Free Radical Biology & Medicine 50 (2011) 892–898

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Interactions of plasmalogens and their diacyl analogs with singlet oxygen in selected model systems

Agnieszka Broniec [a], Radoslaw Klosinski [a], Anna Pawlak [a], Marta Wrona-Krol [a], David Thompson [b], Tadeusz Sarna [a,*]

[a] Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 30-387 Krakow, Poland
[b] Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

ARTICLE INFO

Article history:
Received 30 August 2010
Revised 29 December 2010
Accepted 3 January 2011
Available online 12 January 2011

Keywords:
Plasmalogens
Plasmenylcholine
Singlet-oxygen quenching
Singlet-oxygen phosphorescence
Lipid peroxidation
EPR oximetry
Cholesterol hydroperoxides
Free radicals

ABSTRACT

Plasmalogens are phospholipids containing a vinyl–ether linkage at the *sn*-1 position of the glycerophospholipid backbone. Despite being quite abundant in humans, the biological role of plasmalogens remains speculative. It has been postulated that plasmalogens are physiological antioxidants with the vinyl–ether functionality serving as a sacrificial trap for free radicals and singlet oxygen. However, no quantitative data on the efficiency of plasmalogens at scavenging these reactive species are available. In this study, rate constants of quenching of singlet oxygen, generated by photosensitized energy transfer, by several plasmalogens and, for comparison, by their diacyl analogs were determined by time-resolved detection of phosphorescence at 1270 nm. Relative rates of the interactions of singlet oxygen with plasmalogens and other lipids, in solution and in liposomal membranes, were measured by electron paramagnetic resonance oximetry and product analysis using HPLC-EC detection of cholesterol hydroperoxides and iodometric assay of lipid hydroperoxides. The results show that singlet oxygen interacts with plasmalogens significantly faster than with the other lipids, with the corresponding rate constants being 1 to 2 orders of magnitude greater. The quenching of singlet oxygen by plasmalogens is mostly reactive in nature and results from its preferential interaction with the vinyl–ether bond. The data suggest that plasmalogens could protect unsaturated membrane lipids against oxidation induced by singlet oxygen, providing that the oxidation products are not excessively cytotoxic.

Plasmalogens (Plg's) are widely distributed mammalian phospholipids that contain a *Z*-1′-alkenyl ether linkage at the *sn*-1 position of the glycerophospholipid backbone [1]. They comprise as much as 18% of the total phospholipid mass in humans and are particularly abundant in the brain and cardiac tissue [2]. Despite their prevalent distribution in mammalian tissues, the biological functions of Plg's remain unclear. Nevertheless, it is known that in a group of inherited diseases an impaired function or absence of peroxisomes is correlated with a reduced level of cellular Plg's. Thus in fibroblasts from Zellweger cerebrohepatorenal syndrome patients, the reduction in Plg levels varies between 60 and 80%, and in fibroblasts from the rhizomelic form of chondrodysplasia punctata, the level of plasmalogens can be reduced by 90% [3]. A selective deficiency of ethanolamine plasmalogen was identified in brain samples from patients with senile dementia [4] or Alzheimer disease [5]. Plg's are believed to play a crucial role in ocular development and function [6]. Indeed a variety of ocular abnormalities have been observed in Plg deficiency [7].

It has been postulated that plasmalogens are involved in arachidonate storage and signal transduction [8,9]. Plg's could be responsible for unique membrane protein activities and formation of lipid raft microdomains [10]. The second main hypothesis, seemingly supported by a growing body of experimental evidence, suggests that Plg's are physiological antioxidants, with the vinyl–ether bond serving as a sacrificial trap for reactive oxygen species that would otherwise attack the polyunsaturated fatty acid moieties at the nearby *sn*-2 position or in other lipid molecules [11–16]. Significantly, it was demonstrated that Plg-deficient mutants, derived from the macrophage-like cell line RAW 264.7, exhibited hypersensitivity to chemical hypoxia [17]. The mutants were also much more susceptible to cytotoxicity induced by rose bengal-photosensitized reactions. Restoration of the Plg level resulted in wild-type-like resistance to chemical hypoxia and oxidative stress.

Although the vinyl–ether bond at the *sn*-1 position is relatively resistant to enzymatic degradation, it is quite susceptible to oxidation induced by a variety of oxidizing reagents [18–21]. As a result,

Abbreviations: Plg, plasmalogen; PlgPC, plasmenylcholine; TPP, 5,10,15,20-tetraphenylporphine; RB, rose bengal; MC540, merocyanine 540; LUV, large unilamellar vesicle; EPR, electron paramagnetic resonance; 5α-OOH, 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide; 6α-OOH, 3β-hydroxycholest-4-ene-6α-hydroperoxide; 6β-OOH, 3β-hydroxycholest-4-ene-6β-hydroperoxide; 7α/β-OOH, 3β-hydroxycholest-5-ene-7α/β-hydroperoxide; DMPC, 1,2-dimyristoyl–*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SAPC, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

* Corresponding author. Fax: +48 12 664 6901.
E-mail address: tadeusz.sarna@uj.edu.pl (T. Sarna).

0891-5849/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2011.01.002

plasmalogens undergo oxidative decomposition more readily than their fatty acid ester analogs [22]. The interaction of the vinyl–ether bond with oxidizing radicals and singlet oxygen may be responsible, at least in part, for the postulated antioxidant action of plasmalogens. It was suggested that plasmalogens might interfere with the propagation step rather than the initiation step of lipid peroxidation and, unlike α-tocopherol, plasmalogens did not induce a lag phase during peroxidation of lipids [15]. It seems that products of vinyl–ether oxidation do not effectively propagate the oxidation of polyunsaturated lipids [14], and plasmalogens, being present in the membrane bilayer, may reduce the total membrane oxidizability [16,23].

The product profile of plasmalogen oxidation strongly depends on the type of esterified fatty acids at the *sn*-1 and *sn*-2 positions and the nature of oxidative stress initiators [24–26]. During Plg oxidation, hydrocarbon chains at both the *sn*-1 and the *sn*-2 positions can undergo degradation. Free radical-induced oxidation of plasmalogens yields *sn*-1-lyso-lipids, *sn*-1-formyl-lipids, chain-shortened ω-aldehydes, and γ-hydroxy-α,β-unsaturated aldehydes [27].

The reactivity of singlet oxygen with enol–ether bonds of relatively simple model compounds was studied by product analysis and indirect measurements of the singlet-oxygen lifetime some 30 years ago [28–30]. The results can be summarized as follows: (i) Enol–ethers quench singlet oxygen with a rate constant in the range 10^4–4.2×10^7 M^{-1} s^{-1}, with the rate constant generally being higher in polar solvents compared to nonpolar solvents. (ii) In polar solvents, photooxidation of enol–ethers predominantly occurs via cycloaddition leading to the formation of dioxetanes. (iii) The activation energy for the interaction of enol–ethers with singlet oxygen is very low, and the corresponding entropy change is highly negative, indicating a rapid reversible formation of an exciplex, which then decays via the rate-determining formation of oxidative products.

Products of zinc phthalocyanine-photosensitized oxidation of palmitoyl plasmenylcholine in liposomes were determined by MALDI-MS, ^{1}H NMR, and HPLC-EC [26]. Primary products included a lysolipid species, along with its hydrolysis product, and an allylic hydroperoxide species. Secondary products were ascribed to degradation of allylic hydroperoxides either via Hock rearrangement or by alkenyl ether epoxidation.

Despite this extensive research effort, there are no reliable data on rate constants of the interaction of plasmalogens with singlet oxygen, and it is unclear whether the hydrocarbon chain in the *sn*-2 position affects the interaction of Plg's with singlet oxygen and to what extent the interaction is modified by the microenvironment.

In this study, we determined the rate constants of the interactions of singlet oxygen, generated by photosensitized energy transfer, with various plasmalogens and, for comparison, with their diacyl analogs, employing time-resolved detection of singlet-oxygen phosphorescence at 1270 nm and electron paramagnetic resonance (EPR) oximetry. The kinetic measurements were supplemented by determination of lipid hydroperoxides using the iodometric assay and HPLC-EC for detection of cholesterol hydroperoxides with cholesterol employed as a unique mechanistic reporter molecule. To address the issue of how microenvironment affects the interaction of Plg with singlet oxygen, the measurements were carried out in different model systems—in homogeneous solutions of the lipids in organic solvents and organic solvent mixtures, in Triton X-100 micelles, and in liposomal membranes.

Materials and methods

Reagents

Phospholipids (PlgPC 16:0/18:1, PlgPC 18:0/18:1, PlgPC 18:0/20:4, POPC, SOPC, SAPC, DMPC, DPPC) and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and were used without further purification. Saturated plasmenylcholine (PlgPC 16:0/16:0), 1-*O*-1′-(*Z*)-hexadecenyl-2-oleoyl-*sn*-glycero-3-phosphocholine, was synthesized as described elsewhere [31]. Organic solvents were as follows: Uvasol carbon tetrachloride, LiChrosolv isopropanol, LiChrosolv methanol, LiChrosolv acetonitrile from Merck (Darmstadt, Germany); Riedel-de-Haen carbon disulfide from Sigma–Aldrich (St. Louis, MO, USA); and chloroform from POCH (Gliwice, Poland). Photosensitizers—rose bengal and merocyanine 540—were from Sigma–Aldrich, and tetraphenylporphyrin was from Aldrich Chemical Co. (Milwaukee, WI, USA). 4-Protio-3-carbamoyl-2,2,5,5-tetraperdeuteromethyl-3-pyrroline-1-yloxy (mHCTPO) was a gift from Professor H.J. Halpern (University of Chicago, Chicago, IL, USA) and used as received.

Liposomes preparation

Lipid films composed of DMPC and an additional lipid (PlgPC 16:0/16:0, PlgPC 18:0/18:1, SOPC, POPC, or Chol) were dried from chloroform under a stream of nitrogen and left under vacuum until total evaporation of organic solvent. Hydration was done in pH 7.4 phosphate-buffered solution A (PBSA) in a heated water bath at a temperature over the main-phase transition of DMPC. Afterward, liposomes were pressed through 100-nm-diameter extruder membrane to obtain a large unilamellar vesicle (LUV) suspension. All preparation steps were carried out in darkness.

Micelles preparation

Lipids were solubilized in micelles using 2% w/v Triton X-100 and PBSA (pH 7.4). Final concentration of lipids was 5 mM in a molar ratio of 3:2 of DMPC to the additional lipid (PlgPC 16:0/16:0, POPC, or Chol).

EPR measurements

Photosensitized oxygen consumption was measured by EPR oximetry using mHCTPO as a spin probe and a Bruker EMEX-AA spectrometer (Bruker BioSpin, Germany). Spectral parameters of the mHCTPO spin probe were calibrated for dissolved oxygen concentrations in water solution at room temperature [32]. The micelle solution, in flat quartz cells placed in the EPR resonant cavity, was irradiated with green light derived from a Cermax PE300CE-13FM 300-W lamp in air-cooled housing (PerkinElmer, Palo Alto, CA, USA) using a combination of filters (5 cm of aqueous solution of 5 g/L $CuSO_4$, green dichroic, and a cut-off <500 nm filter). Sample irradiance, measured by a calibrated photodiode (Hamamatsu, Photonics, K.K, Japan), was in the range 151–174 W/m^2. In experiments with liposomes, sample irradiance was ~46 W/m^2.

Singlet-oxygen quenching measurements

To determine rate constants of the interactions of singlet oxygen with plasmalogens and other lipids, time-resolved singlet-oxygen phosphorescence at 1270 nm was measured as a function of the quencher concentration. Under the experimental conditions employed, singlet oxygen was generated within a short time interval after pulse excitation of 2–3 μM tetraphenylporphyrin used as a photosensitizing dye. The experimental setup, described elsewhere [33], was as follows: a Q-switched Nd:YAG laser (Surelite II; Continuum, USA) was used as the excitation source, generating 5-ns pulses of a second (532 nm) or a third (355 nm) harmonic. Singlet-oxygen phosphorescence was detected with a nitrogen-cooled germanium detector (EO-8179P; North Coast Scientific Corp., USA). A silicon cut-off filter for transmitted light above 1100 nm and an interference filter for transmitted light around 1270 nm were used for cutting out stray light. A home-built sequence generator was used to