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Short Communication

Membrane Lipid Oxidation as a Negative Feedback Loop Modulating Cell Response to Oxidative Stress

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Abstract

Reactive oxygen species (ROS) are implicated in originating multiple deleterious processes, including inflammation of the airways, heart diseases, carcinogenesis, age-related neurodegenerative conditions, etc. Less commonly discussed are the beneficial and/or inevitable effects associated with their normal production in healthy cells and tissues. Lipid oxidation by ROS can be viewed as playing a dual role, including upregulation of certain proteins required for the protection of cells and tissues against abnormally high ROS concentrations, thereby constituting a negative feedback loop required for dynamic stability of homeostasis. Analysis of recent publications and the original experimental results provide convincing, if circumstantial, evidence in support of this hypothesis. Gradual decrease of membrane rigidity (bending modulus) accompanied by the change of other mechanical parameters was observed in a model system based on lipid membrane nanotubes (LNT) pulled out of planar lipid bilayers. The experimental system was optimized for real-time measurements of membrane mechanical parameters. The obtained results offer credible explanation to the seemingly paradoxical behavior of certain membrane proteins, such as intracellular divalent cation transporters, ryanodine receptors, or endosomal ion channels in response to oxidative stress; they also suggest novel treatment strategies involving combined use of rare lipid species and photosensitizers, e.g., in order to minimize collateral damage inflicted by phototherapy.

Keywords

Reactive oxygen; photosensitizers; unsaturated lipids; oxidative stress; phototherapy; membrane mechanics; bending modulus; lipid nanotubes

Introduction

Oxidative stress is a well-known driver of diverse pathologies associated with increase of intracellular or tissue concentrations of such molecules as superoxide, hydrogen peroxide, hydroxyl radicals, jointly known as reactive oxygen species (ROS). ROS and peroxidized molecules are normally generated in diverse homeostatic processes, and neutralized by complex antioxidation system including dismutases, catalases, glutathione S-transferases and thioredoxines [1]. In mitochondria, hypoxia and acidosis enhance generation of reactive oxygen species, normally occurring due to electron leak in the electron transfer chain associated with oxidative phosphorylation and trigger a cascade of responses that can ultimately result in actuation of one of the cell death pathways, depending on a combination of known and yet undiscovered factors [2]. The mechanisms of apoptotic pathway activation by ROS are actively investigated both in-vivo and in-vitro, and the cytotoxicity of ROS is practically used, e.g. in cancer phototherapy or septic would treatment with photosensitizers. Easily penetrating through lipid membranes, ROS avidly react with any target, including proteins, lipid headgroups and unsaturated lipid tails. Lipid oxidation by ROS is known to ultimately result in formation of conductive pores in the membrane; however, recent evidence suggests that besides the loss of the membrane barrier functions, as well as DNA peroxidation damage, earlier consequences of cell exposure to elevated ROS concentrations can be of interest for medical purposes. These more subtle, but arguably no less important processes occurring earlier and at lower concentrations of reactive oxygen species can be associated with change of local mechanical properties of lipid membranes caused by lipid oxidation and include regulation of intracellular ion channels, modulation of signal transfer by receptor proteins, and possibly modulation of lateral organization of lipid bilayers (raft formation) and local membrane remodeling resulting in changes of local concentrations of water soluble or surface-associated components [1,3-6]. Thermodynamics of lateral inhomogeneity of lipid distribution in biological membranes strongly depends on the presence of lipids with non-zero spontaneous curvature and even minor change in their concentration can result in coalescence or Ostwald ripening of individual rafts [6,7]. Besides that, generally all the membrane components with non-zero spontaneous curvature strongly affect size distribution of rafts (i.e., such components are linearly active and still - all components with nonzero spontaneous curvature are linearly active, i.e. strongly change the linear tension and the distribution of rafts in size in concentrations in fractions of mole percent. It has been suggested that drug-induced pressure changes in lipid bilayers can change the conformational equilibrium between open and closed states of membrane proteins and thereby cause anesthesia [8]. Changes of lateral pressure profiles caused by ethanol, external mechanical pressure, or other factors can cause similar local anesthetic effects [9]. Indeed, recent experimental study suggested interdigitation of dipalmitoylphosphatidylcholine (DPPC) as a mechanism of local anesthetic action of phenylethanol [10]. Direct test of the hypotheses is complicated by the fact that the lateral pressure profile of membranes or lipid bilayers is difficult to determine in experiments. However, it is to be expected from basic physical principles that oxidation of unsaturated bonds would alter the transversal pressure distribution within the membrane.

Materials, Methods and Results

Photodynamic cancer therapy (PDT) uses non-toxic chemotherapeutic agents, photosensitizer (PS), to initiate a light-dependent and ROS-related cell death. Phthalocyanines (PCs) are third generation and stable PSs with improved photochemical abilities. Their cytotoxic action is known to be related to ROS production, thus investigation of the response of photosensitized membranes to light can elucidate on generic mechanisms of ROS-induced membrane damage and transformation. In the present work

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we report the results of first-time direct measurements of the dynamic effects of lipid oxidation upon the mechanical parameters of a model lipid membrane system based on lipid nanotubes (LNT). Oxidation was triggered by generation of active oxygen species induced by irradiation of the membrane with adsorbed Phthalocyanines derivative photosensitizer with laser light. The experimental and analytical methods we originally suggested in and further refined in were combined with the intramembrane field compensation method (IFC) to achieve real-time measurement of membrane mechanical parameters and verification of absorption of photosensitizers on the membrane of the given lipid composition [11-13]. We used negatively charged lipid membranes (DOPC: DOPE: Cholesterol: DOPS = 40: 10: 30: 20 mol %) and positively charged zinc phthalocyanines complex with choline substitution group as a photosensitizer. This sensitizer proved to have maximal photo induced activity against tumor cells and in treatment of septic and purulent wounds [14,15]. 650 nm 5 mW laser was used for irradiation of the membrane after cholosense adsorption (verified by IFC measurements). In the course of 60-90 second irradiation cycles, the membrane bending modulus monotonously decreased from ~ 20 ± 2 kT (intact membrane, before and after adsorption of the photosensitizer) to ~ 5 ± 1 kT (within 6-7 light exposure cycles to the total duration of ~ 10 minutes). This was accompanied by changes of surface tension of the membrane, which were, however, more difficult to interpret because the initial absorption of the photosensitizer caused substantial changes of this parameter. Further exposure resulted in formation of transmembrane pores, evidenced by characteristic channel-like conductivity changes corresponding to hydrophilic pores with luminal radii of the order of one nanometer, and further verified by release of fluorescent dyes from unilamellar vesicles, and ultimately membrane rupture.

Conclusions

Considerable changes of membrane mechanical properties induced by lipid oxidation precede the loss of the membrane barrier function and can be exploited by cells as early warning signals of the imminent threat, providing a protective negative feedback loop in the ROS homeostasis. Paradoxical upregulation of intracellular ion channels, observed in response to indiscriminate damage dealt by ROS to every target within reach is attributable to changes of transversal membrane mechanical pressure profile colocolized with the active centers, hinge regions, or other functionally important parts of transmembrane domains of various membrane proteins, or to the changes of lateral organization of lipids in the membranes [1]. This mechanism suggests new therapeutic strategies based on ensuring the presence of diverse minor species of [poly] unsaturated lipids in cellular membranes. Protein wetting phenomena can provide the necessary driving force for proper deployment of the necessary lipids in the vicinity of target proteins [7]. A systematic evaluation of the hypothesis is underway, and the underlying principles of protein-lipid interactions are discussed in details in chapter 6 of manuscript [16].

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