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Ubiquinone-10 alters mechanical properties and increases stability of phospholipid membranes

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ABSTRACT

Ubiquinone-10 is mostly known for its role as an electron and proton carrier in aerobic cellular respiration and its function as a powerful antioxidant. Accumulating evidence suggest, however, that this well studied membrane component could have several other important functions in living cells. The current study reports on a previously undocumented ability of ubiquinone-10 to modulate the mechanical strength and permeability of lipid membranes. Investigations of DPH fluorescence anisotropy, spontaneous and surfactant induced leakage of carboxyfluorescein, and interactions with hydrophobic and hydrophilic surfaces were used to probe the effects caused by inclusion of ubiquinone-10 in the membrane of phospholipid liposomes. The results show that ubiquinone in concentrations as low as 2 mol.% increases the lipid packing order and condenses the membrane. The altered physicochemical properties result in a slower rate of release of hydrophilic components, and render the membrane more resistant towards rupture. As judged from comparative experiments using the polyisoprenoid alcohol solanesol, the quinone moiety is essential for the membrane stabilizing effects to occur. Our findings imply that the influence of ubiquinone-10 on the permeability and mechanical properties of phospholipid membranes is similar to that of cholesterol. The reported data indicate, however, that the molecular mechanisms are different in the two cases.

1. Introduction

Ubiquinones constitute an interesting class of fat-soluble compounds present in the plasma and inner membranes of most eukaryotic cells [1], as well as in the membranes of gram-negative bacteria [2]. They consist of a substituted quinone headgroup linked to an isoprenoid chain that is of varying length depending on the species. In humans, the chain contains ten isoprenoid units and human ubiquinone is therefore referred to as ubiquinone-10 (UQ-10), or, simply, Q10 (chemical structure shown in Fig. 1a). Another common name for Q10 is coenzyme Q10 (CoQ10). Q10 is involved in several aspects of the cellular metabolism, including the mitochondrial respiration chain, where it plays an essential role in the electron and proton transport across lipid membranes. Moreover, the fully reduced form of Q10, ubiquinol, acts as a powerful antioxidant that protects the membrane lipids from peroxidation [3–6]. Some less well studied biological aspects of Q10 include its potentially important role in cell growth [7] and certain forms of apoptosis [8,9]. The various confirmed and suggested functions of Q10 (which have been reviewed previously [3,10]) motivate further, more detailed, studies of both the biological and physical roles of Q10 in cell membranes. Such studies are important also from a medical/pharmaceutical point of view, since genetic mutations, disease and ageing can affect Q10 levels in the cells and consequently result in serious health issues [10–13].

The exact location and orientation of Q10 in the lipid membrane, as well as its effect on the membrane physico-chemical properties, is to a great degree still a matter of discussion [10,14,15]. One of the most accepted models states that the isoprenoic chain is embedded in the mid-plane of the apolar region, whereas the quinone ring alternates between the membrane mid-plane and polar head-group region [14]. This central localization of Q10 has been suggested to destabilize the membrane and increase its fluidity and permeability [9,10]. Early studies indicating that the lysis of red blood cells can be prevented by the inclusion of ubiquinone-6 (Q6) in the membrane [16] speak, however, against this hypothesis, although the observations may also suggest radical differences between the effects of Q6 and Q10. More importantly, recently published research has shown that the levels of native ubiquinones in bacteria increase by more than 100 fold when the cells are subjected to prolonged osmotic stress [17]. This has led to speculation concerning a possible mechanical-stability enhancing role of ubiquinones in lipid membranes [18]. Moreover, fluorescence anisotropy measurements performed with a series of different ubiquinones, including Q10, point towards a general pronounced and concentration dependent membrane ordering effect of ubiquinones [15]. Interestingly, the data reported by Jemiola-Rzeminska et al. [15] suggest that Q10 may cause an increase in acyl chain order that is even more pronounced than that observed for cholesterol under comparable conditions [19]. Since cholesterol is well known for its condensing and stabilizing effect in biological lipid membranes [20–22], it is tempting to speculate about a

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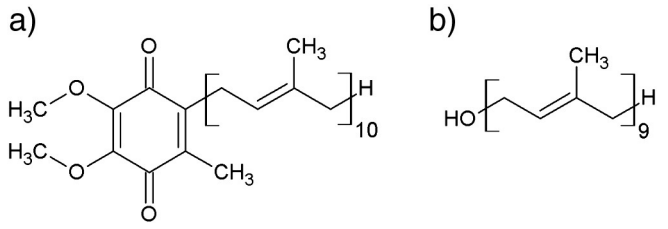

Fig. 1. Molecular structure of a) Q10 and b) solanesol.

similar function for Q10. It is in this context noteworthy that several ubiquinone-rich biological membranes, such as those of mitochondria and aerobic gram-negative bacteria, typically contain very low levels of cholesterol. It is thus plausible that an additional important function of ubiquinone in these membranes is to fulfill the membrane-stabilizing role played by cholesterol in many other types of biological membranes. In order to confirm this hypothesis it remains, however, to verify that the reported Q10 dependent increase in membrane order [15] is indeed coupled to a higher mechanical stability of the membranes.

In this study we characterize the effect of Q10 on several stability related properties of 1-palmitoyl-2-oleyl-sn-glycero-phosphocholine (POPC) liposomes, including their membrane density and permeability, resistance to solubilization by detergents, and promptness to rupture on solid surfaces. We also investigate the effect of Q10 on the degree of order of the acyl chains, as well as on the recently described spontaneous formation of hydrophobic defects, or active sites, in the lipid membrane [23]. The results are compared to what is obtained under similar conditions with liposomes formed by pure lipids, as well as with liposomes supplemented with cholesterol or solanesol. The latter molecule is structurally similar to Q10 but contains 9 instead of 10 isoprene units and the quinone headgroup is replaced by a hydroxyl functional group (see Fig. 1b). Studies with solanesol will help us discern whether the quinone ring is essential for the effects induced by Q10 on the properties of the lipid membrane.

2. Materials and methods

2.1. Chemicals

Dry powder of 1-palmitoyl-2-oleyl-sn-glycero-phosphocholine (POPC) was either purchased from Avanti Polar Lipids (Alabaster, AL), or obtained as a kind gift from Lipoid Gmbh (Ludwigshafen, Germany). No differences between the two providers were observed in the performed experiments. Ubiquinone-10 (Q10), solanesol, cholesterol, polyethylene glycol tert-octylphenyl ether (Triton X-100), 5(6)-carboxyfluorescein (CF), octaethylene glycol monododecyl ether ($C_{12}E_8$), 1,6-diphenyl-1,3,5-hexatriene (DPH), methanol and hexane were purchased from Sigma-Aldrich (Steinheim, Germany). 99.5% spectroscopic grade ethanol was from Kemetyl (Haninge, Sweden), and chloroform was from MERCK (Darmstadt, Germany). Polystyrene nanoparticles, 115 ± 4 nm, 5% *w/v*, were from MicroParticles Gmbh (Berlin, Germany). A phosphate buffer saline (PBS, 10 mM phosphate, 150 mM NaCl, pH 7.4) was used for the measurements performed at a controlled pH value. All aqueous solutions were prepared using deionized water (18.2 M Ω cm) obtained from a Milli-Q system (Millipore, Bedford, USA). Experiments were performed at room temperature (~22 °C) unless indicated otherwise.

2.2. Liposome preparation

Liposomes were prepared by either extrusion or sonication, depending on the preferred final size distribution. For both methods, the desired amount of lipid was first weighted and dissolved in chloroform. For Q10 or solanesol containing liposomes the necessary amount from a stock solution (Q10 stock: 1 mg/mL in 1:1 chloroform:ethanol, solanesol stock: 1 mg/mL in ethanol) was added to obtain the desired composition of the lipid mixture. The solvent was then evaporated under a constant stream of nitrogen until a homogenous film was obtained. Remaining traces of solvent were removed by placing the samples under vacuum overnight (Squaroid vacuum oven, Lab Line Instruments, IL). The lipid film was thereafter suspended in the desired aqueous solution (PBS if not otherwise indicated).

To produce small unilamellar vesicles (SUVs) the lipid film was hydrated for 40 min in a water bath at 60 °C. The obtained suspension was thereafter sonicated with a tip-sonicator (Soniprep 150, MSE, London, UK) for 45 min with a surrounding ice-bath to finally obtain SUVs. Afterwards, the suspension was centrifuged for 15 min at 10,000 rpm to remove titanium debris produced during the sonication procedure. To create large unilamellar vesicles (LUVs), the lipid suspension obtained after hydration of the lipid film was subjected to five freeze–thaw cycles (freeze in liquid nitrogen, thawing in a water bath at 60 °C). The suspension was thereafter extruded 31 times using a Lipofast extruder (Avestin, Ottawa, Canada) with a 100 nm pore size filter (Watman plc, Kent UK).

After preparation, the suspensions were diluted to the desired concentration and stored at room temperature for 24 h before performing the experiments, with the exception of the spontaneous leakage experiments which are described below. This was done to account for the activation–deactivation phenomenon described in a previous report [23], where we showed that experiments with freshly prepared liposomes may result in irreproducible data. According to this report, after 24 h of incubation the suspensions have equilibrated and the results are more reproducible and reliable.

2.3. Liposome characterization

2.3.1. Size determination

The size distribution of the liposome suspensions was measured by dynamic light scattering (DLS). A uniphase He–Ne laser producing vertically polarized light at 638.2 nm and operating at 25 mW was used as a light source. Data was collected at a 90° angle with a PerkinElmer diode detector (Quebec, Canada) connected to a ALV-5000 multiple digital autocorrelator (ALV-laser Vertriebsgellschaft mbH, Germany). The lipid concentration in the samples was between 0.5 and 1 mM.

2.3.2. Cryo-TEM characterization

The structure and morphology of the formed structures were characterized with cryogenic transmission electron microscopy (Cryo-TEM). Analyses were performed with a Zeiss TEM Libra 120 instrument (Carl Zeiss AG, Oberkochen, Germany) operating at 80 kV, in a zero-loss bright-field mode. The digital images were recorded under low-dose conditions with a BioVision Pro-SM Slow Scan CCD camera (Proscan elektronische systeme GmbH, Scheuring, Germany). All the investigated samples had a lipid concentration of 1–10 mM. The sample preparation method before microscopy has been described in detail by Almgren et al. [24]. Briefly, a small drop (~1 μL) of sample is deposited on a copper grid, reinforced with a holey polymer film, and gently blotted to remove the excess of fluid. This thin film of sample is then quickly vitrified in liquid ethane and transferred to the electron microscope. The sample preparation was made in a custom-built climate chamber at 25 °C and at ~98–100% relative humidity. Samples were kept below −165 °C and protected against atmospheric conditions during the transfer from the climate chamber and the sample examination.

2.3.3. Determination of Q10 and solanesol content

In order to determine the proportion of Q10 and solanesol present in the samples, the compounds were first extracted from the suspended lipid phase according to a slightly modified version of the protocol described by Kroger [25]. More specifically, an aliquot of the liposome

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