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Ca^{2+}-independent phospholipase A2 participates in the vesicular transport of milk proteins

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Abstract

Changes in the lipid composition of intracellular membranes are believed to take part in the molecular processes that sustain traffic between organelles of the endocytic and exocytic transport pathways. Here, we investigated the participation of the calcium-independent phospholipase A2 in the secretory pathway of mammary epithelial cells. Treatment with bromoenol lactone, a suicide substrate which interferes with the production of lysophospholipids by the calcium-independent phospholipase A2, resulted in the reduction of milk proteins secretion. The inhibitor slowed down transport of the caseins from the endoplasmic reticulum to the Golgi apparatus and affected the distribution of p58 and p23, indicating that the optimal process of transport of these proteins between the endoplasmic reticulum, the endoplasmic reticulum/Golgi intermediate compartment and/or the cis-side of the Golgi was dependent upon the production of lysolipids. Moreover, bromoenol lactone was found to delay the rate of protein transport from the trans-Golgi network to the plasma membrane. Concomitantly, membrane-bound structures containing casein accumulated in the juxtanuclear Golgi region. We concluded from these results that efficient formation of post-Golgi carriers also requires the phospholipase activity. These data further support the participation of calcium-independent phospholipase A2 in membrane trafficking and shed a new light on the tubulo/vesicular transport of milk protein through the secretory pathway.

Keywords: Epithelial cell; Ca^{2+}-independent phospholipase A2; Milk protein; Secretion; Membrane dynamics

1. Introduction

Milk is a complex biological fluid comprising several phases, the casein micelles, the fat globules and an aqueous phase that contains whey proteins including immunoglobulins, hormones, and whey acidic protein (WAP) in rodent and lagomorphs, but also lactose and minerals in ionic or bound forms [1]. Depending of their origin, newly synthesised (caseins, WAP) or blood plasma-derived (immunoglobulins, hormones) milk proteins travel to the apical side of the mammary epithelial cell (MEC) via either the secretory pathway or via the transcytotic route. Milk secretion is obviously of interest due to its importance in providing nutrients to the neonates, but also as a model to study a high-efficiency transport pathway in a physiological cell system. While the secretory pathway of MEC is known in general outline (for review see [2–4]), a limited amount of data is available on the membrane events participating in the intracellular trafficking of milk proteins. Over the past two decades, most research efforts concerning the identification of the molecular machinery that controls transport of cargo molecules between intracellular compartments of eukaryotic cells, and hence the formation of transport carriers, focused on the participation of protein-based coats (COPs, clathrin) that assemble on the donor membranes and bend them, allowing vesicles containing cargo protein to form. For example, the initial membrane trafficking event in the secretory pathway requires vesicles that bud from the

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endoplasmic reticulum (ER) and fuse with the ER/Golgi intermediate compartment (ERGIC) at the cis-side of the Golgi apparatus. These anterograde-directed vesicles are coated with a set of proteins termed COPII coatomers [5–7]. In contrast, COPI-coated vesicles would move bi-directionally between the ER and the Golgi, mediating the anterograde transport from the ERGIC to the cis-most cisternae of the Golgi or the recycling from the Golgi and ERGIC to the ER.

By contrast, membrane lipids were for a long time consigned to a passive role until the recent findings that highlight lipid-based mechanisms coupling membrane dynamics and cell trafficking. In a recent review, Pomorski and collaborators underlined the participation of lipid flippases in membrane budding [8]. First known to maintain the asymmetric arrangement of lipids between the two leaflets of biological membranes, these enzymes now appear to participate in cell trafficking by creating a lipid mass imbalance between the two leaflets. Moreover, the enrichment in aminophospholipids that flippases can induce in the cytosolic leaflet may facilitate the recruitment of proteins such as ARF, clathrin and/or endophilin, effector proteins well recognised as critical players in the dynamic events of cell trafficking [8]. On the other hand, a role for phosphoinositol lipids in recruiting these effector proteins to the target membranes has been clearly demonstrated (for review see [9]). Increasing information also concerns the participation of phospholipases, the largest group of lipid-modifying enzymes. The implication of two distinct phospholipases D enzymes in several processes of the membrane traffic, including vesicle coat recruitment, budding from the Golgi apparatus, exocytosis and endocytosis, has been reported (for review see [10,11]). Such roles are thought to be linked to the generation of negatively charged phosphatidic acid that may participate either in the regulation of PI(4)P5-kinase required for the synthesis of PIP2 [12,13] or in the modulation of membrane curvature [14,15]. Work from our group also supports a role for phospholipase D in milk protein transport in the secretory pathway of MEC [16].

Phospholipases A2 (PLA2s), which constitute the largest class of cellular phospholipases, catalyse the hydrolysis of the sn-2 ester bond of cellular glycerophospholipids, generating lysophospholipids and free fatty acids. They fall into several classes, including the secretory PLA2 (sPLA2), the cytosolic Ca^{2+}-dependent PLA2 (cPLA2) and the intracellular Ca^{2+}-independent PLA2 (iPLA2). Historically, PLA2s have been extensively studied in the context of cellular signalisation in response to specific stimuli, via the study of the generation of signalling molecules, notably arachidonic acid [17]. Of all members of this vast family of phospholipases, only cPLA2 has been shown to play an important role in lipid messenger biosynthesis and is therefore the most relevant enzyme for intracellular signalling (for review see [18]). By contrast, iPLA2 does not seem to actively participate in the stimulation of arachidonic acid release due to an apparent lack of specificity for arachidonate-containing substrate; most probably, this family of enzymes interferes with arachidonic acid metabolism via their involvement in the lipid deacylation/reacylation cycle. Recent innovative investigations highlight the participation of iPLA2 in the formation of membrane tubules that appear to emanate from various intracellular organelles. By means of live cell imaging, Lippincott-Schwartz and colleagues have established the unexpected prevalence of tubules in the traffic between organelles of eukaryotic cells [19]. Such tubules were found between the ER and the Golgi apparatus, between the Golgi stacks, but also emanating from the TGN [20–22]. A role for iPLA2 in the generation of tubules was further supported by data recently published by de Figueiredo et al. This group demonstrated that iPLA2 inhibitors prevented Golgi membranes from tubulating in response to BFA [23] and interfered with the dynamic maintenance of the Golgi architecture [24]. It also appears that these tubules are likely to function in various trafficking pathways, including the transport of cargo proteins in the anterograde pathway of secretion [25].

To gain further insights into the potential role of iPLA2 in the anterograde protein traffic, we have studied the effect of bromoenol lactone (BEL), a highly selective iPLA2 inhibitor, on the transport of milk proteins in the secretory pathway of MEC. The potential membrane targets of the enzyme were searched for by first exploiting the fact that caseins undergo phosphorylation, a post-translational modification that occurs in the Golgi apparatus, allowing one to selectively study the ER-to-Golgi transport step. Additional information on the role of iPLA2 in this early step of the secretory pathway were obtained by conducting labelling with antibodies that label the ER-to-Golgi intermediate compartment (ERGIC) and the cis-side of the Golgi apparatus. Finally, data supporting an involvement of iPLA2 in the TGN-to-plasma membrane step mainly stemmed from electron microscopic observations.

2. Materials and methods

2.1. Main reagents

Bromoenol lactone (BEL), 2-(*p*-amylcinnamoyl)amino-4-chlorobenzoic acid (ONO-RS-082) and arachidonyl trifluoromethyl ketone ($AACOCF_3$) were supplied by Biomol Research Laboratories Inc. (Plymouth Meeting, PA, USA). BEL, ONO-RS-082 and $AACOCF_3$ were added to the incubation medium from 1000-fold concentrated stock solutions in DMSO. L-3-Phosphatidylcholine, 1-stearoyl-2-[1-^{14}C]arachidonyl (1.85 MBq/mmol) and [^{35}S]methionine/cysteine mix (>37 TBq/mmol) were purchased from Life Technologies (Les Ulis, France). Monoclonal anti-rat TGN38 (2F7.1), polyclonal anti-rat p58 and polyclonal anti-p23 peptide (CT1 p23) were kindly provided by Dr. G. Banting (Bristol, UK), Dr. J. Saraste (Bergen, Norway) and