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Oligomeric state regulated trafficking of human platelet-activating factor acetylhydrolase type-II

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ABSTRACT

The intracellular enzyme platelet-activating factor acetylhydrolase type-II (PAFAH-II) hydrolyzes platelet-activating factor and oxidatively fragmented phospholipids. PAFAH-II in its resting state is mainly cytoplasmic, and it responds to oxidative stress by becoming increasingly bound to endoplasmic reticulum and Golgi membranes. Numerous studies have indicated that this enzyme is essential for protecting cells from oxidative stress induced apoptosis. However, the regulatory mechanism of the oxidative stress response by PAFAH-II has not been fully resolved. Here, changes to the oligomeric state of human PAFAH-II were investigated as a potential regulatory mechanism toward enzyme trafficking. Native PAGE analysis *in vitro* and photon counting histogram within live cells showed that PAFAH-II is both monomeric and dimeric. A Gly-2-Ala site-directed mutation of PAFAH-II demonstrated that the N-terminal myristoyl group is required for homodimerization. Additionally, the distribution of oligomeric PAFAH-II is distinct within the cell; homodimers of PAFAH-II were localized to the cytoplasm while monomers were associated to the membranes of the endoplasmic reticulum and Golgi. We propose that the oligomeric state of PAFAH-II drives functional protein trafficking. PAFAH-II localization to the membrane is critical for substrate acquisition and effective oxidative stress protection. It is hypothesized that the balance between monomer and dimer serves as a regulatory mechanism of a PAFAH-II oxidative stress response.

1. Introduction

PAFAH-II is an intracellular enzyme expressed in a variety of cell types, including human platelets, lymphocytes, neutrophils [1], skin [2], liver and kidney cells [3,4]. PAFAH-II is a widely conserved protein, found in organisms from *Schizosaccharomyces pombe* [5] and *Caenorhabditis elegans* to higher invertebrates [6], such as mammals. As a member of the phospholipase A_2 (PLA_2) superfamily, this enzyme hydrolytically cleaves the *sn*-2 position of a phospholipid, resulting in a free fatty acid and lysophospholipid [7]. PAFAH-II, which is a Ca^{2+} independent serine hydrolase, was originally named for its ability to hydrolyze and inactivate platelet-activating factor (PAF) [3,6]. The substrate specificity of PAFAH-II also includes phospholipids structurally resembling PAF, such as oxidatively fragmented phospholipids [4,8,9] as depicted in Fig. 1.

PAFAH-II has been observed simultaneously distributed in the cytoplasm and localized to intracellular membranes [3,10,11]. Upon the addition of oxidative stress, PAFAH-II distribution becomes more heavily membrane associated [2,11,12]. Previous research in our laboratory has shown that following oxidative stress, PAFAH-II is specifically localized to the membranes of both the endoplasmic reticulum (ER) and Golgi apparatus [12]. Conversely, when anti-oxidants were added to stressed cells, the distribution of PAFAH-II returned to a pre-stressed distribution with the majority of protein being cytosolic [11].

A number of studies have confirmed the importance of this enzyme in the prevention of oxidative stress induced apoptosis [13–15]. It is believed to function by cleaving the oxidatively fragmented portion of a damaged phospholipid, thereby starting the reparative process [2–4,6,8,11,16–18]. Its substrate specificity for shortened chains at the *sn*-2 position prevents PAFAH-II from hydrolyzing intact phospholipids, making this enzyme an essential component of homeostasis [4,11]. By trafficking from the cytoplasm to the membrane, PAFAH-II can be localized to the areas containing damaged phospholipids.

Previously we constructed a PAFAH-II homology model (Fig. 2) from the plasma PAFAH crystal structure to further understand this enzyme's

Abbreviations: CPSM, counts per second per molecule; eGFP, enhanced green fluorescent protein; eGFP-pCEP4, enhanced green fluorescent protein monomer; eGFP-eGFP-pCEP4, enhanced green fluorescent protein dimer mimic; ER, endoplasmic reticulum; FFS, fluorescent fluctuation spectroscopy; G2A, glycine-2 to alanine mutant; OPM, Orientations of Proteins in Membranes; PAGE, polyacrylamide gel electrophoresis; PAF, platelet-activating factor; PAFAH-II, platelet-activating factor acetylhydrolase type II; PCH, photon counting histogram; PLA_2, phospholipase A_2; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; TBS-T, Tris buffered saline with Tween 20; WT, wild-type

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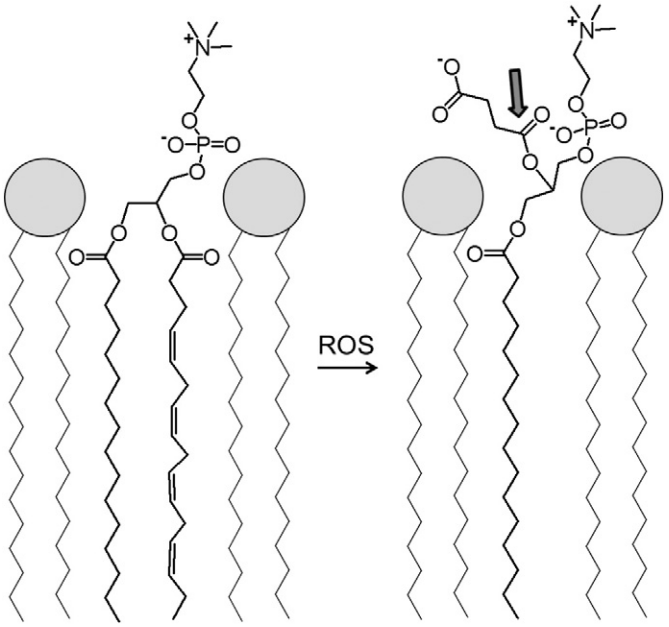

Fig. 1. Model of a phospholipid monolayer depicts an unsaturated phospholipid on the left. The unsaturated chain of this phospholipid can be oxidatively fragmented by a reactive oxygen species (ROS), as shown on the right. Shown on the right is one of the many possible "whisker" products. The shortened and more polar chain depicted from this fragmentation is shown pointing toward the aqueous phase, and the ester bond targeted for esterolysis by PAFAH-II is indicated with an arrow.

structure [12] and interactions at the membrane surface as predicted by the Orientations of Proteins in Membranes (OPM) database computational approach [19,20]. Specific PAFAH-II regions involved in membrane binding were predicted and then tested using site directed mutagenesis and localization experiments in live HEK293 cells [12]. The location of the active site above the hydrophilic–hydrophobic interface is consistent with the known substrate specificity and model of PAFAH-II targeting "whisker" acyl chains of an oxidized phospholipid [21]. The whisker acyl chain of an oxidatively fragmented phospholipid is shown in Fig. 1 projecting away from the hydrophobic portion and toward the aqueous phase. Furthermore, our previous work demonstrated the role played by both the myristoyl group and the hydrophobic patch of PAFAH-II in directing the enzyme to membrane surfaces [12].

The translocation of myristoylated proteins between the cytoplasm and membrane is regulated by a number of different mechanisms. In general, these select proteins are functionally influenced by the presence of a myristoyl group or by the oligomeric state of the protein. The role of the myristoyl group in protein oligomerization can vary. In Visinin-like protein, the myristoyl group is not directly involved in this protein–protein interaction [22]. For the protein Nef from HIV-1 the myristoyl group prevents formation of higher order oligomers [23,24]. While for some proteins, the myristoyl group is required to form oligomers, as is the case for NAP-22 [25]. Previous studies had concluded that PAFAH-II purified from bovine liver is monomeric [4]. The oligomeric state of this myristoylated protein has not been studied further, especially for its behavior in a native-like environment. Therefore it is possible that PAFAH-II exists in alternate oligomeric states and that its localization and function may be regulated by changes in this oligomerization.

In the present study we have looked more closely at the oligomeric state of PAFAH-II *in vitro* and in live human kidney cells. We followed PAFAH-II oligomerization in living cells using fluorescent fluctuation spectroscopy (FFS), and specifically, we utilized the photon counting histogram (PCH) method. PCH data is collected by monitoring the fluorescence counts of molecules as they move in and out of a specified observation volume [26]. Data for a specific amount of time is compiled as a histogram of photon counts and their frequency. The resulting

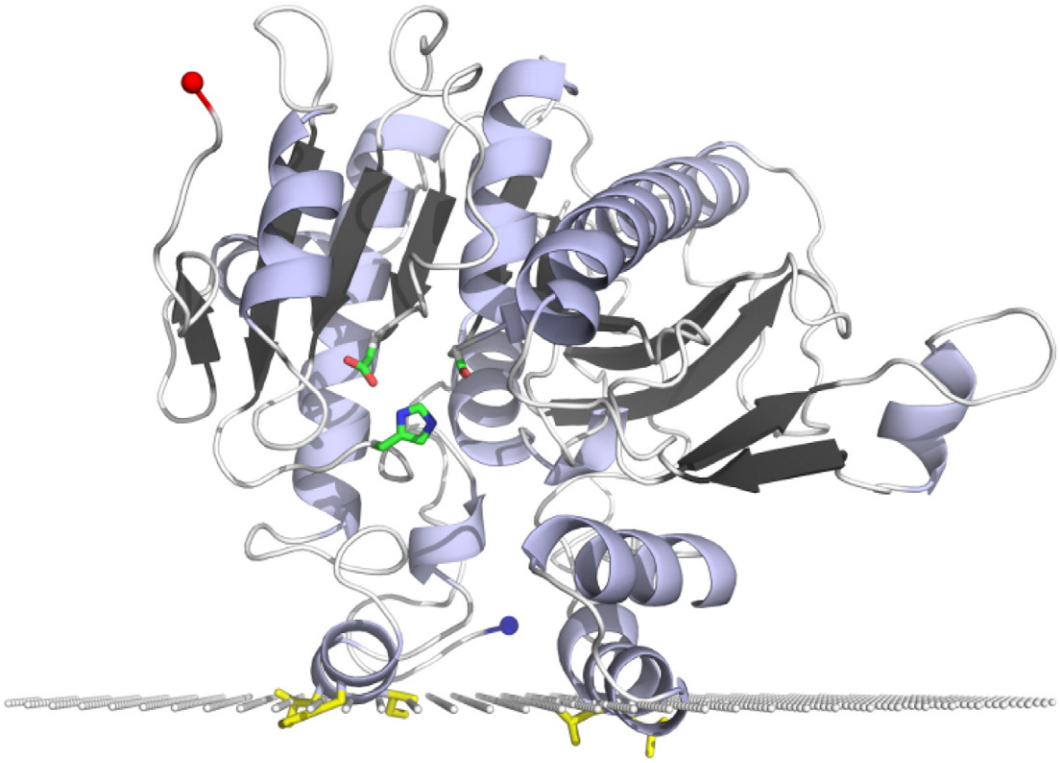

Fig. 2. The PAFAH-II homology model is shown with the active site Ser, His, Asp catalytic triad in green, hydrophobic patch residues (L76, L79, L327, I328, and F331) in yellow, C-terminus depicted with a red sphere and N-terminus (location of myristoyl group) depicted with a blue sphere [12]. The plane of white spheres represents the hydrophilic–hydrophobic interface of the enzyme's predicted membrane binding interface [19].