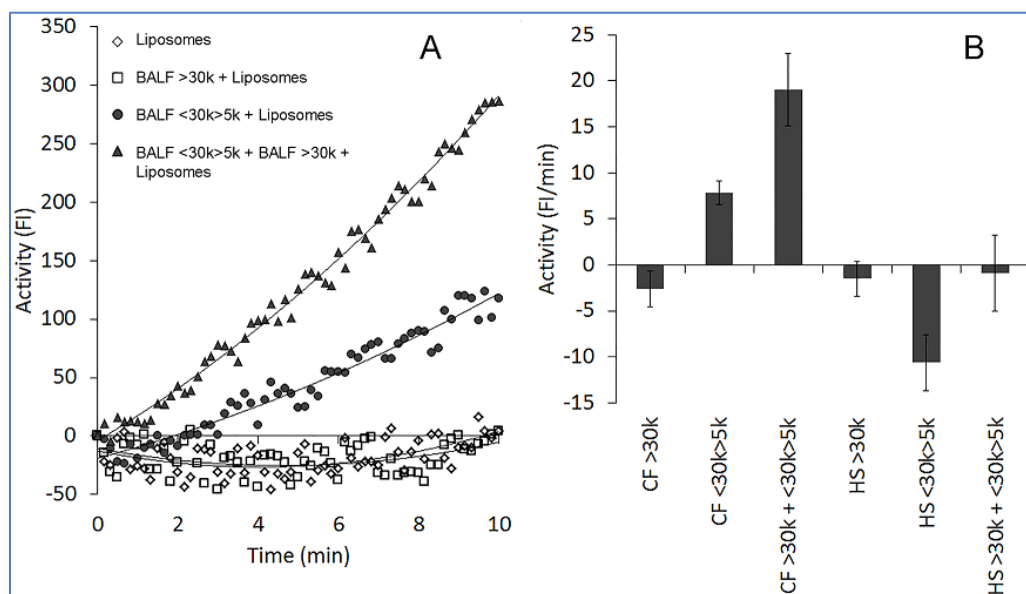


Figure 3: Fluorescent assay of the effect of Nt-A1AT on endogenous sPLA₂ activity in CF BALF. Fractions of MW >30 kDa which contained Nt-A1AT and fractions of MW <30 kDa >5 kDa which contained sPLA₂-IIA were isolated from the BALF of patients with CF and healthy subjects (HS). The activities of Nt-A1AT and sPLA₂ were determined in a 96-well microplate in a fluorescent plate reader. **(A)** Examples of the real time measurement of the activity of Nt-A1AT (BALF >30 kDa + Liposomes), sPLA₂ (BALF <30 kDa >5 kDa + Liposomes), and the combination of Nt-A1AT and sPLA₂ (BALF <30 kDa >5 kDa + BALF >30 kDa + Liposomes), as compared to the measurement of liposome alone. **(B)** Comparison of the activities of Nt-A1AT (>30kDa), sPLA₂ (<30 kDa >5 kDa), and Nt-A1AT + sPLA₂ in the fractions isolated from CF BALF and HS BALF. The results are mean \pm SEM of n=6 CF BALF samples, n=4 HS BALF samples. The fraction of Nt-A1AT in CF BALF had no detectable sPLA₂ activity (CF >30k), but in combination with the sPLA₂ fraction, it significantly stimulated the sPLA₂ activity (CF >30k + <30 kDa >5 kDa compared to CF <30 kDa >5 kDa) (p=0.023). There was no detectable endogenous sPLA₂ and Nt-A1AT stimulation in the HS BALF.

Continuous fluorescent assay of PLC and effect of Nt-A1AT on PLC activity

To determine the mechanism underlying the stimulation of sPLA₂ activity by Nt-A1AT, we studied the effect of Nt-A1AT on PLC activity under the fluorescent DOPC-PG liposome assay conditions, except the exogenous sPLA₂ was replaced with PLC. In contrast to the sPLA₂ assay, the PLC reaction produced a time-dependent and PLC-dose dependent decrease in FI (Figure 4A). PLC hydrolyzed liposome phospholipids to yield diacylglycerols (DG) that caused fluorescence quenching. When pancreatic lipase (0.14 unit) was added to the PLC (0.06 unit) reaction mixture 2 min later, FI markedly increased with time due to the hydrolysis of fluorescently labeled DG to yield the fluorescence-labeled fatty acid and the aqueous soluble monoacyl glycerol which caused an increase in FI (Figure 4B). When 0.6 μ g Nt-A1AT was added to the reaction mixture prior to the addition of PLC and lipase, Nt-A1AT effectively inhibited PLC activity as determined by less FI decrease in the PLC reaction and less FI increase in the lipase reaction (Figure 4B). The presence of 0.6 μ g or 3 μ g human serum albumin had no effect on the PLC and lipase activities (Figure 4B).

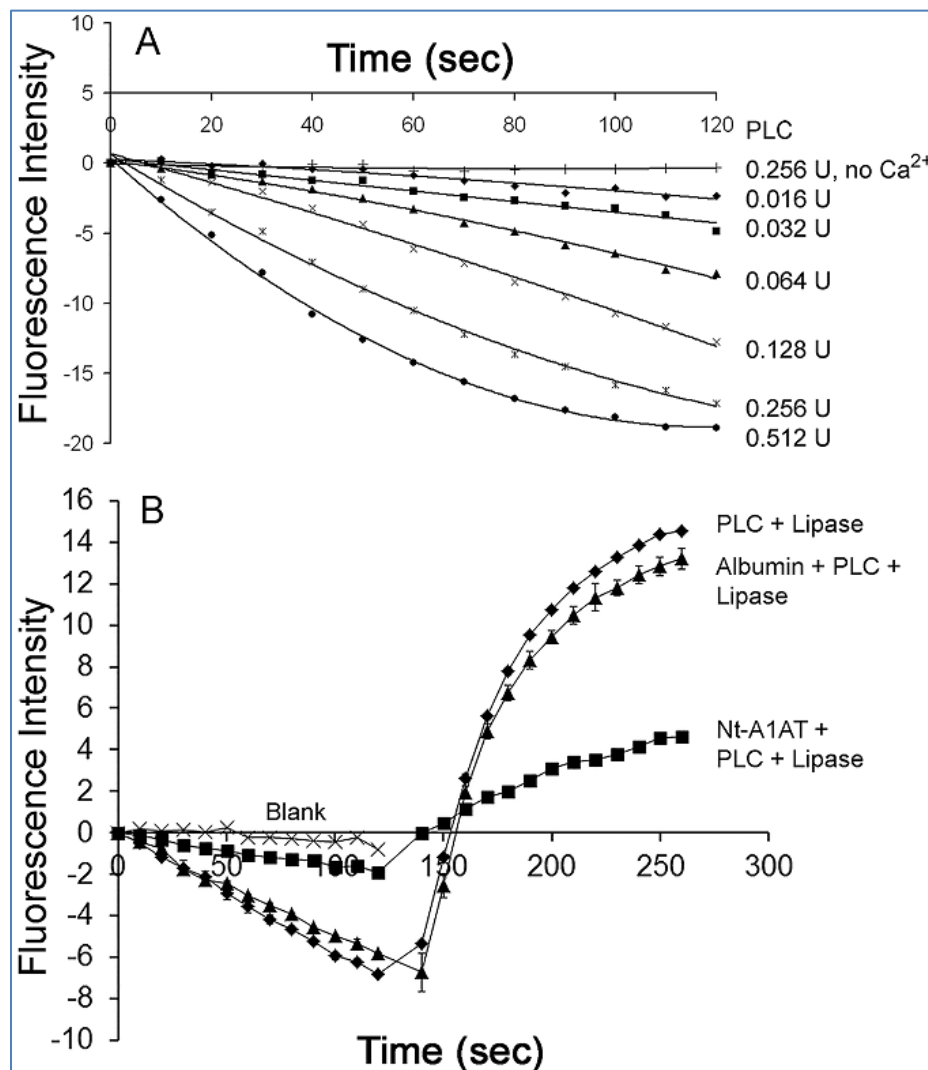


Figure 4: (A) Fluorescent assay of PLC. Similar to the sPLA₂ fluorescent assay, the PLC reaction mixture contained 20 μ g liposomes (50% DOPC-50%PG) labeled with fluorescent BODIPY-PC, 10 mM calcium chloride, and specified amount of PLC unit (U) in 3 ml 0.01 M Tris-HCl, pH 7.4. The reaction was conducted at room temperature for 2 min, and FI was recorded every 10 secs after addition of PLC. PLC decreased FI in a dose-dependent manner. (B) Effect of Nt-A1AT and albumin on PLC activity. An amount of 0.06 unit PLC (◆) was added to the reaction mixture and the reaction was conducted at room temperature for 2 min, then, an amount of 0.14 unit pancreatic lipase was added to the PLC reaction mixture and the reaction was continued for another 2 min (Control). In a separate reaction, an amount of 0.6 μ g Nt-A1AT (■) or 0.6 and 3 μ g albumin (▲) was added to the reaction mixture prior to the addition of 0.06 unit PLC and 0.14 unit lipase. The results are an average of triplicates (◆) or duplicates (■ ▲) assays and bars represent SD.

Discussion

Both radiometric and fluorometric assays showed that CF BALF stimulated the sPLA₂ catalytic activity, regardless of whether the sPLA₂ was exogenous or endogenous. The N-terminal truncated 50 kDa A1AT (Nt-A1AT) was identified as the stimulating factor. A1AT is a 52 kDa glycoprotein that is a member of the serpin superfamily and functions as a protease inhibitor. The prime function of A1AT is to inhibit NE and protect tissues from destruction by unopposed NE. When A1AT interacts with NE in a 1:1 molar ratio, the elastase cleaves A1AT at the active site of the C-terminus at the Pro357-Met358 position, and cleaved A1AT binds to NE to form an inactive A1AT-NE complex, resulting in inhibition of NE enzymatic activity [18-20]. Various proteolytically cleaved A1AT products have been found in bronchial secretions or sputa of patients with smoking-associated emphysema, asthma, chronic bronchitis, adult respiratory distress syndrome, bronchopulmonary dysplasia, and CF [21,22]. In addition to functioning as an endogenous elastase inhibitor, A1AT may also promote monocyte recruitment, activate pro-inflammatory pathways, and affect cell migration and proliferation [23,24]. Additionally, proteolytically cleaved and inactivated A1AT can be a potent chemotactic factor for neutrophils [19].

This study showed that significant amounts of A1AT in CF respiratory secretions were degraded to Nt-A1AT, which was not only an inactive product of proteolytic cleavage but could act as a sPLA₂ cofactor to enhance sPLA₂ catalytic activity on membrane phospholipid degradation. We previously observed that a significant amount of NE in BALF from patients with CF was not adequately inhibited [13]. The heat stable property of Nt-A1AT is consistent with the observations that proteolytically cleaved A1AT is folded into a highly ordered and stable structure that is more thermodynamically stable than the native form of A1AT [25,26].

This study demonstrated that Nt-A1AT stimulates sPLA₂ activity with liposome substrates containing negatively charged PG. In contrast, Nt-A1AT inhibited PLC activity with PC-PG liposome substrates. Inhibition of PLC suggests that Nt-A1AT must interact with the head group of negatively charged PG and blocks PLC from cleaving the head groups of phospholipids. This notion is consistent with the finding that less diacylglycerol was available for the action of lipase in the reaction mixture containing Nt-A1AT and PLC. Thus, interaction of Nt-A1AT with negatively charged PG may also widen the space between phospholipid molecules of the outer layer of liposomes and allows sPLA₂ to penetrate into the bilayer to cleave the *sn*-2 fatty acyl groups of phospholipids at the lipid-water interface. Additionally, Nt-A1AT-membrane interactions may provide an ideal physical environment for sPLA₂ catalytic action on phospholipid substrates.

Neutrophil elastase can be inhibited by A1AT, and sPLA₂ activity can be restrained by native sPLA₂ inhibitors such as annexin 1 in inflamed airways [13]. However, in CF respiratory secretions annexin 1 is extensively degraded by unopposed NE [13]. Based on the current study and previous findings [13], we suggest that it is possible that degradation of A1AT to Nt-A1AT in the airways of CF patients not only fails to inhibit NE but may also lead to the augmentation of neutrophil necrosis and bioactive lipid mediator generation in the presence of sPLA₂ and absence of intact annexin 1. Preventing A1AT degradation to Nt-A1AT may be of central importance in the treatment of CF lung disease. We speculate that it is possible that exogenous A1AT replacement therapy given in an attempt to diminish NE-mediated lung tissue damage may lead to increased amounts of Nt-A1AT if NE catalytic activity cannot be adequately blocked in the CF airspace inflammatory milieu. Additionally, we suggest that such augmentation therapy (e.g. inhaled A1AT) given as a treatment for CF lung disease may have the potential to promote rather than ameliorate airway inflammation if unopposed NE continues to be present in the airspaces and degrades A1AT to Nt-A1AT, which may enhance sPLA₂ catalytic activity and membrane phospholipid degradation.

Acknowledgments

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