

# Lantibiotics as probes for phosphatidylethanolamine

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**Abstract** Phosphatidylethanolamine (PE) is a major component in the mammalian plasma membrane. It is present mainly in the inner leaflet of the membrane bilayer in a viable, typical mammalian cell. However, accumulating evidence indicates that a number of biological events involve PE externalization. For instance, PE is concentrated at the surface of cleavage furrow between mitotic daughter cells and is correlated with the dynamics of contractile ring. In apoptotic cells, PE is exposed to the cell surface, thus providing a molecular marker for detection. In addition, PE is a cofactor in the anticoagulant mechanism, and a distinct distribution profile of PE has been documented at the blood–endothelium interface. These recent discoveries were made possible using PE-specific probes derived from duramycin and cinnamycin, which are members of type B lantibiotics. This review provides an account on the features of these PE-specific lantibiotics in the context of molecular probes for the characterization of PE on a cellular and tissue level. According to the existing data, PE is likely a versatile chemical species that plays a role in the regulation of defined biological and physiological activities. The utilities of lantibiotic-based molecular probes will help accelerate the characterization of PE as an abundant, yet elusive membrane component.

**Keywords** Duramycin · Cinnamycin · Phosphatidylethanolamine · Anticoagulant · Apoptosis · Cytokinesis · Imaging probe

## Overview

A ubiquitous phospholipid in mammalian cellular membranes, PE is among the highly abundant molecules in the biological system (Rouser et al. 1971; Spector and Yorek 1985). In a viable, typical mammalian cell, PE is predominantly a constituent in the inner leaflet of the plasma membrane. Emerging evidence indicates that, besides being a structural element, PE is translocated or redistributed across the membrane bilayer in a number of distinct biological events: (1) PE is externalized transiently at the cleavage furrow in dividing cells. (2) In apoptotic cells, where the membrane asymmetry is compromised, PE is exposed to the extracellular milieu and provides a molecular marker for the detection of cell death. (3) PE is a critical cofactor in the protein C anticoagulant mechanism, and recent evidence indicates that PE is present at the luminal surface of endothelial cells. Given the versatility in its biological roles, PE as a chemical species constitutes a distinct molecular target among aminophospholipids and other membrane phospholipid components. It is critical that PE-selective probes are available for characterizing this phospholipid in its natural settings. To this end, molecular probes derived from the lantibiotics, duramycin (PA48009, MW = 2,013 Da) and cinnamycin (Ro 09-0198, MW = 2,041 Da), bind specifically to PE with relatively high affinity, and provide an important means in the investigation of PE distribution in target cells and tissues. These findings, in turn, will help better unveil the biological functions of PE in the living systems.

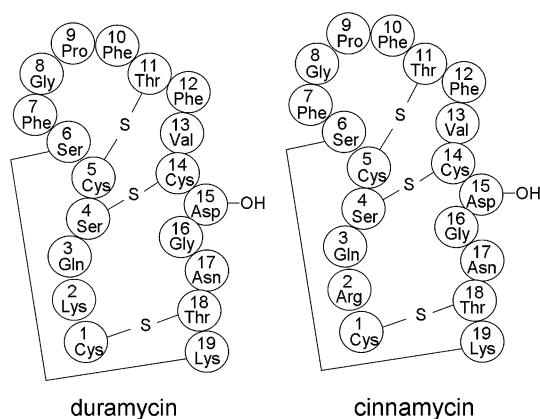
## Biosynthesis and structural features

Bacteriocins duramycin and cinnamycin are members of type B lantibiotics produced by streptomyces (Sahl and

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Bierbaum 1998). These peptides are synthesized as pre-peptides in the ribosome and extensively modified post-translationally by enzymes encoded in gene clusters. Among documented structural genes of lantibiotics, the full-length cinnamycin gene encodes for 77 amino acids, of which the N-terminal 58 residues is the leader sequence (Kaletta et al. 1991). The lantibiotic gene clusters encode for multiple enzymes, where the consortium of these functional factors enables the streamlined posttranslational modifications of the lantibiotic product (Sahl and Bierbaum 1998; Widdick et al. 2003). The enzymatic reactions include the formation of lanthionines, the ATP-dependent export of lantibiotics, and a peptidase that cleaves the leader peptide from the finished product. The cloning and characterization of the lantibiotic gene clusters make it possible to manipulate the structural compositions and to synthesize recombinant libraries for pharmacological screening/selection processes (Pag and Sahl 2002; Widdick et al. 2003).

The primary structures of duramycin and cinnamycin are shown in Fig. 1. Differing by a single amino acid at position 2 (Lys-2 for duramycin and Arg-2 for cinnamycin), the two peptides are closely related, and share a high degree of sequence and structural homology. One of the most striking features in type B lantibiotics is the extent of internal crosslinking and the number of uncommon amino acids derived from posttranslational modifications (Sahl and Bierbaum 1998; Widdick et al. 2003). The term lantibiotic refers to “lanthionine-containing antibiotic peptides” for the presence of lanthionines and methyllanthionines. During the synthesis of cinnamycin, and likely duramycin, the formation of lanthionines involves the selective dehydration of Ser-3, Ser-6, Thr-11, and Thr-18 (Kaletta et al. 1991). The dehydrated intermediates for Ser and Thr are  $\alpha,\beta$ -unsaturated amino acids didehydroalanine



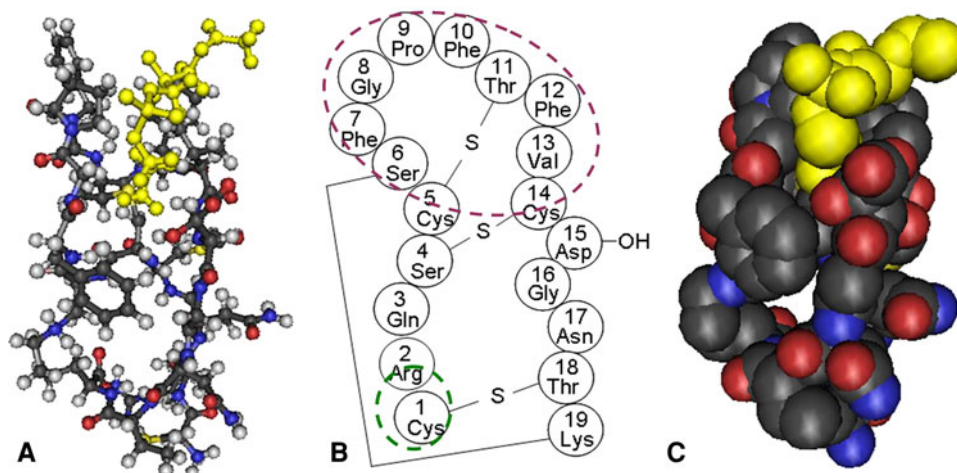
**Fig. 1** Primary structures for duramycin and cinnamycin. The two lantibiotics differ by a single amino acid at position 2. Extensive intramolecular bridges include one lanthionine, two methyllanthionines and one lysinoalanine

and didehydrobutyryne, respectively. Subsequently, nucleophilic addition from the Cys side chain to didehydroalanine forms the lanthionine, and to didehydrobutyryne results in methyllanthionine. The reaction between the C-terminal Lys and the didehydroalanine at residue-6 forms lysinoalanine. In all, the peptide backbones of duramycin and cinnamycin are each stabilized by four covalent bridges, including one lanthionine, two methyllanthionines and one lysinoalanine. Another uncommon amino acid is a  $\beta$ -hydroxyaspartic acid located at Asp-15. With extensive intramolecular crosslinking, these lantibiotics form rigid, stable structures, and are among the smallest known polypeptides that have a defined three-dimensional-binding site.

### Binding to PE

Duramycin and cinnamycin bind the head group of PE with high affinity at a molar ratio of 1:1, with the dissociation constant toward PE-containing lipid membranes in the low nanomolar range (Hayashi et al. 1990; Seelig 2004; Zimmermann et al. 1993). The PE-binding site contains lipophilic side chains from Phe-7 to Cys-14, which are all conserved between duramycin and cinnamycin. The site resembles a hydrophobic pocket-shaped surface that fits around the PE headgroup with defined physicochemical interactions (Fig. 2). The binding of the ethanolamine headgroup is stabilized by an ionic interaction pair between the ammonium group of PE and the carboxylate of Asp-15. This overall tight fitting with the ethanolamine headgroup confers thermodynamic stability and specificity for PE. The binding interactions in the binding pocket are in such a way that no structures other than that of a glycerophosphoethanolamine head group are able to fit the binding pocket (Hosoda et al. 1996). This binding specificity is substantiated by experimental data where only PE-containing liposomes are able to competitively diminish the target binding of lantibiotic probes, and none of the closely related phospholipid species is effective (Zhao et al. 2008). In addition, the hydrophobic side chains protruding from the peptide are likely to stabilize the peptide-membrane interactions by anchoring toward the hydrophobic core region of the membrane bilayer. The preference for membrane-bound PE, as opposed to soluble phosphoethanolamine analogs, is reflected in the requirement for at least one alkyl chain. The binding is augmented by longer alkyl chains with up to eight-carbon long, suggesting that the hydrophobic interactions between the peptide and the hydrophobic region of the membrane bilayer may be limited to the superficial-to-intermediate depth beyond the headgroup and the glycerol backbone (Machaidze and Seelig 2003).

**Fig. 2** The binding interactions between cinnamycin and a molecule of lyso-PE. The PE head group is embedded in the binding pocket of cinnamycin. The amino acid residues that contribute to the PE-binding site are mainly between Ser-4 and Asp-15. The N-terminal, which is situated away from the PE-binding pocket, provides a convenient location for site-directed conjugation



### Lantibiotics as molecular probes

A number of structural features of these lantibiotics contribute favorably to their being molecular probe candidates. (1) The peptides bind PE with high affinity and, importantly, high specificity. This feature makes it possible to examine the membrane localization of PE in the presence of other closely related phospholipid species, including phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylglycerol (PG) and phosphatidylinositides (PIs). (2) At  $\sim 2$  kDa, the relatively low molecular weight of PE-specific lantibiotics is more suitable for glomerular filtration, and is likely to translate into a faster clearance and lower background for *in vivo* imaging applications. (3) Compared with typical linear peptides and larger proteins, the lantibiotics are extraordinarily stable. Both duramycin and cinnamycin are crosslinked by four covalent intramolecular bridges, and have no free peptide termini. These features confer a greater stability and resistance to proteolytic degradation *in vivo*. (4) The availability of primary amines at the N-terminal, which is at the distal end away from the PE binding pocket, provides convenient and ideal configuration for site-directed bioconjugation and labeling, where the binding activities of the peptides will be minimally affected. This structural feature is supported by NMR analyses of cinnamycin where the proton resonances of the five N-terminal amino acids were not shifted when the peptide binds to PE, indicating that these residues are not involved in interacting with PE (Wakamatsu et al. 1990).

### Derivatization

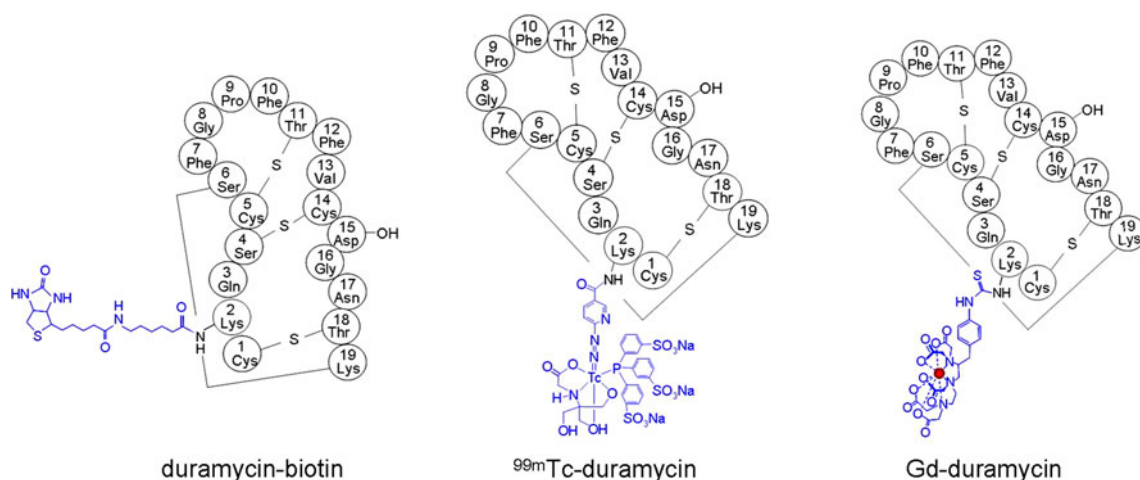
Duramycin and cinnamycin carry two and one primary amines, respectively, at the N-terminal. The locations of these functional groups provide convenience for covalent reactions in a site-specific fashion without significantly

interfering with the PE-binding activity of the peptides. A number of lantibiotic-based molecular probes for PE have been reported, while other forms of derivatization for imaging are plausible at least in theory.

Biotinylated duramycin and cinnamycin can be synthesized using chemically activated biotin derivatives, such as the N-hydroxysuccinimide (NHS) ester-form of biotin (NHS-biotin) (Fig. 3a). The bioconjugation to cinnamycin is more straightforward since the peptide contains a single primary amine at Cys-1. For duramycin, because of the presence of two primary amines (Cys-1 and Lys-2), the conjugation product in saturation reaction conditions contains an isomeric mixture of three chemical species. The isomers, duramycin-(Lys2)biotin, duramycin-(Cys1)biotin and duramycin-(biotin)<sub>2</sub> can be resolved and isolated using reverse phase high-performance liquid chromatography. The biotinylated lantibiotics are suitable for a two-step detection system using labeled avidin with relatively high specificity (Aoki et al. 1994; Li et al. 2009).

Radiolabeled PE-binding probes can be synthesized using established radiochemistry. An example is the Technetium-99 m (<sup>99m</sup>Tc)-labeled duramycin. The radiopharmaceutical was synthesized in two steps: duramycin was first derivatized with hydrazinonicotinamide (HYNIC), before being labeled with <sup>99m</sup>Tc using the tricine/phosphine coligands (Fig. 3b). Experiments using <sup>99m</sup>Tc-duramycin in rodents demonstrated a fast renal clearance with a blood half-life of about 3 min. The radiopharmaceutical has extraordinary chemical and radiochemical stability *in vivo*, where it was recovered in the urine without being metabolized or derivatized (Zhao et al. 2008). Given the diverse redox chemistry for <sup>99m</sup>Tc, many alternative radiochemistry options are available with different coordination complexes and coligands (Bleeker-Rovers et al. 2004; Liu and Edwards 1999).

Liposome-based probes have been reported consisting of duramycin attached to the surface of polyethylene glycol



**Fig. 3** Schematic diagrams of molecular probes derived from duramycin: **a** duramycin-biotin, **b** the radiochemical structure of  $^{99m}\text{Tc}$ -duramycin, and **c** gadolinium-labeled duramycin

(PEG)-derivatized, fluorescent liposomes. After thiolation using 2-iminothiolane, duramycin is covalently conjugated to the liposome surface preactivated by maleimide (Mărcănescu and Thorpe 2008). Given that multiple duramycin molecules are incorporated to the surface of a single liposome, the PE-binding affinity could be enhanced by the multivalency effect.

Other forms of lantibiotic-based imaging probes are being synthesized and investigated. We have recently produced Gadolinium (Gd)-labeled duramycin (Gd-duramycin) for the investigation of PE distribution in the vasculature using high-resolution magnetic resonance imaging (Fig. 3c). The chelation of Gd can be accomplished using bifunctional linkers/chelators. Similar principle of chelation chemistry can be applied to the labeling with other paramagnetic ions or radioisotopes, including Manganese, Indium-111 and Copper-64. The magnetically labeled probes and gamma emitting radiopharmaceuticals will be applicable to the noninvasive characterization of PE on a tissue level.

### Potential toxicity

The type B lantibiotics, duramycin and cinnamycin, are amphiphilic PE-specific peptides. Comparatively, these peptides are less disruptive to lipid membranes than linear peptide antibiotics. The antibiotic activities of duramycin and cinnamycin are likely exerted in the inhibition of enzyme-dependent cell wall synthesis in prokaryotes (Brötz et al. 1998). Nonetheless, potentials for toxicity must be observed closely to ensure that the discoveries made using these molecular probes are of physiological nature and not secondary artifacts. At sufficiently high concentration, the lantibiotics can cause membrane

distortion and leakage. The concentration of cinnamycin to induce 50% hemolysis of human erythrocytes in 4 min of incubation at 37°C is 5  $\mu\text{M}$  (Choung et al. 1988). The dosages that cause cytotoxicity vary over a broad range for different cell types and for the same cell type in different animal species, but the underlying mechanism for lantibiotic-induced cytotoxicity is unknown. However, complex formation between cinnamycin and avidin abolishes the toxicity, thereby suggesting that structural configuration and/or molecular orientation of the lantibiotics on the membrane surface may play a role (Aoki et al. 1994). For imaging purposes, the typical concentration of lantibiotic-derived probes is much lower than the known threshold of cytotoxicity for the target tissue/cell type.

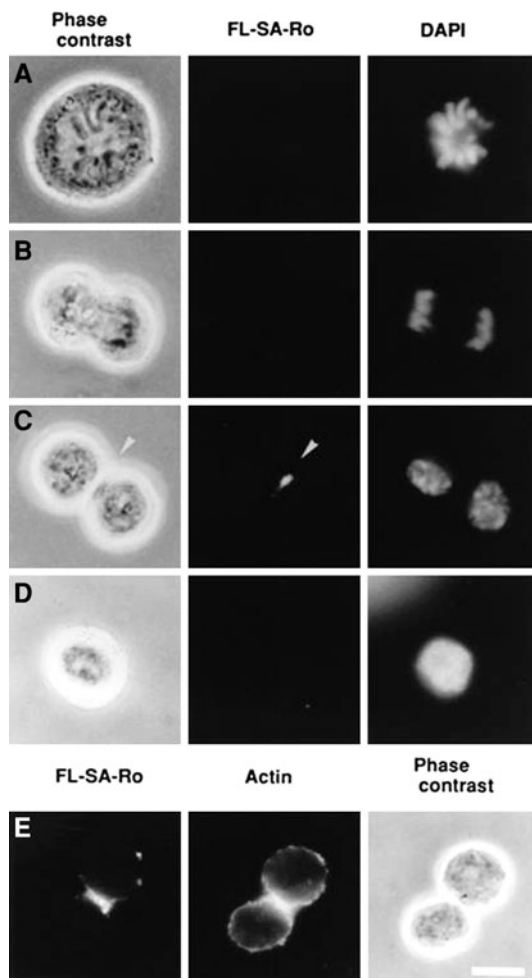
### Biological applications

As a major membrane phospholipid species, PE is predominantly and typically in the inner leaflet of the plasma membrane, maintained by energy-dependent enzymes. However, accumulating evidence has documented the translocation of PE to the cell surface in a number of distinct physiological events. These discoveries, made using PE-specific lantibiotic probes, shed light on the potentially versatile biological roles of this membrane component.

### PE externalization in cytokinesis

Mitotic cell division involves extensive reorganization/redistribution of cellular contents. An intriguing phenomenon is the transient externalization of PE in dividing mammalian cells. Using biotinylated cinnamycin as a probe, it was discovered that PE was exposed to the surface

of the plasma membrane specifically at the cleavage furrow in the late telophase of dividing cells (Emoto et al. 1996) (Fig. 4). The transbilayer relocation of PE is selective and no other phospholipid exhibits a change in distribution (Umeda and Emoto 1999). Although the exact roles of PE externalization remain to be determined, this event is associated with the dynamics of actin assembly. When the surface PE was sequestered using avidin-bound cinnamycin, cytokinesis was blocked at the late telophase, where the disassembly of actin filament and the fusion of plasma membrane were inhibited (Emoto et al. 1996; Umeda and Emoto 1999). Similarly, mutant cells that are deficient in PE biosynthesis exhibited attenuated cytokinesis, which was overcome using a PE supplement (Emoto and Umeda 2000; Emoto et al. 2005). These data underscored the essential, yet to be fully defined, roles of PE in the normal progression of mammalian cell division.



**Fig. 4** Transient externalization of PE at the cleavage furrows of Chinese Hamster Ovarian cells during cytokinesis. **a** Prometaphase, **b** anaphase, **c** late telophase, **d** G<sub>1</sub> phase. The double staining for PE and actin is shown in **e** (Copy right Emoto et al. 1996)

Transient PE externalization is observed also in dividing or budding yeast cells, suggesting a degree of conservation among eukaryotic species (Iwamoto et al. 2004). Using biotinylated cinnamycin, localized PE exposure was detected at polarized cytokinetic ends in *Saccharomyces cerevisiae*. Similarly to the mammalian cells, immobilization of PE using the avidin-duramycin complex was associated with F-actin accumulation at the site of cell division. It was subsequently discovered that a membrane glycoprotein, Ro-sensitive 3 (Ros3p) plays a key role in the PE-specific translocation across the membrane bilayer (Kato et al. 2002).

PE deficiency causes truncated cytokinesis in prokaryotes as well. The protein filamenting temperature-sensitive mutant Z (FtsZ) elongates and assembles into a ring structure at the septum of bacterial cell division, where the process is analogous to the contractile ring in mitotic mammalian cells (Bi and Lutkenhaus 1991). In *Escherichia coli* cells deficient in PE synthesis, the cytokinesis fails to complete and the dividing cells remain attached as filamentation. Microscopy studies indicate that FtsZ proteins are localized at the septum, but the FtsZ ring does not undergo the normal contraction that drives cell division (Mileykovskaya et al. 1998). Comparatively, none of the deficiencies in phospholipids other than PE results in filamentation.

The above data strongly indicate an essential role for membrane PE in the normal progression and completion of cell division in prokaryotes and eukaryotes alike. However, neither the signaling pathway for PE enrichment at the cell surface nor the biological roles of PE in cytokinesis is well understood. It is possible that the PE-rich membrane provides the necessary structural features that regulate the attachment, organization, and assembly of membrane-bound proteins and enzymes (Emoto et al. 2005). Continued investigation is likely to yield insights regarding the PE-mediated protein-membrane interactions in cytokinesis.

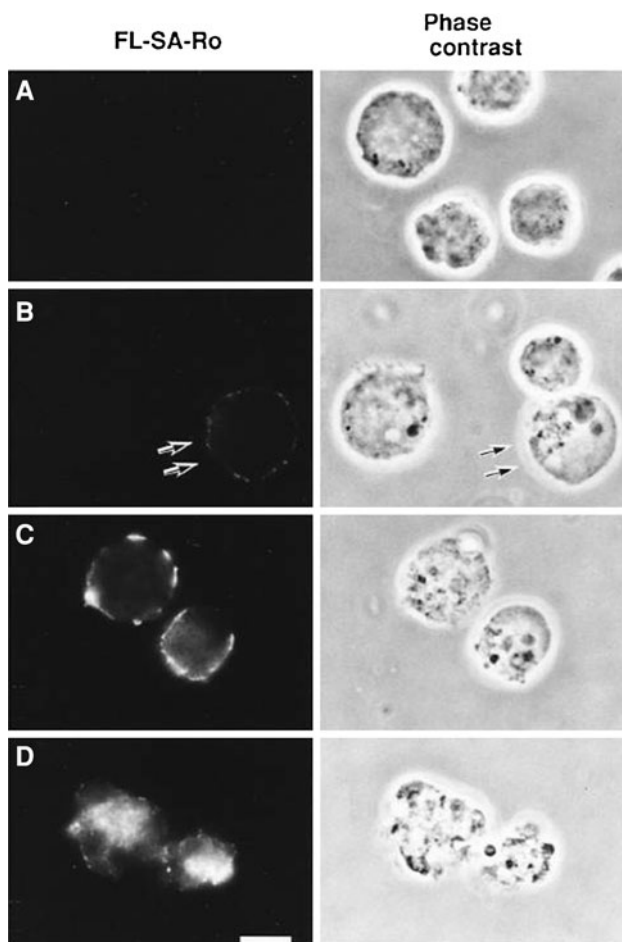
### Detection of cell death

Apoptosis and necrosis are major, but distinct, modes of cell death. Apoptosis, or programmed cell death, is an intracellular, energy-dependent self-destruction (Song and Steller 1999; Wyllie 1997). Once committed, the execution phase of apoptosis is carried out by caspases, and is accompanied by distinct molecular markers (Beverly et al. 1999). Necrosis is a form of passive cell death characterized by swelling and plasma membrane damage.

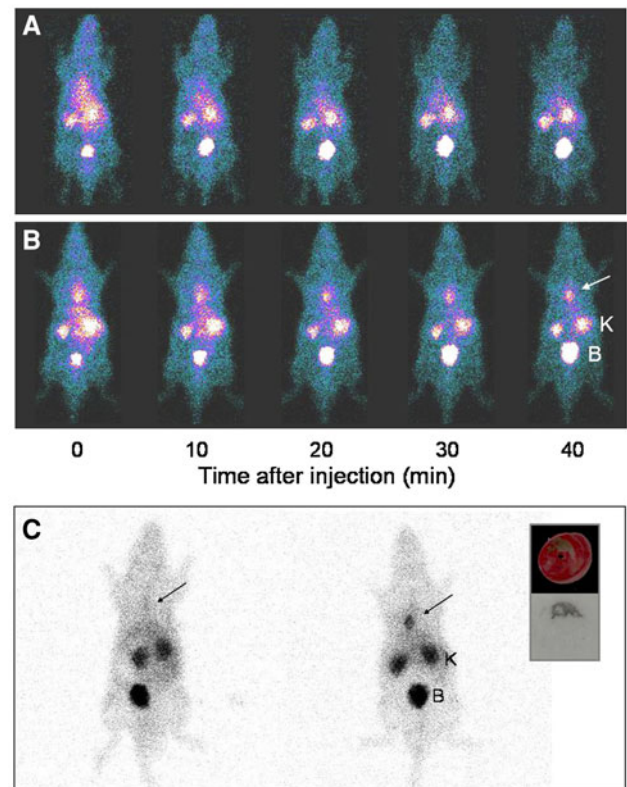
An important molecular marker for apoptosis is the redistribution of phospholipid species across the bilayer of the plasma membrane. In viable cells, aminophospholipids

such as PE and PS are predominantly constituents of the inner leaflet of the plasma membrane. The asymmetry of the lipid bilayer is maintained by the actions of energy-dependent enzymes, including aminophospholipid translocase and floppase (Beyers et al. 1996; Sahu et al. 2007). During apoptosis, the inhibition of translocase and floppase is accompanied by the activation of scramblase, and the redistribution of phospholipids across the bilayers is facilitated (Williamson and Schlegel 2002). In vitro evidence has demonstrated a synchronized externalization between PE and PS in apoptotic cells (Emoto et al. 1997) (Fig. 5). It is noteworthy that although the redistribution of membrane phospholipids is widely regarded as a marker by the general consensus, exceptions have been documented, where the externalization of PE and PS precedes the onset of apoptosis in ischemic conditions and after ionizing irradiation (Maulik et al. 1998; Marconescu and Thorpe 2008). In necrotic cells, the compromised integrity of the

plasma membrane renders intracellular components, including PE and PS, accessible to extracellular milieu. Because PE and PS are major phospholipid species in the plasma membrane, their externalization provides ample molecular targets for the detection of cell death. In this regard, a greater abundance of PE gives rise to an even higher number of targets, which is likely to lead to improved image quality. The noninvasive imaging of cell death using PS-binding probes has been demonstrated in a wide range of applications (Blankenberg et al. 1998; Johnson et al. 2005; Kiestelaer et al. 2004; Liu et al. 2007; Narula et al. 2001; Sosnovik et al. 2005; Thimister et al. 2003; Zhao et al. 2001; Zhao et al. 2006). As a viable alternative, PE-binding probes have been shown with selectivity in detecting dead and dying cells (Emoto et al. 1997; Zhao et al. 2008), while in vivo experiments (Fig. 6) demonstrated favorable biodistribution, clearance and pharmacokinetic profiles (Zhao et al. 2008).



**Fig. 5** Exposure of PE at the plasma membrane surface in apoptotic cells at 0 h (a), 6 h (b), 12 h (c) and 48 h (d) after the induction of apoptosis (Copy right Emoto et al. 1997)



**Fig. 6** In vivo radionuclide imaging of acute cardiac cell death using  $^{99m}\text{Tc}$ -Duramycin in a rat model of myocardial ischemia and reperfusion. **a** Whole-body dynamic planar imaging of  $^{99m}\text{Tc}$ -Duramycin uptake in a rat with acute myocardial infarction. **b** Non-color-enhanced, raw counts static planar images of sham-operated (left) and infarcted rat (right) acquired at 120 min after the intravenous injection of  $^{99m}\text{Tc}$ -Duramycin. The infarct site is marked by arrows. In autoradiography, the radioactivity uptake in the myocardium co-localizes with the infarct (inset). *K* kidneys, *B* bladder (Copy right Zhao et al. 2008)

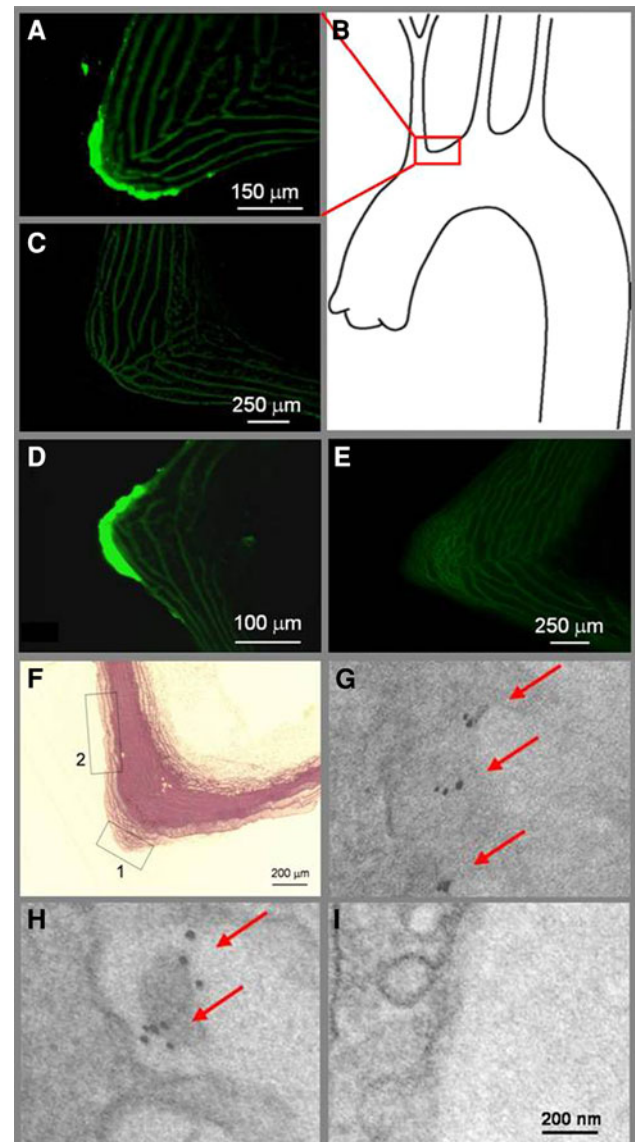
## Vascular PE as a critical anticoagulant

Accumulating evidence indicates that PE is a critical anticoagulant. However, little is known regarding the physical and dynamic distribution of PE and how it is regulated in the vasculature. The availability of PE-specific probes will contribute critically to its characterization.

The bulk of *in vivo* evidence on vascular PE is indirect and comes from its depletion or deficiency (Smirnov et al. 1995; Esmon et al. 1997a, b, 1999). The presence of anti-PE autoantibodies, also known as aPEs, is strongly associated with clinical cases of unexplained thrombosis and recurring fetal loss among pregnancies in humans (Berard et al. 1996; Hachulla et al. 2007; Sanmarco et al. 2001; Sugi et al. 1999, 2004; Vinatier et al. 2001). The scenario is supported by genetic knockout studies in mice, where a deficiency in ethanolamine kinase 2, which is a key enzyme in PE biosynthesis, results in placental thrombosis and fetal loss (Tian et al. 2006). In a reconstituted coagulation system *in vitro*, the proteolytic inactivation of factor Va by activated protein C is amply enhanced by PE in a concentration-dependent manner (Smirnov et al. 1999). The specificity to PE is attributed to the membrane-binding Gla domain of protein C (Smirnov and Esmon 1994). These existing data provide evidence that PE is an essential component in hemostasis, particularly, the protein C anticoagulant mechanism. An underlying implication is that the PE's roles as an anticoagulant necessitate a physical presence at the luminal vascular surface in order to directly interact with blood components.

Experiments were carried out to examine the presence and distribution profile of PE in the aorta using biotinylated duramycin. In the rat aortic arch, intense duramycin binding was initially detected at the flow dividers that bifurcate the blood flow (Fig. 7) (Li et al. 2009). The prominent duramycin binding is seen also at the flow dividers in the mouse aortic arch, suggesting that this pattern of distribution is conserved at least to some degree (Fig. 7). On the ultrastructural level, transmission electron microscopy confirmed that significant duramycin binding at the flow dividers was localized on the luminal surface of the endothelial membrane (Fig. 7).

These recent data offer new insights on PE in the vasculature. Flow bifurcations in major arteries generate high blood flow velocity, shear stress and turbulence, which pose a constant source of prothrombogenic risk factors (Chandran 1993). The heavy presence of PE is consistent with an anticoagulant role in vascular regions exposed to hemodynamic stress. Additionally, these findings have implications in a physical link between anti-PE autoimmunity and idiopathic thrombosis, and suggest that aPE likely promotes thrombogenesis by masking PE in the vasculature, particularly at regions under significant hemodynamic burden (Li et al.



**Fig. 7** Prominent binding of duramycin at the luminal endothelial surface. **a** Fluorescent microphotographs of aortic flow divider cryosections from the rat stained with duramycin-biotin and avidin-FITC. **b** A diagram illustrating the anatomic structure of the aorta with the flow divider highlighted. **c** Negative control with avidin-FITC. **d** Duramycin binding at the flow divider of a mouse. **e** Double-infusion using duramycin and Annexin V-FITC. **f** Ultra-thin section of a rat aortic flow divider before trans EM examination, where regions 1 and 2 are marked at the apex and lateral portion of the flow divider. **g, h** Electron micrographs taken from region 1. The presence of 6 nm gold particles is marked with arrows. **i** Typical electron micrograph taken from region 2 (Copy right Li et al. 2009)

2010). The interfering effects of anti-PE autoimmunity are consistent with *in vitro* studies, where the presence of aPE severely attenuates the PE-dependent protein C activities (Smirnov and Esmon 1994).

Duramycin binding at the vascular luminal surface was not accompanied by the presence of PS (Fig. 7) (Li et al. 2009). It is likely that the exposure of PE at the flow

dividers may involve a distinct mechanism of externalization. PE and PS are known substrates for aminophospholipid translocases, flippases, floppases and scramblases (Bever et al. 1996; Sahu et al. 2007). However, in the asymmetrical distribution of membrane phospholipids between the bilayers, it has been reported that PE is more slowly transported to the inner membrane leaflet (Devaux 1991). At least in the yeast, selectivity is known for PE, but not PS, in the transbilayer movement mediated by defined transmembrane proteins (Kato et al. 2002). Since various coagulation complexes exhibit different PE/PS dependences, the composition of the phospholipid membrane luminal surface in blood vessels may be a way of fine-tuning the coagulation potential (Smirnov et al. 1999). Further investigations are warranted to fully understand the functional roles of PE in hemostasis.

### Conclusion and remarks

PE as a membrane component has been known for decades, yet new discoveries on its biological functions continue to emerge. A multi-modality imaging approach, combined with cellular and molecular-level investigations, will provide a system-level understanding of PE in its physiological roles. In terms of lantibiotic-based molecular probes for investigating the spatial distribution of PE, advances are anticipated in the following aspects: (1) fine tuning of physicochemical properties of lantibiotic derivatives for various imaging needs, (2) labeling with different tags for multi-modality detection, (3) modulating binding affinity by structural manipulations; and (4) understanding the probe–target interactions in the context of *in vivo* imaging. These technical progresses will contribute critically to the development of new biomarkers and, ultimately, the translational applications of PE imaging.

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