HHS Public Access

Author manuscript

Trends Biotechnol. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

Trends Biotechnol. 2017 August ; 35(8): 686–690. doi:10.1016/j.tibtech.2017.03.015.

How does Membrane Oxidation affect Cell Delivery and Cell Killing?

M. Daben J. Libardo[1,†], **Ting-Yi Wang**[2,†], **Jean-Philippe Pellois**[2,3,*], and **Alfredo M. Angeles-Boza**[1,*]

[1]Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269, United States

[2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, United States

[3]Department of Chemistry, Texas A&M University, College Station, Texas 77843, United States

Abstract

The biophysical properties of cellular membranes intimately influence the delivery of cargoes into cells by cell-penetrating peptides and the bactericidal activity of antimicrobial peptides. We discuss how lipid oxidation creates important chemical and biophysical changes in membranes and hypothesize about the observed synergy between oxidized membranes and membrane-active peptides.

Keywords

Membrane oxidation; cell delivery; cell-penetrating peptides; cell killing; antimicrobial peptides; lipid peroxidation

Cell delivery or killing applications often involve permeating or irreversibly damaging the structure of biological membranes. In this context, understanding the parameters that influence the biophysical properties of membranes is critical to improving cargo delivery and/or enhancing target cell death. Notably, recent evidence suggests that the often-overlooked phenomenon of membrane oxidation plays an important role in a variety of biotechnologies [1]. Membrane oxidation takes place when cells are exposed to various levels of oxidative stress. Lipids and proteins can, for instance, react with reactive oxygen species to generate compounds with altered structures and properties [2, 3]. Oxidized lipids and proteins consequently do not behave in the lipid bilayer as their non-oxidized precursors do, and they often contribute to increasing the membrane's permeability. Electroporation [1],

*To whom correspondence should be addressed: Prof. Alfredo M. Angeles-Boza, Department of Chemistry, University of Connecticut, Unit 3060, 55 North Eagleville Road, Storrs, Connecticut 06269, Telephone: (860) 486-6718; FAX: (860) 486-2981; alfredo.angeles-boza@uconn.edu. Prof. Jean-Philippe Pellois, Department of Biochemistry and Biophysics, Texas A&M University, Biochemistry and Biophysics Building, Room 430, 300 Olsen Boulevard, College Station, Texas 77843-2128, Telephone: (979) 845-0101; FAX: (979) 862-4718; pellois@tamu.edu.

†These authors contributed equally to this work.

gas plasma [4], and photodynamic techniques [5–7] have already been shown to take advantage of membrane oxidation to achieve cell penetration or cell killing.

Cell-penetrating peptides (CPPs) and antimicrobial peptides (AMPs) are biomolecules commonly used for cellular delivery and bactericidal applications, respectively. CPPs and AMPs, which are often rich in cationic and hydrophobic residues, interact with biological membranes to effect either membrane disruption or translocation [8]. Here, we highlight recent evidence that suggests an under-appreciated synergy between oxidized membrane and membrane-active CPPs and AMPs, with a particular focus on oxidized lipids. In order to promote conversation on the importance of lipid membrane oxidation in biotechnological applications, we attempt to explain this synergy and propose several ways to exploit membrane oxidation to improve delivery or killing outcomes.

What are the potential effects of membrane oxidation on lipid-peptide interactions?

The current consensus holds that CPPs achieve cytosolic penetration either through direct cellular membrane translocation (where peptides passively diffuse through the bilayer) or via endocytosis (where CPPs are surrounded by a portion of the plasma membrane to form an intracellular vesicle) [8]. However, the membrane-crossing efficiency has remained controversial, as several laboratories have reported performances that differ for the same peptide. As a result, mechanistic studies are ambiguous and our ability to predict the behavior of CPPs remains impaired. Recent evidence suggests that CPPs gain new functions in the presence of oxidized lipids within a lipid bilayer (Box 1). CPPs and AMPs share many chemical properties (e.g., they are short polypeptides that are positively charged at neutral pH). Like CPPs, AMPs strongly interact with lipid membranes. Interestingly, membrane oxidation also seems to influence the interaction of AMPs with bacterial membranes (Box 1).

Oxidation of membrane lipids results in their hydroxylation, peroxidation, and carbonylation. Although the extent of the effect of these changes in the lipid membrane has not been fully explored, the newly created functional groups within the lipid membrane could provide enough driving force to change the biophysical properties of the membrane and introduce stress [3, 9]. For example, upon oxidation of a fatty-acid chain, cylinder-shaped lipids, which are critical to the formation of the bilayer structure, could turn into inverted-cone-shaped lipids that prefer to arrange into non-bilayer structures (Figure 1).

In addition to physical changes of the bilayer structure originating from the change in shape of the lipids, the presence of aldehyde groups formed upon oxidation can modify the nature of the membrane association by peptides [10]. In particular, peptides bearing nucleophilic groups can react with the aldehyde groups from the oxidized lipids to form adducts (i.e. imines) affecting the biophysical properties of the membrane [10]. In addition, if several lipids are oxidized simultaneously, or in a very short period of time, domains composed largely of oxidized lipids can form [11]. The addition of oxidized lipids (5–15 mol%) on lipid bilayers or the *in-situ* oxidation of bilayers in giant vesicles result in phase separation [11]. This effect probably results from the hydrophilic functional groups on the fatty acids

migrating towards the membrane surface, with an accompanying increase in the area occupied by the oxidized lipid (Figure 1). The behavior of these domains is difficult to assess given the scarcity of information on the biophysics of phase co-existence when oxidized lipids are present. Nevertheless, the formation of oxidized lipid domains could directly recruit cationic peptides to the cell surface and facilitate the transfer of the peptide across the bilayer by forming structures such as inverted micelles. It could also add stress to the bilayer, seeding the formation of either transient or stable pores. Finally, it is not impossible that a decrease in the bilayer thickness, brought about by a reduction in the fatty acid length upon oxidation, would result in a steeper membrane potential gradient and an increased pull toward the cell interior for cationic peptides. Arguably, oxidation of membrane proteins and carbohydrates could also contribute to the activities of CPPs and AMPs. [12]. Whether these oxidation products synergize with oxidized lipids and modulate the behavior of membrane-active peptides, directly or indirectly, is however still an open question.

How can oxidation be controlled and exploited?

Drawing on these previous observations, we hypothesize that the more oxidized a membrane is, the more susceptible it may be to the activity of CPPs or AMPs. Excessive oxidation of cellular membranes results in cell death, a problem for delivery applications. In principle, un-targeted oxidation is also not desirable for cell killing applications as killing should be restricted to disease-causing cells and not affect healthy cells. Whether the membrane oxidation dose and selectivity can be tuned to achieve a desirable outcome is unclear. However, the notion of exploiting oxidation in biotechnological applications may not be implausible. In particular, a new study highlights how dendritic cells utilize membrane oxidation to enhance the endosomal release and cytosolic delivery of endocytosed antigenic proteins [13]. The endosomal release process is mediated in part by of NOX2, a ROS-producing endosomal enzyme. By causing local lipid peroxidation, activation of NOX2 enhances the permeability of the endosomal membrane, thereby favoring the escape of antigens from the endosomal lumen to the cytosol. Notably, the use of the ROS-generating fluorescent protein KillerRed to mimic NOX2 activity also resulted in an increase in antigen release from endosomes [13]. If cells modulate the permeability of their membranes with oxidation, it may be worth learning how to do the same.

In principle, there are many ways to increase the extent of membrane oxidation in cells to improve the outcome of delivery or killing processes involving CPPs and AMPs. The extent of oxidation could be improved by using a hyperoxic environment (e.g. a 20% oxygen incubator), by supplementing cells with lipophilic oxidants, or by stimulating oxidizing cellular enzymes. Alternatively, minimizing the ability of cells to protect themselves from oxidation, by depleting cells or cell culture media from antioxidants (*e.g.* polyphenols, carotenoids) or by inhibiting ROS detoxifying enzymes, may lead to similar effects. Notably, all these processes are biologically reversible, and cells can repair oxidative damage. Mild and transient membrane destabilization may therefore be envisioned. This is of particular importance for CPP-mediated translocation, as cells should ideally recover after a delivery protocol is performed.

Concluding Remarks

Overall, a number of pertinent studies support the hypothesis that lipid oxidation enhances the activity of CPPs and AMPs. As more becomes known about the interaction between peptides and oxidized lipids, it may become possible to rationally design CPPs or AMPs with enhanced activities. For instance, as addition of an oxidizing copper-binding sequence to existing AMPs improves antimicrobial killing, conjugating oxidants to CPPs may also enhance cell penetration performance. Alternatively, it may be possible to introduce moieties or domains to AMPs and CPPs that will enhance their binding to oxidized lipids. Finally, reagents that permit the controlled oxidation of membranes with organelle or cell specificities could also be used in combination with CPPs and AMPs to direct where and when these peptides exert their membrane-induced effects.

References

1. Vernier PT, et al. Electroporating fields target oxidatively damaged areas in the cell membrane. PLoS One. 2009; 4(11):e7966. [PubMed: 19956595]
2. Milic I, et al. Simultaneous detection of low and high molecular weight carbonylated compounds derived from lipid peroxidation by electrospray ionizationtandem mass spectrometry. Anal Chem. 2013; 85(1):156–62. [PubMed: 23186270]
3. Runas KA, Malmstadt N. Low levels of lipid oxidation radically increase the passive permeability of lipid bilayers. Soft Matter. 2015; 11(3):499–505. [PubMed: 25415555]
4. Xu D, et al. Intracellular ROS mediates gas plasma-facilitated cellular transfection in 2D and 3D cultures. Sci Rep. 2016; 6:27872. [PubMed: 27296089]
5. Meerovich I, et al. Photodamage of lipid bilayers by irradiation of a fluorescently labeled cell-penetrating peptide. Biochim Biophys Acta. 2014; 1840(1):507–15. [PubMed: 24135456]
6. Johnson GA, et al. Photoinduced membrane damage of E. coli and S. aureus by the photosensitizer-antimicrobial peptide conjugate eosin-(KLAKLAK)2. PLoS One. 2014; 9(3):e91220. [PubMed: 24608860]
7. Dosselli R, et al. Synthesis, characterization, and photoinduced antibacterial activity of porphyrin-type photosensitizers conjugated to the antimicrobial peptide apidaecin 1b. J Med Chem. 2013; 56(3):1052–63. [PubMed: 23231466]
8. Reissmann S. Cell penetration: scope and limitations by the application of cell-penetrating peptides. J Pept Sci. 2014; 20(10):760–84. [PubMed: 25112216]
9. Khandelia H, Mouritsen OG. Lipid gymnastics: evidence of complete acyl chain reversal in oxidized phospholipids from molecular simulations. Biophys J. 2009; 96(7):2734–43. [PubMed: 19348756]
10. Stemmer U, Hermetter A. Protein modification by aldehydophospholipids and its functional consequences. Biochim Biophys Acta. 2012; 1818(10):2436–45. [PubMed: 22450235]
11. Haluska CK, et al. Photo-activated phase separation in giant vesicles made from different lipid mixtures. Biochim Biophys Acta. 2012; 1818(3):666–72. [PubMed: 22172805]
12. Cumpstey I. Chemical modification of polysaccharides. ISRN Org Chem. 2013; 2013:417672. [PubMed: 24151557]
13. Dingjan I, et al. Lipid peroxidation causes endosomal antigen release for cross-presentation. Sci Rep. 2016; 6:22064. [PubMed: 26907999]
14. Wang TY, et al. Membrane Oxidation Enables the Cytosolic Entry of Polyarginine Cell-penetrating Peptides. J Biol Chem. 2016; 291(15):7902–14. [PubMed: 26888085]
15. Libardo MD, et al. Central Role of the Copper-Binding Motif in the Complex Mechanism of Action of Ixosin: Enhancing Oxidative Damage and Promoting Synergy with Ixosin B. ACS Infect Dis. 2016; 2(1):71–81. [PubMed: 27622949]

Box 1

Oxidized lipids as targets for CPPs and AMPs

Two recent studies highlight the importance of membrane lipid oxidation within the context of CPPs and AMPs. First, cytosolic penetration of linear polyarginine peptides containing an increasing number of arginine residues (from 5 to 13) depends on the oxidation state of the cellular membrane [14]. Polyarginine peptides were less successful at entering cells grown with 2% dioxygen as compared to cells grown at the more oxidizing condition of 20% dioxygen. Direct measurement of oxidized lipid products with the fluorescent probe diphenyl-1-pyrenylphosphine showed a decrease in lipid peroxidation when cells are grown in hypoxic conditions. The presence of antioxidants in the growth medium also limited the internalization of the polyarginines, whereas treatment with the lipophilic oxidant cumene hydroperoxide or with hydrogen peroxide positively affected the cell penetrating abilities of these CPPs. Coupling hypoxic culture conditions with antioxidants in the growth medium resulted in a 12-fold reduction in penetration efficiency, almost completely abolishing peptide translocation. Moreover, pretreatment of cells with the E06 monoclonal antibody, known to bind several by-products of the oxidation of phosphatidylcholine, also caused a severe reduction in cytosolic penetration highlighting the key role of oxidized lipids in polyarginine penetration. Notably, the polyarginine peptide R9, along with other cationic peptides such as TAT (GRKKRRQRRRG-NH_2, a CPP) and $(KLAKLAK)_2$ (an AMP), were previously shown to promote the leakage and aggregation of mildly oxidized liposomes [5, 6]. Altogether, it appears that peptides gain new functions in the presence of oxidized lipids within a lipid bilayer.

A second case study comes from the AMP literature. The peptides ixosin (GLHKVMREVLGYERNSYKKFFLR-NH_2) and ixosin B (QLKVDLWGTRSGIQPEQHSSGKSDVRRWRSRY-NH_2), isolated from the salivary glands of the tick *Ixodes sinensis*, act synergistically by exploiting bacterial membrane oxidation [15]. The first three amino acids in the sequence of ixosin, Gly-Lys-His, have high affinity for copper ions. As the copper-ixosin complex forms, it oxidizes the bacterial membrane through the production of reactive oxygen species (ROS) as measured by a standard thiobarbituric acid-reactive substances assay (TBARS, test for presence of malonyldialdehyde, one of the products of lipid peroxidation). The ROS directly produced by copper bound to this binding motif has a strong oxidizing capacity and reacts with a large variety of lipids, including saturated phospholipids [2]. Metal chelators that outcompete ixosin for copper ions inhibit the oxidation of the lipid membrane. Once the lipids are oxidized, ixosin B exhibits an increased affinity for the membrane resulting in a synergistic effect (assessed via fractional inhibitory concentration, or FIC, indices) between the two ixosins [15]. It should be noted that FIC indices corresponding to synergistic interactions represent a minimum of 4-fold increase in potency.

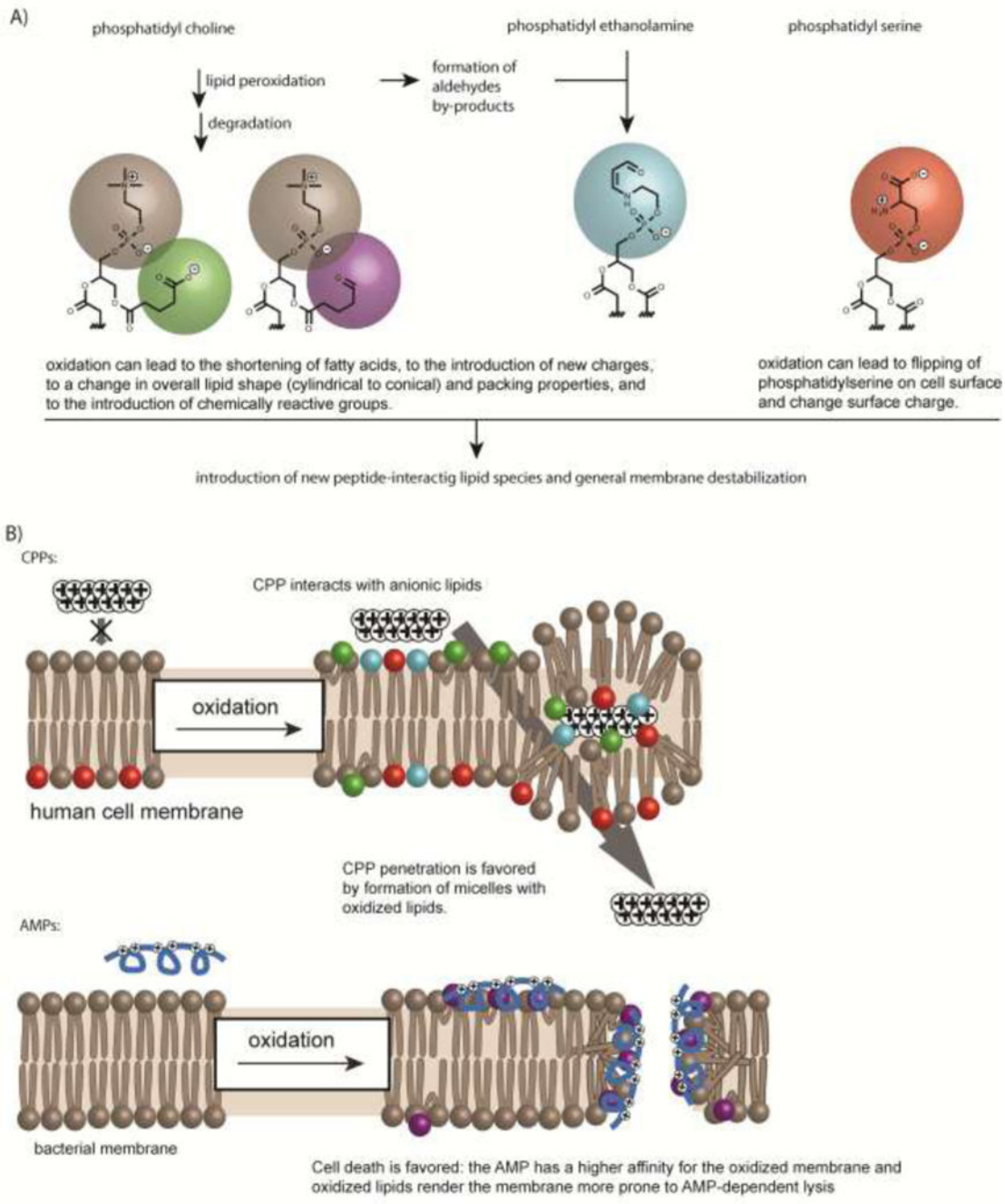

Figure 1.

A) Examples of how oxidation changes lipid structures properties. Lipid oxidation leads to complex and heterogeneous mixtures. Only a subset of possible oxidation products from glycerol phospholipids is represented. These examples highlight how polar head charge and reactivity, and how fatty acid length and hydrophobicity are modified upon oxidation. Overall, these oxidized lipids will impact the properties of a bilayer. B) Plausible effects of oxidation on peptide/membrane interactions. The structures highlighted in A) (based on polar head color-scheme) are used to postulate how CPPs and AMPs cross or lyse oxidized

membranes. The combined effects of various oxidized lipids can increase the affinity of peptides for the bilayer. Conversely, the peptides may more readily form inverted micelles or pores with oxidized lipids than with their non-oxidized counterparts (note that these mechanisms are speculative and that other non-mutually exclusive translocation or permeation processes are possible). Overall, conditions of mild membrane oxidation are sufficient to observe dramatic changes in peptide behavior.