Minireview

Oxidative signaling pathway for externalization of plasma membrane phosphatidylserine during apoptosis

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Abstract Active maintenance of membrane phospholipid asymmetry is universal in normal cell membranes and its disruption with subsequent externalization of phosphatidylserine is a hallmark of apoptosis. Externalized phosphatidylserine appears to serve as an important signal for targeting recognition and elimination of apoptotic cells by macrophages, however, the molecular mechanisms responsible for phosphatidylserine translocation during apoptosis remain unresolved. Studies have focused on the function of aminophospholipid translocase and phospholipid scramblase as mediators of this process. Here we present evidence that unique oxidative events, represented by selective oxidation of phosphatidylserine, occur during apoptosis that could promote phosphatidylserine externalization. We speculate that selective phosphatidylserine oxidation could affect phosphatidylserine recognition by aminophospholipid translocase and/or directly result in enzyme inhibition. The potential interactions between the anionic phospholipid phosphatidylserine and the redox-active cationic protein effector of apoptosis, cytochrome *c*, are presented as a potential mechanism to account for selective oxidation of phosphatidylserine during apoptosis. Thus, cytochrome *c*-mediated phosphatidylserine oxidation may represent an important component of the apoptotic pathway.

1. Introduction

The 'raison d'etre' for the plethora of different molecular species of phospholipids in membranes is not clearly understood at the molecular level. Neither are cellular functions of individual phospholipid species well described (with perhaps the notable exceptions of phosphoinositol-derived signaling products and precursors of bioactive eicosanoids). Despite that, asymmetric distribution of major phospholipid classes across membranes has been established as a fundamental feature of all cells whose disturbance is incompatible with physiological functions of membranes and with cell viability [1,2]. Although membrane lipid asymmetry has been known for many years, the mechanisms for maintaining or regulating the transbilayer lipid distribution are still not completely understood. Recently, three major enzymatic pathways involved in phospholipid transbilayer asymmetry have been identified: (1) an inward-directed pump, an ATP-dependent transporter specific for aminophospholipids (phosphatidylserine (PS) and phosphatidylethanolamine (PE)), known as aminophospholipid translocase (APT) or 'flippase'; (2) a phospholipid scramblase, which facilitates bi-directional migration of all phospholipid classes independent of the polar headgroup across the bilayer; and (3) an outward-directed pump referred to as 'floppase' with little selectivity for the polar headgroup of the phospholipid [3]. The actual participation of the latter in transport of phospholipids has not been firmly established.

The concerted action of APT and 'floppase' is believed to account for the maintenance of lipid asymmetry in intact cells [3]. The surveillance function of APT provides for the rapid inward translocation of aminophospholipids, while 'floppase' may facilitate the transbilayer movement of phospholipids to replace aminophospholipids. APT inhibition by itself, however, does not lead to spontaneous redistribution of lipids [4,5]. Conditions of cellular activation characterized by elevated levels of intracellular Ca^{2+} can cause collapse of lipid asymmetry by activation of an ATP-independent scramblase and subsequent bi-directional movement of all phospholipid classes [6]. Thus, both inhibition of the APT and activation of the scramblase are necessary for a collapse of lipid asymmetry, manifested by exposure of PS on the cell surface [3]. The latter phenomenon, known as PS externalization, has been identified as one of the early and prominent features of programmed cell death or apoptosis [7,8]. PS exposure on the outer leaflet of the plasma membrane is a surface change common to many apoptotic cells and has several potential biological consequences, one of which is recognition and removal of the apoptotic cell by phagocytes [9]. It is still not

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Abbreviations: PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PnA, *cis*-parinaric acid; APT, aminophospholipid translocase; PMC, 2,2,5,7,8-pentamethyl-6-hydroxy-chromane; AMVN, 2,2′-azobis(2,4-dimethylisovaleronitrile); t-BuOOH, *tert*-butylhydroperoxide

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clear which receptors mediate PS recognition on apoptotic cells; however, several candidates have been proposed. These include the class B scavenger and thrombospondin receptor (CD36), an oxidized LDL receptor (CD68), CD14, annexins, β_2 glycoprotein I, gas-6, and a novel protein expressed on macrophages stimulated with digestible particles such as β-glucan. Whether PS is the sole ligand recognized by phagocytes or whether it is associated with other molecules to form a complex ligand is unknown [10].

If inhibition of APT (along with the activation of scramblase) is critical for aminophospholipid externalization, then one would expect that both PS and PE should be exposed on the outer surface of the plasma membrane in apoptotic cells. Numerous data, however, indicate that PS, rather than PE, is predominantly externalized on plasma membranes of apoptotic cells. To some extent, this conclusion may be based on the specificity of the assay for PS externalization. A negatively charged PS can specifically bind fluorescently labeled annexin V, the most commonly used reagent for flow cytometric measurements of apoptosis [11,12]. In other words, the annexin V binding assay ignores any potential contribution of PE to aminophospholipid externalization. High-performance thin-layer chromatography (HP-TLC) assays of aminophospholipids chemically labeled on the cell surface by non-permeating reagents, however, demonstrated that the degree of PS externalization far exceeds that of PE during apoptosis in different cells [9,13,14]. This suggests that some, as yet unidentified, factor(s) may be responsible for predominant translocation of PS in plasma membranes of apoptotic cells.

2. Measurements of peroxidation in different classes of phospholipids in live cells

Oxidative stress is a frequent trigger of apoptosis in a variety of cells and is also thought to be involved as a component of the common pathway in execution of apoptosis [15–21]. While effects of oxidative stress on apoptotic machinery, such as caspases [22,23], have been well characterized, the information on selective oxidation of specific classes of phospholipids is scarce. This is mainly due to the fact that quantitative assays for oxidation of different classes of phospholipids are not readily available. One of the major reasons for this is a very effective system of remodeling and repair of oxidatively modified phospholipids [24] that interferes with their accurate measurement.

In our attempts to characterize phospholipid oxidation during oxidative stress-induced apoptosis, we metabolically labeled cellular phospholipids with a natural oxidation-sensitive and highly fluorescent fatty acid, *cis*-parinaric acid (PnA). This reagent has been extensively used in its free (non-esterified) form for structural measurements in membranes as well as for assays of oxidative stress in simple model systems [25,26]. We recently developed and optimized conditions that yielded cells containing the major phospholipid classes (phosphatidylcholine (PC), PE, PS, phosphatidylinositol (PI), diphosphatidylglycerol and sphingomyelin) fluorescently labeled with PnA and an extremely low intracellular concentration of free PnA [27]. The level of PnA labeling of endogenous phospholipids ($\approx$1–3 mol%) was low enough to have minimal effects on cell viability and functions yet sufficient to permit quantitative detection of oxidative stress [27]. Since free PnA was not available for phospholipid repair resolution

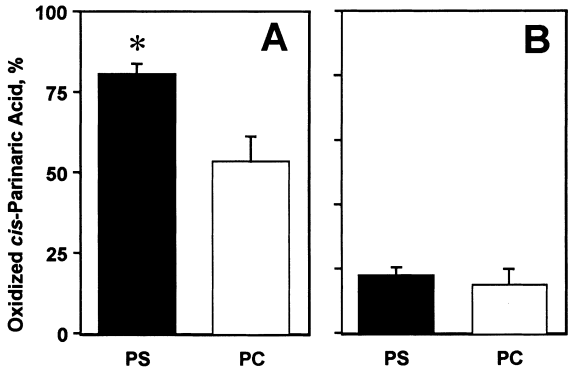


Fig. 1. Cumene hydroperoxide-induced oxidation of PnA-labeled PS and PC in intact live normal human epidermal keratinocytes (NHEK) (A) and cell-free PnA-labeled liposomes derived from NHEK. PnA was incorporated into normal human epidermal keratinocyte phospholipids as described earlier [27]. Intact living PnA-labeled NHEK (A) were exposed to cumene hydroperoxide (200 μM) for 1 h at 37°C. PnA-labeled liposomes (B) were prepared from PnA-loaded NHEK as described earlier [38] and similarly treated with 200 μM cumene hydroperoxide at 37°C for 1 h. At the end of the incubations, total lipids were extracted by the Folch procedure and resolved by HPLC [27]. PS, phosphatidylserine; PC, phosphatidylcholine. Data represent means ± S.E.M., $n = 3$, $^*P < 0.02$.

of major phospholipid classes by fluorescence high-performance liquid chromatography (HPLC) can be used to quantify their oxidative damage (as a decreased content of fluorescent PnA residues in respective phospholipid classes). Importantly, the PnA-based assay can identify the selectivity of phospholipid oxidation based on their polar head groups and it is obviously independent of the fatty acid composition of phospholipids [27,28]. In addition, the proximity of PnA-labeled phospholipids to the sites of radical generation will also determine the degree of oxidation.

3. Non-random oxidation of different classes of phospholipids during oxidative stress-induced apoptosis

We have found that apoptosis is associated with selective oxidation of specific phospholipid classes, most notably PS. For example, paraquat, a known inducer of oxidative stress via CYP_{450} reductase-mediated redox-cycling and subsequent production of reactive oxygen species, caused apoptosis in 32D cells as evidenced by characteristic changes in nuclear morphology, DNA fragmentation, and PS externalization revealed by annexin V binding. When 32D cells containing PnA-labeled phospholipids were exposed to paraquat, only two classes of phospholipids, PS and PI, underwent significant peroxidation during a 2 h incubation [13]. Importantly, neither apoptosis nor oxidation of PS and PI was induced by paraquat in 32D cells overexpressing the bcl-2 gene product. These initial experiments posed several questions: (i) whether selective oxidation of PS is typical of apoptosis induced by other agents, (ii) what is the major intracellular compartment where PS is oxidatively modified during apoptosis, (iii) whether PS oxidation is an early or late event in executive pathways of apoptosis, (iv) whether PS oxidation is required for PS externalization (and other apoptotic mechanisms), (v) what may act as an endogenous catalyst for selective PS oxidation during apoptosis?

4. Oxidation of PS is selective and precedes its externalization in cells during apoptosis

Our initial experiments with paraquat-treated 32D cells clearly showed that PS oxidation preceded PS externalization as measured by annexin V binding and DNA fragmentation [13]. Similarly, in keratinocytes exposed to cumene hydroperoxide, selective PS oxidation occurred prior to its detection on the cell surface by either non-permeant amino-reagents or annexin V and in the absence of DNA laddering [29]. Fig. 1 compares the oxidation of PnA-labeled PS and PC in intact cells (Fig. 1A) and in keratinocyte-derived liposomes (Fig. 1B) treated with cumene hydroperoxide. Note the preferential oxidation of PS is observed only in intact living cells and not in the cell-free liposome preparation. In several other cell types such as human leukemia HL-60 cells [18], pheochromocytoma PC12 cells [30], rat cardiomyocytes [31], selective oxidation of PS preceded or coincided with the appearance of very early biomarkers of the apoptotic execution pathway, caspase 3 activation and PS externalization. A summary of these and other studies is presented in Table 1.

The fundamental association of PS oxidation with apoptosis was strengthened by experiments in which we used a vitamin E homologue, 2,2,5,7,8-pentamethyl-6-hydroxy-chromane (PMC). Here we employed the lipophilic azo-initiator of radicals, 2,2′-azobis(2,4-dimethylisovaleronitrile) (AMVN), to generate membrane-confined oxidative stress and induce apoptosis in HL-60 cells [18]. As an effective radical scavenger, PMC was able to completely protect all phospholipids against oxidation with the remarkable exception of PS. Furthermore, PMC failed to protect HL-60 cells against apoptosis following AMVN (Table 1). An exclusive resistance of PS to antioxidant protection by PMC implies a unique molecular mechanism responsible for its oxidation during apoptosis.

5. Oxidation of PS is predominantly confined to plasma membrane during oxidative stress-induced apoptosis

The temporal sequence of PS oxidation and externalization is compatible with a casual link between these two events. If so, PS oxidation should occur within the plasma membrane where PS translocation events during apoptosis are known to occur. We performed subcellular fractionation experiments in PnA-labeled cells challenged with *tert*-butylhydroperoxide (t-BuOOH). We recently documented that t-BuOOH induced apoptosis and prominent PS oxidation in whole cell lipid extracts. Most importantly, we found that plasma membrane PS was the largest source of oxidized PS compared to other organelles such as mitochondria, microsomes, nuclei and lysosomes [32]. In these cells, we also established that apoptosis resulted in a selective externalization of PS as compared to PE. Thus, the time course and the intracellular location of PS oxidation support our hypothesis regarding the mechanistic link between PS oxidation and subsequent externalization.

6. The hypothetical role of PS oxidation in its externalization

There are several potential pathways through which PS oxidation may mediate its externalization. As has been pointed out, maintenance of PS asymmetry is mainly due to the activity of APT. Inhibition of APT and activation of phospholipid scramblase are required for apoptotic PS externalization (see above). Therefore, it is of interest to explore the relationships between PS oxidation and externalization and these enzymatic activities. APT has been reported to be sensitive to oxidative stress [4,41,42]. In particular, oxidation of APT cysteines is a target for reactive oxygen species resulting in amelioration of the enzyme activity. While scramblase is not activated directly by oxidants, influx and elevations of cytosolic Ca^{2+} (that could indirectly follow oxidant challenge [43,44]) are responsible for enzyme activation during apoptosis [45–47]. The failure of APT to internalize PS may be caused by either direct enzyme inhibition or the enzyme's inability to recognize oxidized PS. In the latter case, oxidized PS has to be located in the outer leaflet of the plasma membrane. It is noteworthy that oxidized phospholipids undergo spontaneous 'flip–flop' more readily than their non-oxidized counterparts [48]. Thus, the requirement of having oxidized PS on the cell surface does not seem to be unrealistic. In our experiments, however, direct inhibition of APT, as measured by 7-nitro-

Table 1
Comparison of PS oxidation, PS externalization and apoptosis in various cells exposed to oxidants

Cell line	Stimuli	Apoptosis	PS externalization	PS oxidation	References
HL-60	AMVN	+	+	+	[18]
HL-60	AMVN+PMC	+	+	+	[18]
HL-60	AMVN+NO*	+	+	−	[14]
HL-60	t-BuOOH	+	+	+	[32]
HL-60	H_2O_2	+	N.D.	+	[33]
HL-60	Cu-NTA+NO	+	+	+	Liu et al. (unpublished)
HL-60	Cu-NTA	+	+	+	[34], Kawai et al. (unpublished)
32D	Paraquat	+	+	+	[13]
32D/bcl-2	Paraquat	−	−	−	[13]
PC12	Neocarzinostatin	+	+	+	[30]
PC12/bcl-2	Neocarzinostatin	−	−	−	[30]
PC12	AMVN	+	N.D.	+	[35]
PC12/bcl-2	AMVN	−	N.D.	+	[35]
PC12	Glutamate	−	N.D.	−	[36]
MCF-7	AMVN	+	N.D.	+	[37]
NHEK	Phenol	−	N.D.	−	[38]
NHEK	Hydroperoxide	+	+	+	[29]
RCm	Hydroperoxide	+	N.D.	+	[31,39,40]

HL-60 = human leukemia cells, 32D = mouse hematopoietic progenitor cells, PC12 = rat pheochromocytoma cells, NHEK = normal human epidermal keratinocytes, RCm = rat cardiomyocytes. N.D. signifies not done. Asterisk denotes that APT activity was also inhibited by both AMVN and NO.

2,1,3-benzoxadiazol-4-yl-PS internalization, occurred at the same time as PS oxidation [14]. This implies that enzyme inhibition itself can play a role in PS externalization. The activity of the enzyme towards oxidized PS is not known and further studies are necessary to determine the relative affinity of APT for oxidized and non-oxidized PS, as well as the nature of the reactive species responsible for enzyme modification.

It should also be mentioned that PS oxidation may be sufficient but not necessary for APT inhibition and PS externalization during apoptosis. Indeed, we have observed that AMVN-induced apoptosis and PS externalization in HL-60 cells occurred even after exposure to nitric oxide, which completely blocked oxidation of all membrane phospholipids, including PS [14]. APT cysteines, however, are potential targets for S-nitrosylation and enzyme inhibition by NO [49,50]. In fact, we observed a decreased content of SH-groups in the molecular weight region corresponding to APT (115 kDa) on SDS–PAGE gels of HL-60 proteins stained by a fluorescent maleimide thiol reagent [14]. It should also be noted that direct caspase-driven inactivation of APT is not likely. Using different inhibitors of mitochondrial electron transport, Zhuang et al. could dissociate PS externalization from caspase activation in THP-1 cells [51]. Similarly, Fadeel et al. showed that oxidants generated via NADPH oxidase were essential for PS exposure during neutrophil apoptosis despite their potential to inhibit caspase activity [52]. Recent studies demonstrated that overexpression of scramblase in Raji cells, which exhibit low constitutive expression of this enzyme, by retroviral transduction of phospholipid scramblase or treatment of the cells with interferon-α, failed to confer the capacity to expose PS in response to apoptotic stimuli [53]. However, PS exposure in cells overexpressing scramblase could be reconstituted only in the presence of thiol reactive agents, such as *N*-ethylmaleimide, disulfiram and diamide [53]. Thus, oxidative and/or nitrosative modification of APT is likely to be critical for PS externalization and subsequent recognition of apoptotic cells by phagocytes. This poses the question as to what potential molecular mechanisms are responsible for PS oxidation/externalization.

7. Cytosolic cytochrome *c* as a catalyst for plasma membrane PS peroxidation during apoptosis

It is generally accepted that release of cytochrome *c* from mitochondria is one of the very early events in the development of an apoptotic program that is a prerequisite for subsequent engagement of caspase cascades and PS externalization [54–56] (although some workers reported that PS exposure during apoptosis preceded release of cytochrome *c* and decrease in mitochondrial transmembrane potential [57]). Using green fluorescent protein (GFP)-tagged cytochrome *c*, Goldstein et al. [58] found that the release of cytochrome *c*-GFP always preceded exposure of PS and the loss of plasma membrane integrity, characteristics of apoptotic cells. Moreover, p53 activates the apoptotic machinery through induction of the release of cytochrome *c* from the mitochondrial intermembrane space [58].

The role of cytochrome *c* in the initiation of caspase cascades is well established and is presumed to be independent of its redox activity [54–56,59,60]. In fact, cytochrome *c* containing Zn or Cu in place of Fe is sufficient for interactions within

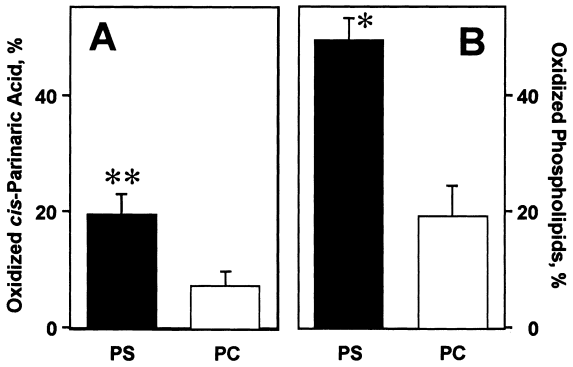
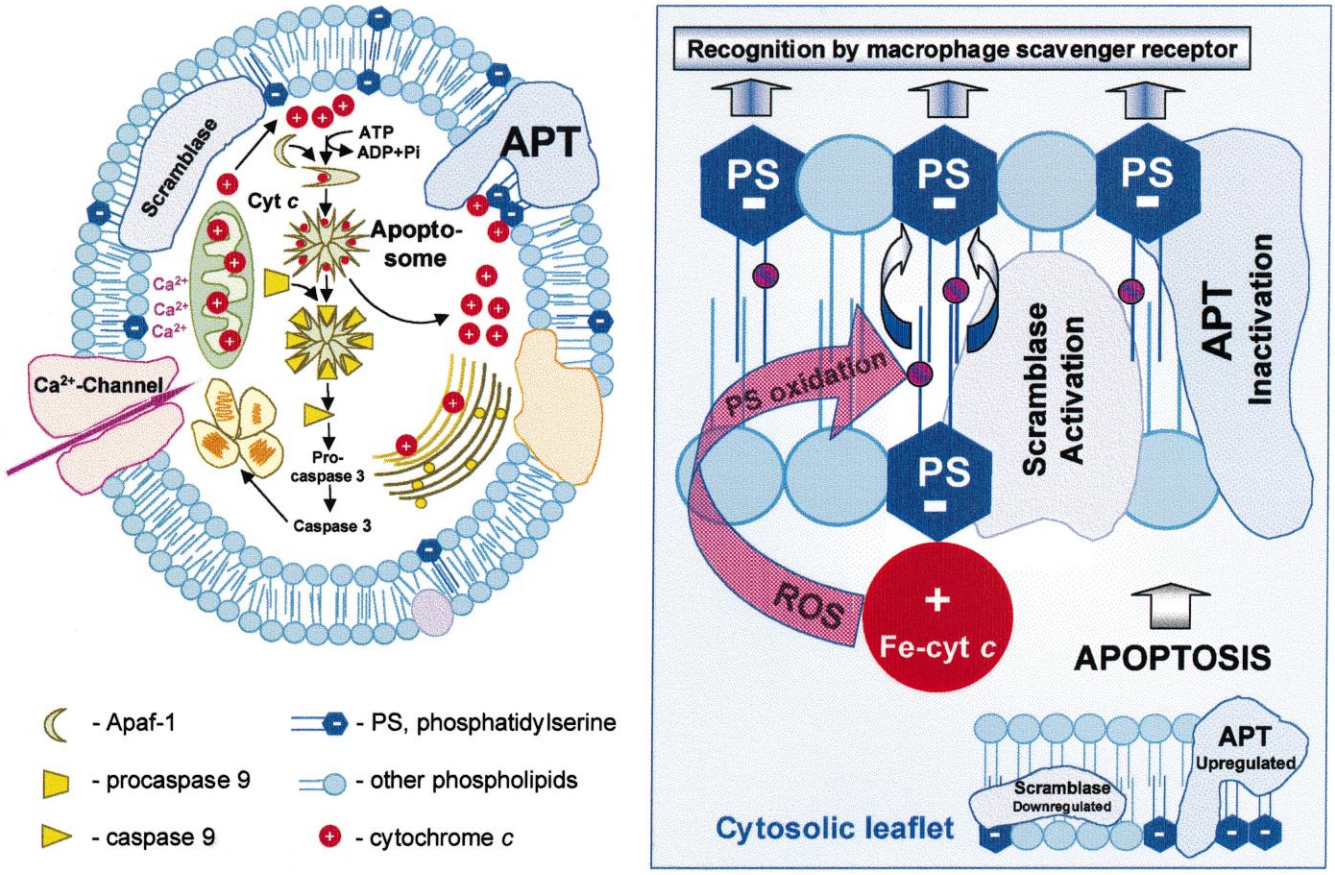


Fig. 2. Cytochrome *c*-induced oxidation of PS and PC in intact living HL-60 cells (A) and liposomes (B). PnA was incorporated into HL-60 cell phospholipids as previously described [27] and then incubated in the presence of cytochrome *c* for 20 min at 37°C (A). At the end of this time, total lipids were extracted by the Folch procedure and resolved by HPLC [27], and analyzed for fluorescent content of PnA. Cytochrome *c* was incorporated into PnA-labeled cells by mild sonication. The amount of incorporated cytochrome *c* was determined spectrophotometrically and estimated as 0.44 ± 0.03 nmol/mg protein. PS, phosphatidylserine; PC, phosphatidylcholine. Data are means ± S.E.M., $n = 14$, $^{**}P < 0.04$. Multilamellar dispersions of 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine (PC) or 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phospho-L-serine (PS) (2 mg/ml) in 50 mM phosphate buffer pH 7.4 were incubated in the presence of cytochrome *c* (5 μM), ascorbate (500 μM), H_2O_2 (400 μM), desferroxamine (100 μM) during 1 h at 37°C (B). At the end of incubation, phospholipids were extracted and HP-TLC was performed [27]. Oxidized phospholipid products could be resolved as a distinct 'tailing' following the native unoxidized lipid spot. The percent of oxidation was estimated by determining the phosphorus content of the tail relative to the total lipid phosphorus available. Data are means ± S.E.M., $n = 3$, $^{*}P < 0.02$.

procaspase protein complex and subsequent caspase activation [61]. However, the redox repercusions of cytochrome *c* release from mitochondria into cytosol cannot be ignored. Recently, Cai and Jones [21] demonstrated that departure of cytochrome *c* from electron transport chains in mitochondria is accompanied by a dramatic increase in production of reactive oxygen species. This is in keeping with the antioxidant role for cytochrome *c* during electron transport demonstrated by Skulachev [62,63]. It is well known, however, that antioxidants may readily be subverted to prooxidants when highly orchestrated redox conditions in the environment are dysregulated, e.g., during apoptosis. For example, one of the major water-soluble antioxidants, vitamin C (ascorbate), is routinely used for induction of oxidative stress in conjunction with free transition metals (such as Cu or Fe) or with hemoproteins (such as hemoglobin, cytochrome *c*) [44]. Indeed, cytochrome c^{2+} is approximately 20 times more effective at catalyzing hydroxyl radical production from H_2O_2 than is free iron [64].

A series of papers have recently described the molecular nature of the apoptosome, the multimeric molecular complex of Apaf-1/cytochrome *c*/procaspase 9 [65–67]. Cytochrome *c* appears necessary for the initial binding to Apaf-1, which in the presence of ATP/dATP assembles into a multimeric complex. Once this multimeric complex is formed, the recruitment and cleavage of caspase 3 appear independent of cytochrome *c* or ATP hydrolysis, however, some cytochrome *c* can still be found associated with the apoptosome and could serve to further stabilize this structure. The affinity of Apaf-1 for cytochrome *c* is extremely high ($\approx 10^{11}$ M^{-1}) [68] implying that



Scheme 1. Proposed mechanism for oxidative signaling of PS externalization during apoptosis. The left side of the scheme illustrates the common pathways of apoptotic program via mitochondrial permeability transition, cytochrome *c* release, apoptosome formation and caspase activation. This leads to a number of apoptotic endpoints including nuclear fragmentation and PS externalization. The right side details our proposed model by which redox-active and positively charged cytochrome *c* interacts with negatively charged PS on the cytosolic side of the plasma membrane. Cytochrome *c* catalyzed reactive oxygen species attack PS to form its hydroperoxide. Oxidatively modified PS then undergoes spontaneous and/or scramblase-assisted externalization. The surveillance function of APT then is disrupted either by direct inhibition of enzyme activity by reactive electrophilic PS oxidation products or failure to recognize the oxidatively modified PS. Recognition and phagocytosis of apoptotic cells is then facilitated by interaction of PS with the macrophage scavenger receptor, which may preferentially bind oxidized PS.

only a few molecules of cytochrome *c* per cell are necessary for apoptotic execution, especially in light of the amplifying function of the apoptosome. Yet, cytochrome *c* is fairly abundant and presumably released into the cytosol in excess of that needed for Apaf–caspase interaction. In addition, it may not be required for apoptosome function once formed. Therefore, cytochrome *c* is potentially available for interactions with other molecular targets. It should be kept in mind that cytochrome *c* is a basic protein (p*I* 10.3) [69]. As a result of this, it would more readily interact with negatively charged molecules. Outside of mitochondria, PS is one of the two (along with PI) negatively charged phospholipids. Physiological localization of PS to the inner leaflet of plasma membranes renders it particularly suitable for interaction with cationic cytochrome *c* in the cytosol. In fact, apocytochrome *c*/PS interactions have been implicated in the mitochondrial import of cytochrome *c* [70,71]. Moreover, apocytochrome *c* has been shown to have a very high affinity for PS-containing vesicles (dissociation constant K_d less than 1 μM) [72,73]. The initial electrostatic interaction of apocytochrome *c* with PS is followed by penetration of the protein in between the acyl chains [74]. ^{31}P nuclear magnetic resonance studies demonstrated that conformational changes occur in the protein on binding to PS [75]. Numerous studies [76–78] demonstrated the potential of cytochrome *c* to catalyze peroxidation of different phospholipids in both liposomes and membranes fragments. Combined, these data suggest that cytosolic cytochrome *c* may be involved in specific interaction with PS located in the cytosolic leaflet of plasma membrane and be responsible for selective PS oxidation during apoptosis. In fact, incorporation of cytochrome *c* into PnA-labeled HL-60 cells by gentle sonication resulted in preferential oxidation of PS as compared to other phospholipids [32]. Furthermore, in cell-free model systems, PS proved to be selectively oxidized by a cytochrome *c*/ascorbate/H_2O_2 catalytic system as compared to PC [32]. Fig. 2 shows that PS is preferentially oxidized relative to PC when intact HL-60 cells were loaded with cytochrome *c* (Fig. 2A) and when multilamellar dispersions of PS or PC were incubated with cytochrome *c*/ascorbate/H_2O_2 (Fig. 2B).

8. Concluding remarks

Since APT inhibition is a prerequisite for PS externalization during apoptosis, it is important to understand the mechanisms by which the inhibition occurs. We speculate that PS

oxidation acts as a sufficient (if not necessary) component of apoptotic machinery ultimately contributing to PS externalization. Our proposed model for the lipid oxidation signaling pathway in apoptosis is shown on Scheme 1. A variety of apoptotic stimuli cause mitochondrial permeability transition and release of cytochrome *c* from mitochondria into cytosol. While a fraction of cytochrome *c* is transiently involved in interactions with Apaf-1 within the apoptosomal complex to initiate the caspase cascade, non-bound cytochrome *c* or that released from the apoptosome is free to interact with other membrane and cytosolic targets. Among those, the electrostatic interaction of cationic cytochrome *c* with anionic phospholipids, such as PS, would be predicted. The extreme juxtaposition of PS fatty acid residues with a redox-active heme-site would favor selective oxidation of PS as opposed to other phospholipids. Oxidized PS can then be externalized spontaneously and/or enzymatically (via scramblase-assisted mechanism). Oxidized PS on the cell surface may be recognized as a signal by macrophage scavenger receptor and serve to direct phagocytosis of apoptotic cells. This may only happen if oxidized PS, in contrast to non-oxidized PS, is not recognized by APT. Alternatively, oxidized PS could be utilized by APT as a substrate but the electrophilicity and high reactivity of the hydroperoxy group on oxidized PS make it a potential suicidal substrate resulting in enzyme inactivation. Thus, cytochrome *c*-catalyzed oxidation of PS may represent an important component of the final common pathway associated with signaling and recognition of apoptotic cells and their safe elimination by professional phagocytes.

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