A novel antioxidant action of ethanolamine plasmalogens in lowering the oxidizability of membranes

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Abstract
We have demonstrated a novel antioxidant action of ethanolamine plasmalogens both in protecting cholesterol from oxidation by free radicals and in lowering the oxidizability of membranes, along with the action of scavenging radicals, by the oxygen-uptake method using large unilamellar vesicles and the water-soluble azo-radical initiator, AAPH [2,2′-azobis-(2-amidino-propane) dihydrochloride].

It is important to protect cholesterol from oxidation by free radicals in vivo, because its oxidation products, i.e., oxysterols, cause various pathological events, such as atherosclerosis [1]. Cholesterol oxidation is characterized by a low ability to propagate radical chain reactions, differing from other oxidizable lipids, such as polyunsaturated fatty acids, and therefore the most effective way to minimize the oxidative damage seems to be to lower the susceptibility of cholesterol to attack by free radicals rather than breaking cholesterol chain oxidation. We have tried to search for a physiological antioxidant for cholesterol by making use of a system to assess the oxidizability of cholesterol in phospholipid (PL) bilayers using large unilamellar vesicles (LUVs) and a water-soluble radical initiator, 2,2′-azobis-(2-amidino-propane) dihydrochloride (AAPH) [2]. Cholesterol oxidation in this system follows the classical rate law for autoxidation without any significant interference from oxidizable PL present with cholesterol, and therefore the oxidizability of cholesterol in PL bilayers can be experimentally determined using the mathematical expression $R_p/[LH]^{1/2}$ (3) where $R_p$ is the rate of oxidation, $R_p^{1/2}$ is the square root of rate of radical initiation and [LH] is the concentration of substrate) by measuring the rate of cholesterol oxidation with GLC.

We have noticed that 1-alkenyl-2-acyl-sn-glycero-3-phosphoethanolamine (ethanolamine plasmalogen) could potentially act as a physiological antioxidant for cholesterol in biomembranes, because ethanolamine plasmalogen is abundant in biomembranes containing large amounts of cholesterol, such as nervous-system myelin and red-blood-cell membranes [4]. These biomembranes appear to possess structures capable of resisting oxidative stress, especially cholesterol oxidation, because these tissues have long life spans, despite high levels of oxygen consumption and frequent exposure to oxygen. Plasmalogens are glycerophospholipids with vinyl–ether double bonds (CH$_2$–CH=CH–) at the sn-1 position of the glycerol backbone, and are widely distributed in most mammalian cells and tissues [5]. The physiological role of plasmalogens is not fully understood, but recent studies on plasmalogen-deficient mutant cells led to the proposal that these ether lipids serve to protect cells from oxidative stress as endogenous antioxidants by scavenging radicals at the vinyl–ether linkage [6–8].

With our system using LUVs and AAPH, it was found that the incorporation of bovine brain ethanolamine plasmalogen (BBEP) into LUVs decreases the oxidizability of cholesterol in PL bilayers dose-dependently, and more efficiently than bovine heart choline plasmalogen (BHCP) and its diacyl counterpart, egg-yolk phosphatidylethanolamine (EYPE) (Figure 1) [2]. Effects of these phospholipids on the time course of cholesterol oxidation were compared with that of a typical radical scavenger, α-tocopherol. All of these phospholipids (BBEP, BHCP and EYPE) reduced the rate of cholesterol oxidation dose-dependently, but α-tocopherol only introduced a lag time, while having no effect on the rate of cholesterol oxidation [2], which suggested a novel antioxidant mechanism of ethanolamine plasmalogen differing from the action of scavenging radicals.

To verify this point, total membrane oxidizability of various LUVs, including those containing BBEP, EYPE, cholesterol and α-tocopherol, were measured by the oxygen-uptake method with the water-soluble radical initiator, AAPH, and an inhibitor, Trolox (2-carboxy-2,5,7,8-tetramethyl-6-chromanol) [9]. We have previously developed a convenient procedure for assessment of the relative oxidizability of phospholipids in bilayers using a mixed-bilayer system with an inert saturated phospholipid [10]. According to this procedure, the oxidizability of each phospholipid [soyabean phosphatidylcholine (SPC), BBEP and EYPE] were respectively determined as $1.7 \times 10^{-2}$, $1.9 \times 10^{-5}$ and $1.7 \times 10^{-2} (M/s)^{1/2}$ from the expression $R_p/[LH]^{1/2}$ (as described above) [11]. These values imply that BBEP is more susceptible to oxidation than SPC and EYPE. As the total membrane oxidizability of LUVs comprising several

Key words: cholesterol, ethanolamine plasmalogen, free radical, oxidation, oxidizability.
Abbreviations used: AAPH, 2,2′-azobis-(2-amidino-propane) dihydrochloride; BBEP, bovine brain ethanolamine plasmalogen; BHCP, bovine heart choline plasmalogen; EYPE, egg-yolk phosphatidylethanolamine; LUV, large unilamellar vesicle; PL, phospholipid; SPC, soyabean phosphatidylcholine; Trolox, 2-carboxy-2,5,7,8-tetramethyl-6-chromanol.

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Figure 1 | Dose-dependent effects of BBEP, BHCP and EYPE on the oxidizability of cholesterol in bilayers

The oxidizability of cholesterol was determined by measuring the rate of cholesterol oxidation in LUVs with 10 mM AAPH in PBS containing EDTA at 37°C. Each LUV is composed of cholesterol (CH), SPC and test lipids BBEP, BHCP or EYPE in variable amounts at approximately the equivalent molar ratio of total phospholipids to cholesterol. The oxidizability values are plotted against the molar ratio of test lipid to cholesterol in LUVs. Symbols and bars represent means ± S.D. respectively.

Figure 2 | Effects of (A) EYPE, (B) BBEP, (C) cholesterol (CH) and (D) α-tocopherol on total membrane oxidizability

The total membrane oxidizability of LUVs was evaluated as the $R_p/R_i^{1/2}$ value, measured by the oxygen uptake method with AAPH and Trolox. The effects of BBEP, EYPE, cholesterol and α-tocopherol on the total membrane oxidizability were examined by changing the content of each test lipid and antioxidant in LUVs. The total membrane-oxidizability measurements of SPC/EYPE-mixed LUVs were almost constant, independent of the mixed ratio, while those of SPC/BBEP-mixed LUVs decreased greatly.

oxidizable lipids is dependent on the oxidizability and concentration of each lipid in the vesicles, this can be conveniently expressed as:

$$\left\{ \left( \frac{R_p}{[LH]} \right)^{1/2} \times [LH]_1 + \left( \frac{R_p}{[LH]} \right)^{1/2} \times [LH]_2 \cdots + \left( \frac{R_p}{[LH]} \right)^{1/2} \times [LH]_{n-1} + \left( \frac{R_p}{[LH]} \right)^{1/2} \right\} = \frac{R_p}{R_i^{1/2}}$$

Accordingly, the total membrane oxidizability of LUVs was evaluated as the $R_p/R_i^{1/2}$ value, measured by the oxygen uptake method with AAPH and Trolox. The values are plotted against the constituent molar ratio of test lipid to total phospholipids (TPL) in LUV (A)–(C), or the mol% of α-tocopherol (Toc.) in LUVs in (D).
with an increasing content of BBEP in the vesicles, despite the high oxidizability of BBEP itself (Figures 2A and 2B) [11]. The incorporation of cholesterol into LUVs also led to a dose-dependent lowering of the total membrane oxidizability (Figure 2C) [11]. On the other hand, the incorporation of α-tocopherol into LUVs did not affect the membrane oxidizability (Figure 2D) [11], but prolonged an induction period dose-dependently. These results strongly suggest the presence of a novel antioxidant mechanism for ethanolamine plasmalogen in lowering the oxidizability of membranes.

Ethanolamine plasmalogen reduced the total membrane oxidizability more efficiently in the presence of cholesterol in LUVs [11]. This was confirmed visually, using electron microscopy to show that the collaborative action of ethanolamine plasmalogen and cholesterol in reducing the membrane oxidizability serves the oxidative stability of LUV [12]. This action may be important in contributing towards the resistance to oxidative stress of the biomembranes of nervous-system myelin and red blood cells, which have long life spans.

References

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