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A role for diacylglycerol in annexin A7-mediated fusion of lung lamellar bodies

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Abstract

Lung surfactant secretion in alveolar type II cells occurs following lamellar body fusion with plasma membrane. Annexin A7 is a Ca^{2+}-dependent membrane-binding protein that is postulated to promote membrane fusion during exocytosis in some cell types including type II cells. Since annexin A7 preferably binds to lamellar body membranes, we postulated that specific lipids could modify the mode of annexin A7 interaction with membranes and its membrane fusion activity. Initial studies with phospholipid vesicles containing phosphatidylserine and other lipids showed that certain lipids affected protein interaction with vesicle membranes as determined by change in protein tryptophan fluorescence, protein interaction with *trans* membranes, and by protein sensitivity to limited proteolysis. The presence of signaling lipids, diacylglycerol or phosphatidylinositol-4,5-bisphosphate, as minor components also modified the lipid vesicle effect on these characteristics and membrane fusion activity of annexin A7. In vitro incubation of lamellar bodies with diacylglycerol or phosphatidylinositol-4,5-bisphosphate caused their enrichment with either lipid, and increased the annexin A7 and Ca^{2+}-mediated fusion of lamellar bodies. Treatment of isolated lung lamellar bodies with phosphatidylinositol- or phosphatidylcholine phospholipase C to increase diacylglycerol, without or with preincubation with phosphatidylinositol-4,5-bisphosphate, augmented the fusion activity of annexin A7. Thus, increased diacylglycerol in lamellar bodies following cell stimulation with secretagogues may enhance membrane fusion activity of annexin A7.

Keywords: Membrane fusion; Surfactant secretion; Protein fluorescence; Proteolysis; Membrane insertion; Membrane binding; Signaling lipids

Lung surfactant is essential for normal lung function in air-breathing mammals because it is required for lowering surface tension at the air–liquid interface during end-expiration (reviewed in [1,2]). This lipoprotein-like complex of phospholipid and proteins is synthesized and secreted by the alveolar type II cells (reviewed in [3]). The phospholipid and some of the protein components are stored in lamellar bodies, the secretion organelles unique to type II cells. Surfactant secretion occurs through fusion pores that are formed following lamellar body fusion with plasma membrane (reviewed in [4,5]). Several studies have demonstrated that several agents increase surfactant secretion in isolated perfused lung and in isolated type II cells by increasing cell Ca^{2+}, cAMP, and protein kinase C (PKC) activity [4,5]. Some of these agents also increase the number of surface fusion pores suggesting that the membrane fusion activity is increased to allow elevated secretion of surfactant [6,7].

Previous studies have suggested a role for annexin proteins in membrane fusion during surfactant secretion [8–10]. Annexin A7 is postulated to promote membrane fusion during exocytic secretion in some cell types [8,11]. We have previously demonstrated that annexin A7 could promote membrane fusion between isolated lamellar bodies and lung plasma membrane fractions [9], or increase surfactant secretion in semi-intact type II cells [8]. Annexin A7 binding to lamellar bodies is higher than to the plasma membrane or the cytosol fraction [12] suggesting that the lamellar body membrane characteristics could contribute

Abbreviations: DAG, diacylglycerol; DPE, dansyl phosphatidylethanolamine; F_{345}, fluorescence at 345 nm; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PIP_2, phosphatidylinositol-4,5-bisphosphate; PI, phosphatidylinositol; PS, phosphatidylserine; PKC, protein kinase C; TAE, Tris-acetate-EGTA

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to the higher binding. Although annexin A7 binding protein was detected in both the lamellar bodies and the plasma membrane fractions [12], the lipids in the lamellar body membrane could also contribute to annexin A7 binding. This study also showed that the protein binding to lamellar bodies and plasma membrane can be further enhanced followed cell treatment with calcium ionophore or phorbol myristate acetate (a direct activator of PKC). Since intracellular membranes show differences in lipid composition [13–16], it is also likely that these differences contribute to specificity and membrane binding and fusion activities of annexin A7.

Annexin A7, like other annexin proteins, binds to phospholipid membranes in a Ca^{2+}-dependent manner through the highly homologous COOH– (core) domain (reviewed in [17–19]). The unique NH_2- (tail) terminus is short for most annexin proteins except for annexin A7 and A11 that have long tails. The unique nature of the NH_2-terminus is postulated to contribute to specificity of annexin function [20]. Several previous studies employing annexin A5 as a model annexin protein have demonstrated high affinity binding to acidic phospholipid like phosphatidylserine (PS) or phosphatidic acid (reviewed in [17]). This is also supported by the presence of postulated PS binding sequences in the annexin molecule [21]. Even though PS binding sites are present in the core domain, our studies with recombinant wild type and deletion mutant annexin A7 proteins suggest that the NH_2-terminus could modify the core domain and its Ca^{2+}-dependent interaction with phospholipid vesicle (PLV) membranes [22]. Although annexin proteins bind poorly to major membrane phospholipids like phosphatidylcholine (PC) and phosphatidylethanolamine (PE) [17], a comparison of the Ca^{2+}-dependent binding of annexin A7 and its mutants to PC:PS (3:1) and PE:PS (3:1) PLV indicated that change in major lipid could influence annexin A7 interaction with membranes [23]. Further, annexin A7 preferably binds to specific biological membranes [12,24–26] alluding to the possibility that the membrane lipid composition could have a role in affecting protein interaction with these membranes. Others have demonstrated [27–29] preferred binding of annexin A2 to specific cellular membranes like plasma membrane or to phospholipid vesicles (PLV) enriched with phosphatidylinositol-4,5-bisphosphate (PIP_2). This important phospholipid is also implicated in the action of synaptotagmin, a Ca^{2+}-binding synaptic vesicle protein, which showed preference for PIP_2-containing domains in membranes [30]. These observations suggest that other phospholipid species (besides PS) could also control protein interaction with membranes. Such regulation of protein binding by specific lipid could contribute to site-specific physiological regulation of protein function [14,16], since significant differences in phospholipid composition of intracellular membranes have been reported [13–16]. Another possible implication of observations with annexin A2 and synaptotagmin lies in the involvement of PIP_2 in the signal transduction pathways (reviewed in [31–33]), since its hydrolysis by phospholipase C (PLC) would generate inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) that increase cell Ca^{2+} and protein kinase C (PKC) activity, respectively. In alveolar type II cells, as in most other cell types, Ca^{2+} and PKC are implicated in stimulation of secretion by exocytosis [34–37]. Thus, modulation of annexin A7 properties by DAG or PIP_2 would suggest that stimulation of cells with appropriate secretagogues would regulate annexin A7 function for augmented secretion.

In this study, we tested the hypothesis that the PIP_2 and DAG levels would significantly modulate the properties of annexin A7, since increased membrane fusion activity would be needed in type II cells stimulated with surfactant secretagogues [34,36,37]. We utilized PLV prepared from various lipid mixtures to show that lipid composition, PIP_2 and DAG in particular, affected the molecular characteristics (protein fluorescence and protease sensitivity) and the membrane fusion function of annexin A7. In parallel, altering the PIP_2 or DAG content in isolated lung lamellar bodies modulated the membrane binding and membrane fusion activities of annexin A7. We propose that these signaling lipids in biological membranes could contribute to the specificity of annexin A7 action and regulation of membrane fusion during surfactant secretion.

1. Materials and methods

L-α-phosphatidylcholine (brain), L-α-phosphatidylserine (Brain, bovine), L-α-phosphatidylinositol-4,5-bisphosphate (brain, Porcine), 1,2,dioleoylglycerol, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-(5-dimethylamino-1-naphthalenesulfonyl) (dansylPE, DPE), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine were obtained from Avanti Polar Lipids (Alabaster, AL) and were used without further purification. Recombinant trypsin (Promega, Madison, WI) was used to cleave recombinant fusion protein for the release of free annexin A7. Chymotrypsin, PC-PLC, PI-PLC, cholesterol and other fine chemicals were from Sigma Chemical Co. (St. Louis, MO). Polyclonal antibodies to PIP_2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). All standard chemicals and glassware were from VWR Scientific (Rochester, NY) unless indicated otherwise. Silica gel G coated thin layer chromatography plates were by Whatman Inc and obtained from Fisher Scientific (Philadelphia, PA). Recombinant annexin A7 protein was expressed in *Escherichia coli* as described [22]. The purified protein was stored in small aliquots at −70 °C in 50 mM Tris-Acetate–1 mM EGTA (TAE) buffer (pH 8.3).

1.1. Preparation of phospholipid vesicles

Lipid mixtures of indicated composition in chlroform:methanol (20:1, v/v) were evaporated to dryness under a stream of N_2. The dried lipids were suspended by vortexing in TAE buffer and passed through a membrane extruder (LIPEX, Northern Lipids, Inc., Vancouver, Canada) fitted with a 100-nm filter to prepare lipid vesicles. Lipid suspensions were passed (×4) through a 200-nm filter and then (×4) through the 100-nm filter. The passage of lipid suspension through the filter causes formation of lipid vesicles of approximately 100 nm in diameter. The vesicle suspension was stored at 4 °C and brought to experimental temperature before use. If any small unilamellar vesicles (<30 nm) were present, they would spontaneously fuse to form larger vesicles during storage at 4 °C, which may be below the phase transition temperature of vesicle lipid mixtures.

1.2. Isolation of lamellar bodies

Lungs of anesthetized (Nembutal, 50 mg/kg, ip) and exsanguinated male Sprague–Dawley rats (~200 ×*g*) were ventilated and cleared of residual blood by perfusion through the pulmonary artery with phosphate buffered saline containing 10 mM glucose. The visibly cleared lungs were instilled with sucrose (1 M, unbuffered), harvested, and homogenized in 1 M sucrose. Lamellar bodies from lung homogenate (10%) were isolated by upward flotation on a discontinuous sucrose density gradient, as described previously [38]. We