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Protective Effects of Mitochondria-Targeted Antioxidant SkQ in Aqueous and Lipid Membrane Environments

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Abstract The antioxidant activity of mitochondria-targeted small molecules, SkQ1 and MitoQ (conjugates of a lipophilic decyltriphenylphosphonium cation with an antioxidant moiety of a plastoquinone and ubiquinone, respectively), was studied in aqueous solution and in a lipid environment, i.e., micelles, liposomes and planar bilayer lipid membranes. Reactive oxygen species (ROS) were generated by azo initiators or ferrous ions with or without *tert*-butyl-hydroperoxide (t-BOOH). Chemiluminescence, fluorescence, oxygen consumption and inactivation of gramicidin peptide channels were measured to detect antioxidant activity. In all of the systems studied, SkQ1 was shown to effectively scavenge ROS. The scavenging was inherent to the reduced form of the quinone ($SkQ1H_2$). In the majority of the above model systems, SkQ1 exhibited higher antioxidant activity than MitoQ. It is concluded that $SkQ1H_2$ operates as a ROS scavenger in both aqueous and lipid environments, being effective at preventing ROS-induced damage to membrane lipids as well as membrane-embedded peptides.

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Keywords SkQ · MitoQ · Mitochondria-targeted antioxidant · Liposome · Bilayer lipid membrane · Reactive oxygen species · Gramicidin

Abbreviations

MitoQ	10-(6′-Ubiquinonyl)decyltriphenyl-phosphonium
SkQ1	10-(6′-Plastoquinonyl)decyltriphenyl-phosphonium
CoQ1	Ubiquinone-1
BLM	Bilayer lipid membrane
ROS	Reactive oxygen species
ML	Methyl ester of linoleate
AAPH	2,2′-Azobis(2-amidinopropane)-dihydrochloride
AlPcS3	Aluminum phthalocyanine trisulfonate
BR	Rose bengal
MB	Methylene blue
DPhPC	Diphytanoylglycerophosphocholine
DPhPG	Diphytanoylphosphatidylglycerol
TBARS	Thiobarbituric acid-reactive species
t-BOOH	*Tert*-butyl-hydroperoxide

Introduction

Unraveling the crucial role of reactive oxygen species (ROS) in pathophysiology has led to growing attention to antioxidants and their application for treating a variety of diseases. Recently, the efficiency of antioxidants was strongly increased by linking an antioxidant moiety to a residue, which addresses the compound to a specific intracellular target. As mitochondria represent a major source of ROS in eukaryotic cells and mitochondrial oxidative damage contributes to a wide range of pathologies,

including cardiovascular disorders and neurodegenerative diseases (Papa and Skulachev 1997; Orrenius 2007), mitochondria-targeted antioxidants were synthesized. In 2001, Murphy and coworkers began reporting on the synthesis of 10-(6′-ubiquinonyl)decyltriphenylphosphonium (MitoQ), which proved to be effective at preventing oxidative stress in isolated mitochondria and in ischemic heart (Kelso et al. 2001; Adlam et al. 2005; Murphy and Smith 2007). In 2007, a newly synthesized mitochondria-targeted antioxidant, 10-(6′-plastoquinonyl)decyltriphenylphosphonium (SkQ1), was reported by our group to be a very efficient agent, preventing a variety of degenerative processes and prolonging the life span (Skulachev 2007a).

Both MitoQ and SkQ1 contain a lipophilic decyltriphenylphosphonium cation covalently attached to an antioxidant moiety of a quinone type (ubiquinone in MitoQ and plastoquinone in SkQ1, Fig. 1). As shown in Liberman et al. (1969), tetraphenylphosphonium cations are highly permeable for both artificial and natural membranes, though their permeability is much less than that of lipophilic anions, such as tetraphenylborate (Liberman and Topaly 1969; Laeuger et al. 1981; Zimmermann et al. 2008). Reduced quinones, in particular ubiquinol and plastoquinol, are known to be lipophilic antioxidants, inhibiting lipid peroxidation in both natural (Ernster and Dallner 1995, and refs. therein; Hundal et al. 1995) and artificial (Frei et al. 1990; Landi et al. 1991; Kagan et al. 1990; Shi et al. 1999; Xia et al. 2007) membranes.

The fact that MitoQ prevents lipid peroxidation in mitochondrial membranes (Asin-Cayuela et al. 2004) was considered to be an indication that this compound operates as a ROS scavenger. However, no data have been presented so far on the antioxidant activity of MitoQ in model systems, which are not as complicated as membranes of intact mitochondria. Here, we applied a variety of biophysical methods to characterize the mode of SkQ1 and MitoQ action in aqueous solution, micelles, unilamellar lipid vesicles (liposomes) and planar bilayer lipid membranes (BLMs).

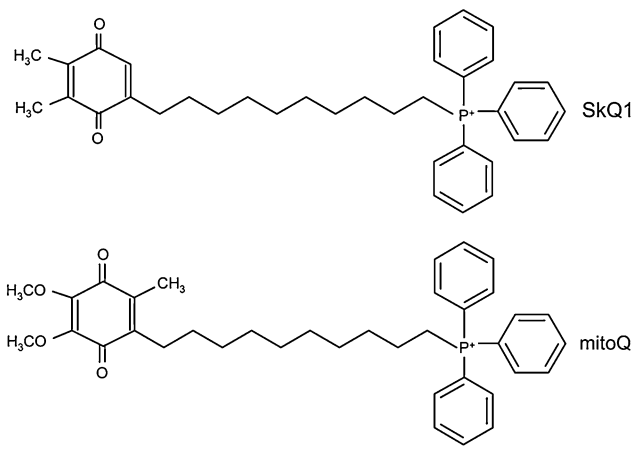

Fig. 1 Chemical structure of SkQ1 and MitoQ

The protective effect of antioxidants in model membranes is usually studied by their ability to prevent lipid peroxidation. On the other hand, oxidative damage to membrane proteins plays an important role in numerous pathologies (Ernst et al. 2004; Mattson 2004; Brame et al. 2004; Fukuda et al. 2005; Choksi et al. 2007). By measuring an electric current across the BLM, we examined the functional consequence of oxidative injury using a membrane peptide, gramicidin A. This pentadecapeptide contains four tryptophan residues that are especially susceptible to damage by ROS (Stark 1991; Strassle and Stark 1992; Rokitskaya et al. 1993, 1996). Gramicidin A is known to form ion channels in BLMs, which results in an increase in electric current across the BLM (Hladky and Haydon 1984). The inhibition of the gramicidin-induced currents caused by ROS has proved to be a convenient procedure for detecting a damaging effect of ROS on membrane proteins (Rokitskaya et al. 1996, 2000; Chernyak et al. 2006). This approach was also applied in our study.

Materials and Methods

BODIPY 581/591 C11 was purchased from Molecular Probes (Eugene, OR), luminol (5-amino-2,3-dihydro-1,4-phthalhydrazinedione) was from MP Biomedicals (Solon, OH), methyl ester of linoleate (ML) and Triton X-100 were from Sigma (St. Louis, MO), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was from Polysciences (Warrington, PA), aluminum phthalocyanine trisulfonate ($AlPcS_3$) was from Porphyrin Products (Logan, UT), rose bengal was from Sigma, methylene blue (MB) was from Serva (Heidelberg, Germany), diphytanoyl-glycero-phosphocholine (DPhPC) and diphytanoyl phosphatidylglycerol (DPhPG) were from Avanti Polar Lipids (Birmingham, AL), thiobarbituric acid (TBA) was from Sigma, t-BOOH was from Sigma and gramicidin A was from Fluka (Buchs, Switzerland). SkQ1 and MitoQ were synthesized by Drs. G. Korshunova and N. Sumbatyan in our group as described by Skulachev (2007b) and Kelso et al. (2001), respectively. Other chemicals were from Sigma and inorganic salts were from Reakhim (Moscow, Russia).

Chemiluminescence Assay

Luminol (0.1 M) was dissolved in dimethyl sulfoxide. 2,2′-Azobis(2-amidinopropane)dihydrochloride (0.1 M), a free radical generator, was dissolved in water. The experiments were performed in buffer containing 100 mM KCl, 20 mM TRIS and 0.2 mM EDTA (pH 8.0). The final concentrations

of luminol and AAPH were 0.1 and 10 mM, respectively. Photons were counted by a homemade luminometer constructed on the basis of a Hamamatsu Photonics (Shizuoka, Japan) H6240-02 photomultiplier operating as a photon-counting device connected to an M7824 counting board and a personal computer.

Oxidation of ML in Triton X-100 Micelles Induced by AAPH

The kinetics of oxygen consumption (Roginsky 2003; Roginsky et al. 2003) was studied with a computerized 5300 Oxygen Biological Monitor (Yellow Springs Instruments, Youngstown, OH) using a Clark electrode as an O_2 sensor. Experiments were performed at 37°C in 50 mM phosphate buffer (pH 7.4) containing 20 mM ML and 50 mM Triton X-100. SkQ1 and MitoQ were reduced by $NaBH_4$ in acidic phosphate buffer (pH 5.0).

Preparation of Liposomes

Liposomes were prepared by evaporation (under a stream of nitrogen) of a 2% solution of soybean phospholipid (asolectin, Sigma) in chloroform, followed by hydration with a buffer solution containing 100 mM KCl and 10 mM KH_2PO_4 (pH 7.0, 20 mg/ml). The mixture was vortexed, passed through a cycle of freezing and thawing and sonicated five times for 15 s with the Virsonic 100 device (Virtis, Gardiner, NY).

TBA Assay for Lipid Peroxidation

Lipid peroxidation was assayed as described by Placer et al. (1966) with some modifications. The liposome suspension (0.5 mg/ml, 0.1 ml) was incubated with 100 μM $FeSO_4$, 1 mM ascorbate and 30 mM t-BOOH. TBA (0.8 ml of 0.06 M) was added with 0.5 ml of 1% Triton X-100 and 0.2 ml of 0.6 M HCl. The tubes were heated at 100°C for 10 min, then placed in water at 25°C for 30 min for cooling. To stabilize the reaction products, 0.2 ml 5 mM EDTA and 2 ml 96% ethanol were added. Fluorescence was recorded at 550 nm (excitation 515 nm). The assay was calibrated using the reaction with 1,1′,3,3′-tetraethoxypropane.

Planar BLMs

BLM was formed on a hole (0.55-mm diameter) in a Teflon partition separating two compartments of a chamber containing aqueous solutions of 100 mM KCl, 10 mM morpholinoethanesulfonic acid (MES) and 10 mM Tris (pH 7.0). The membrane-forming solutions contained 20 mg DPhPC in 1 ml *n*-decane. Gramicidin A was added from ethanol stock solution to the bathing solutions on both sides of the BLM and incubated for 15 min with constant stirring. Experiments were carried out at room temperature (24–26°C). A photosensitizer was added to the bathing solution on the *trans*-side (the *cis*-side is the front side with respect to the flash lamp). The electric current (I) was recorded under voltage-clamp conditions. I_t was measured with a Keithley 428 amplifier, digitized by a LabPC 1200 (National Instruments, Austin, TX) and analyzed on a personal computer using WinWCP Strathclyde Electrophysiology Software, designed by J. Dempster (University of Strathclyde, Strathclyde, UK). Ag-AgCl electrodes were placed into the bathing solutions on both sides of the BLM, and the 30 mV voltage was applied. The BLM was exposed to 20 s continuous illumination with a halogen lamp (Novaflex; World Precision Instruments, New Haven, CT; power density 30 mW/cm^2). Alternatively, single flashes produced by a xenon lamp (flash energy 400 mJ/cm^2, flash duration <2 ms) were used for the light treatment of the BLM. A glass filter cutting off light with wavelengths <500 nm was placed in front of the lamp. To avoid photoelectrical artifacts, the electrodes were covered with black plastic tubes.

Illumination of the BLM by visible light in the presence of a photosensitizer is known to suppress the gramicidin-mediated transmembrane current (Strassle and Stark 1992; Rokitskaya et al. 1993, 1996; Kotova and Antonenko 2005). The photoinactivation of gramicidin A in BLM results from the damage to its tryptophan residues, caused by ROS that are generated upon interaction of an excited photosensitizer molecule with oxygen (Kunz et al. 1995; Sobko et al. 2004; Stark 2005). It has been shown that the light-induced decrease in the gramicidin-mediated current is due to the reduction of the number of open channels, while the single-channel conductance remains unchanged (Rokitskaya et al. 1993). Therefore, the relative decrease in the current, $\alpha = (I_0 - I)/I_0$, induced by illumination is equal to the damaged portion of gramicidin channels. This parameter, "amplitude of photoinactivation," enables us to compare the efficacy of different photosensitizers and antioxidants.

Optical Measurements

Fluorescence emission spectra were recorded with a Panorama Fluorat 02 fluorescence spectrophotometer (Lumex, St. Petersburg, Russia) with excitation and emission slits adjusted to 5 nm. Transient absorbance changes at 680 nm were measured using a homemade single-beam differential spectrophotometer with a resolution time of 1 μs (Drachev et al. 1993). An OSRAM-24-150 lamp (Osram, Munich, Germany) was employed as the source of the measuring beam. Excitation flashes (355 nm, 10 ns, 15 mJ) were

provided by a Quantel (Les Ulis, France) Nd-YAG laser. The signals were recorded by a PMT-84 photomultiplier and converted by the digital oscilloscope card Gage-CS8012A. Each kinetic curve represented the average of 16 measurements.

Results and Discussion

It has been shown that scavenging of peroxyl radicals in aqueous solution can be monitored by the ability of an agent to inhibit the luminol-mediated chemiluminescence induced by a water-soluble azo-initiator, AAPH (Lissi et al. 1992; Krasowska et al. 2000). In this system, the antioxidant activity manifests itself as quenching of chemiluminescence. Disappearance of the chemiluminescence inhibition in time is due to exhaustion of an active form of the antioxidant. Figure 2A shows the effect of 0.5 μM SkQ1 on the time course of the AAPH-induced chemiluminescence. It is seen that attenuation of the chemiluminescence by reduced SkQ1 ($SkQ1H_2$) was much more pronounced and prolonged compared to that of its oxidized form (SkQ1). When added at higher concentrations, $SkQ1H_2$ caused irreversible suppression of chemiluminescence (data not shown). The effect of $MitoQH_2$ was ~50% that of $SkQ1H_2$ (Fig. 2B). As to oxidized MitoQ, it proved to be completely ineffective.

According to Roginsky et al. (2003), antioxidant activity of quinols can be measured by recording the oxygen consumption that accompanies the peroxidation of ML in micelles of Triton X-100. The antioxidant activity of SkQ1 and MitoQ in the reduced form (quinols) was characterized by the rate constant k_1 for the reaction between the peroxy radical of oxygenated linoleate residue ($LO_2^{\bullet}$) and a quinol (QH_2):

$$LO_2^{\bullet} + QH_2 \rightarrow LOOH + QH^{\bullet}$$

which competes with the reaction of the chain propagation (the rate constant k_2):

$$LO_2^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

The experiment was started with determination of the rate of noninhibited oxidation of ML, R_0. The addition of a quinol resulted in a strong decrease in the rate of oxygen consumption and the appearance of the pronounced induction period (Fig. 3A). Importantly, the oxidized forms of SkQ1 and MitoQ were inactive in this system. As the antioxidant was consumed, the rate of inhibited oxidation (R) increased progressively with time, reaching finally the level of R_0 (Fig. 3A). The time course of changes in R was determined by the equation:

$$F = ln\frac{1 + R/R_0}{1 - R/R_0} - \frac{R_0}{R} = \frac{k_1 R_0}{k_2[LH]}t + const \qquad (1)$$

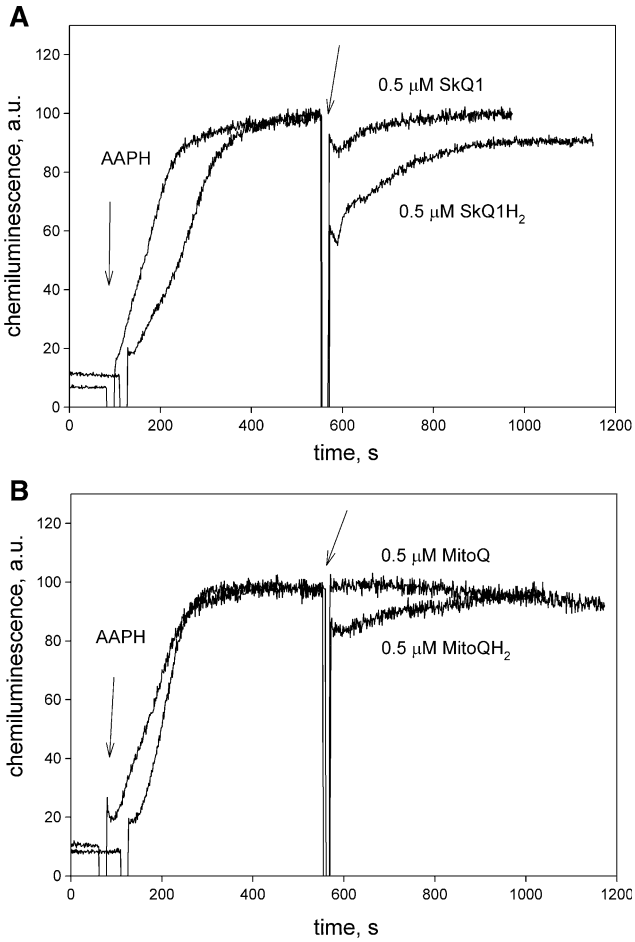

Fig. 2 Effect of the reduced and oxidized forms of SkQ1 (**A**) and MitoQ (**B**) on the intensity of AAPH-induced chemiluminescence of luminol

where [*LH*] is the ML concentration. The k_1/k_2 value was determined as the slope of an F vs. t plot (Fig. 3B). In more detail, this approach was reported elsewhere (Roginsky et al. 2003; Loshadkin et al. 2002).

The absolute values of k_1 were calculated from k_1/k_2 assuming $k_2 = 60\ m^{-1}\ s^{-1}$ (Roginsky et al. 2003). The values of k_1 for $SkQ1H_2$ and $MitoQH_2$ were $2.2 \cdot 10^5$ and $0.58 \cdot 10^5\ m^{-1}\ s^{-1}$, respectively. Thus, the reactivity of $SkQ1H_2$ toward $LO_2^{\bullet}$ was almost four times higher than that of $MitoQH_2$. This is in line with data obtained for more simple analogues of $SkQ1H_2$ and $MitoQH_2$, trimethyl-1,4-hydroquinone (TMHQ) and 2,3-dimetoxy-5-methyl-1,4-hydroquinone (TMOHQ): TMHQ was found to be several times more reactive than TMOHQ in both homogeneous (Loshadkin et al. 2002) and micellar (Roginsky et al. 2003) systems. Most likely, the reason for reduced reactivity of $MitoQH_2$ compared to $SkQ1H_2$ is the same as in the case of TMOHQ and TMHQ (Loshadkin et al. 2002), namely, the increased stabilization of a phenoxy radical produced from $SkQ1H_2$ compared to that formed from $MitoQH_2$.

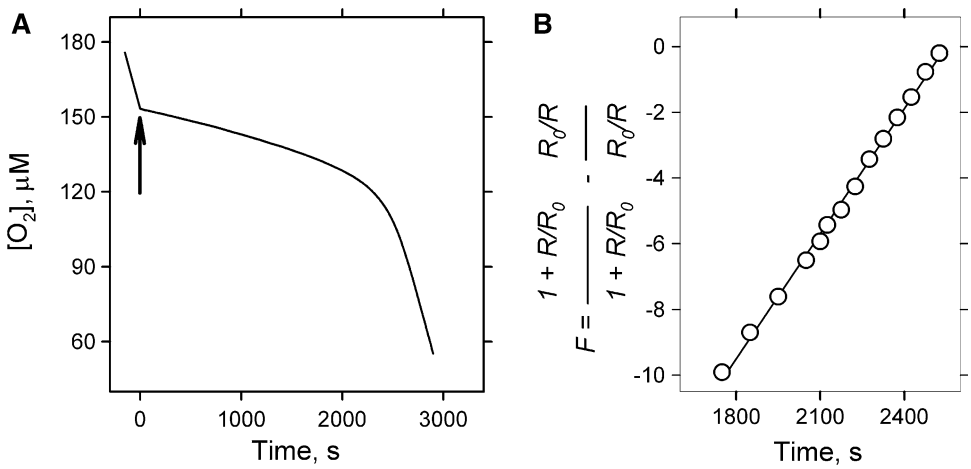

Fig. 3 Effect of $SkQ1H_2$ on the time course of ML oxidation measured by oxygen consumption in Triton X-100 micelles. (**A**) Addition of 6 μm $SkQ1H_2$ is marked by an *arrow*. The concentration of ML was 20 mM. (**B**) Part of the data of **a** presented in the coordinates *F* vs. time following Eq. 1

In a further series of experiments, we examined the antioxidant action of SkQ1 and MitoQ in model lipid membranes. First, we used one of the most conventional methods to assess lipid peroxidation, i.e., TBA-reactive substances (TBARS) assay. Lipid peroxidation in asolectin liposomes was induced by the addition of 100 μM $FeSO_4$, 1 mM ascorbate and 30 mM t-BOOH. Under these conditions, TBARS formation was mainly due to production of malondialdehyde. This process was found to be substantially slowed down in the presence of 10 μM $SkQ1H_2$ compared to the control (Fig. 4A), SkQ1 being much less effective (Fig. 4B).

To directly follow the kinetics of oxidative lipid destruction, we used C11-BODIPY(581/591), a fluorescent lipid peroxidation probe (Naguib 1998; Drummen et al. 2002). From the fluorescence traces depicted in Fig. 5, it is seen that addition of 5 μM Fe^{2+} led to a very strong inhibition of BODIPY fluorescence in asolectin liposomes. The Fe^{2+}-induced fluorescence changes were almost completely prevented in the presence of 0.5 μM $SkQ1H_2$. Oxidized SkQ1 was without any effect.

To model membrane protein damage caused by ROS, we initially applied the method of light-induced inactivation of gramicidin channels developed in our laboratory (Rokitskaya et al. 1996; Kotova and Antonenko 2005). It has been previously shown that illumination of a BLM with visible light (both continuous light and single flashes) in the presence of a photosensitizer (e.g., trisulfonated aluminum phthalocyanine, $AlPcS_3$ (Rokitskaya et al. 1996), or rose bengal (Kotova et al. 2000)) results in a decrease in the gramicidin-mediated current across the BLM. As seen from Fig. 6, 0.4 μM $SkQ1H_2$ or SkQ1 partially prevented a flash-induced decrease in the gramicidin-mediated current occurring if $AlPcS_3$ was added. From the data presented in Fig. 7, it follows that SkQ1 protected gramicidin channels from photoinactivation in the presence of different photosensitizers, i.e., $AlPcS_3$, rose bengal and MB. The

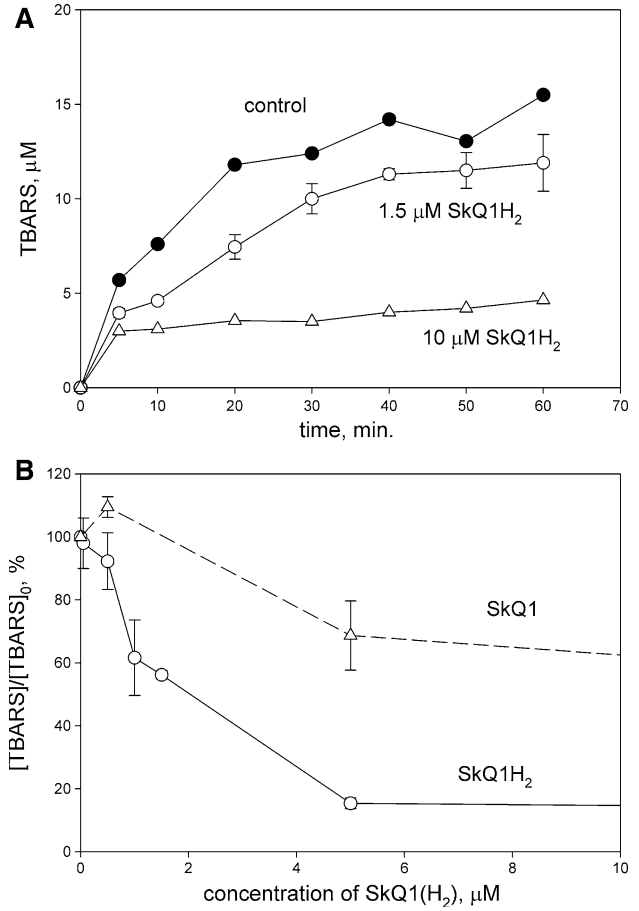

Fig. 4 (**A**) Effect of SkQ1 and $SkQ1H_2$ on the time course of TBARS formation resulting from lipid peroxidation in asolectin liposomes, induced by addition of 100 μM $FeSO_4$, 1 mM ascorbate and 30 mM t-BOOH. The solution was 100 mM KCl and 10 mM KH_2PO_4 (pH 7). (**B**) Dependence of the concentration of TBARS formed during 20 min of lipid peroxidation in asolectin liposomes on the concentrations of $SkQ1H_2$ and SkQ1

protective effect was half-maximal at 0.5 μM SkQ1 with $AlPcS_3$ and at even lower concentration of SkQ1 with rose bengal. MitoQ was less effective than SkQ1 in this system.

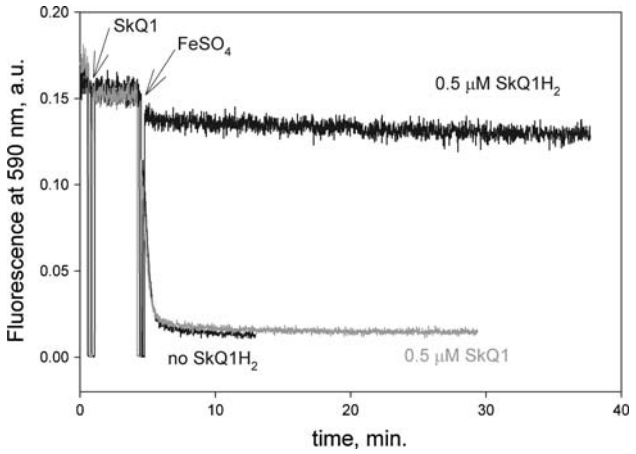

Fig. 5 Time course of the suppression of C11-BODIPY fluorescence at 590 nm induced by the addition of Fe^{2+} (5 μM) in the presence of $SkQ1H_2$ or SkQ1. Liposome and C11-BODIPY concentrations, 40 μg/ml and 10 nM, respectively. Incubation mixture: 100 mM KCl and 10 mM KH_2PO_4 (pH 7.0)

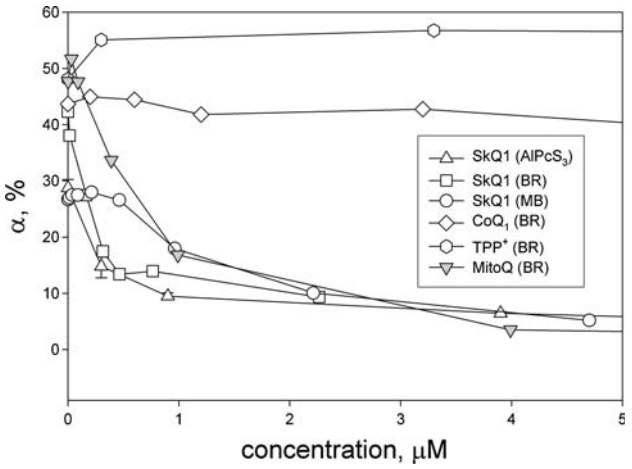

Fig. 7 Dependence of the amplitude of photoinactivation, $\alpha = 100(I_0 - I)/I_0$, on the concentration of SkQ1, MitoQ, CoQ_1 and tetraphenylphosphonium chloride (TPP^+). Photosensitizer: $AlPcS_3$ (1 μM), rose bengal (BR, 1 μM) or MB (0.3 μM)

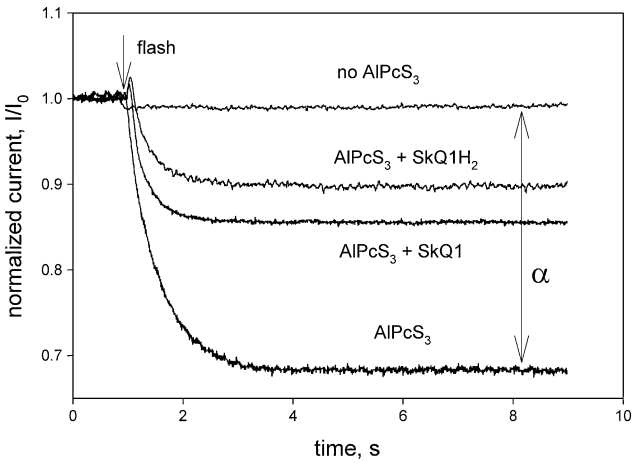

Fig. 6 Protective effect of SkQ1 on the damage of gramicidin A channels in BLM by singlet oxygen. The time course of the light-induced decrease in the gramicidin-mediated current (*I*) across a planar bilayer lipid membrane. *Upper curve* was recorded without a photosensitizer; other records were in the presence of 1 μM $AlPcS_3$. Additions: 0.4 μM SkQ1 or 0.4 μM $SkQ1H_2$

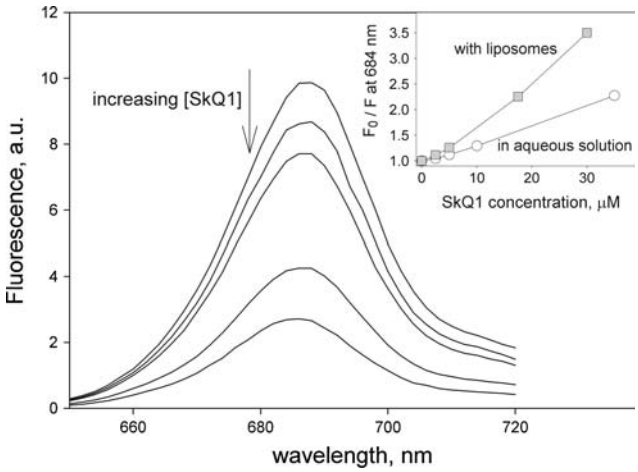

Fig. 8 Fluorescence emission spectra of $AlPcS_3$ at increasing concentrations of SkQ1 in aqueous buffer solution in the presence of egg yolk phosphatidylcholine liposomes (25 μg/ml). $AlPcS_3$ concentration was 1 μM. Excitation was at 375 nm. The solution was 100 M KCl, 10 mM MES, 10 mM Tris (pH 7.0). *Inset* Stern-Volmer plots of the quenching of $AlPcS_3$ fluorescence by SkQ1, i.e., F_0/F vs. the concentration of SkQ1 (where F_0 and F represent fluorescence intensities in the absence and presence of SkQ1, respectively), in the absence (*circles*) and in the presence (*squares*) of liposomes

Ubiquinone-1 (CoQ_1) was effective at much higher concentrations than SkQ1 (15–20 μM). No effect was observed with tetraphenylphosphonium, a close analogue of the charged component of SkQ1.

All of the photosensitizers employed are known to be potent generators of singlet oxygen upon excitation (Nyokong 2007; Gabrielli et al. 2004; Gandin et al. 1983). Therefore, the inhibiting effect of SkQ1 and $SkQ1H_2$ on gramicidin photoinactivation could be ascribed to quenching of singlet oxygen. On the other hand, it has been reported that quinones quench excited states of phthalocyanines (Idowu et al. 2007) and rose bengal (Lambert and Kochevar 1997) in aqueous solution. Thus, quinones may prevent singlet oxygen formation resulting from the energy transfer from a photosensitizer triplet state to oxygen. This mechanism implies the direct interaction between quinone and photosensitizer molecules. Actually, measurements of fluorescence spectra revealed that addition of SkQ1 led to quenching of $AlPcS_3$ fluorescence and a blue shift of the fluorescence maximum both in aqueous solution and in the presence of liposomes (Fig. 8). Both $AlPcS_3$ (Rokitskaya et al. 2000; Shapovalov et al. 2001; Pashkovskaya et al.

2008) and MitoQ (James et al. 2007) are known to bind to liposomes.

In further experiments, we measured transient absorption changes of $AlPcS_3$ at 680 nm after laser excitation at 355 nm. A decrease in absorbance at 680 nm decaying in the submillisecond time range is known to correspond to formation of the triplet state of $AlPcS_3$ (Foley et al. 1997). From the data presented in Fig. 9, it is evident that addition of SkQ1 resulted in the concentration-dependent reduction of the amplitude of the absorption changes both in the absence and in the presence of liposomes, with effective concentrations of SkQ1 being lower in the latter case. Thus, SkQ1 may suppress singlet oxygen formation by decreasing the concentration of $AlPcS_3$ triplet states. It is important, however, that these measurements were made after deaeration of the solution in the experimental cell, i.e., under conditions when singlet oxygen formation via energy transfer from a photosensitizer triplet state is hindered. Our data are consistent with the conclusion of Lambert and Kochevar (1997) that electron transfer quenching of photosensitizer triplet state may not compete with formation of singlet oxygen during photosensitization in natural membranes.

To show the direct protection of a model membrane protein against ROS exerted by mitochondria-targeted quinones, in the last series of experiments we employed a system of ROS generation without photosensitizers. Figure 10A displays time courses of changes in the gramicidin-mediated current across a BLM after addition of Fe^{2+}, ascorbate and t-BOOH. It is seen that these three compounds, when added simultaneously, were able to induce complete inactivation of the gramicidin channels.

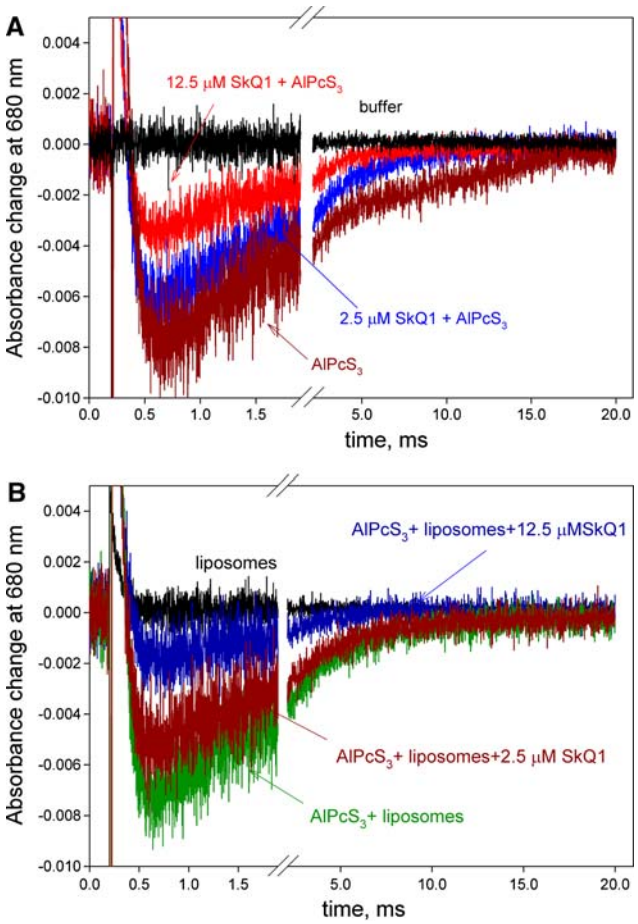

Fig. 9 Quenching of the triplet states of $AlPcS_3$ (1 μM) by SkQ1 in deaerated aqueous buffer solution (**A**) and in liposomes (**B**). Absorbance transients at 680 nm after a laser flash (355 nm) in the presence of two different concentrations of SkQ1. For incubation mixture, see Fig. 7. Egg yolk phosphatidylcholine liposomes, 30 μg/ml

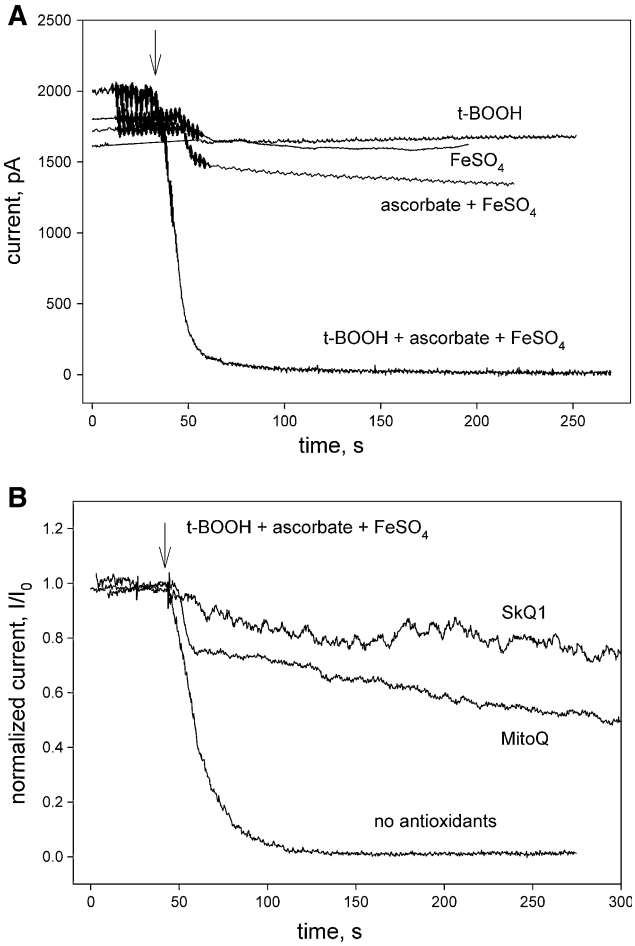

Fig. 10 Protective action of SkQ1 against the inactivation of gramicidin A channels by oxidizing reagents. (**A**) Time course of gramicidin-mediated current across BLM after addition (*arrows*) of 60 μM $FeSO_4$; 60 μM $FeSO_4$ and 1 mM ascorbate; 0.5 mM t-BOOH; 60 μM $FeSO_4$, 1 mM ascorbate and 0.5 mM t-BOOH. (**B**) Protective effect of SkQ1 and MitoQ on gramicidin channels damaged in the presence of 60 μM $FeSO_4$, 1 mM ascorbate and 0.5 mM t-BOOH. Incubation mixture: 100 mM KCl, 10 mM MES, 10 mM TRIS (pH 7). Membrane-forming solution: mixture of DPhPC (20 mg/ml) and DPhPG (10 mg/ml)

Addition of SkQ1 to the bathing solutions on both sides of the BLM markedly decelerated the decrease in the current (Fig. 10B), thus showing the protective effect on gramicidin channels. Again, MitoQ was less efficient. In this experiment, it was difficult to discriminate between the effects of the quinol and quinone forms of SkQ1 (MitoQ) due to the activity of ascorbate as a reductant. It should be noted that the experiments shown in Fig. 10 were performed on a BLM formed of a mixture of neutral DPhPC and negatively charged DPhPG. If the BLM was formed solely of DPhPC, no decrease in the current was obtained, presumably due to poor binding of Fe^{2+} to the neutral BLM.

It is shown here for the first time that mitochondria-targeted quinones (SkQ1 and MitoQ) exhibit antioxidant activity in model systems. Experiments showed that both SkQ1 and MitoQ are able to scavenge different ROS in aqueous solution (Fig. 2), micelles (Fig. 3), membranes of liposomes (Figs. 4 and 5) and planar lipid bilayers (Figs. 7 and 10). In the systems tested, SkQ1 proved to be more efficient than MitoQ. This observation is in line with the finding that chain-breaking antioxidant capability is actually much higher with methyl- compared to methoxy-substituted *p*-hydroquinols (Loshadkin et al. 2002; Kruk et al. 1997). It is noteworthy that plastoquinone forming the quinone moiety of SkQ1 is a component of the photosynthetic electron transport chain in thylakoid membranes of chloroplasts. This organelle, due to oxygen-evolving activity and light absorption by photosynthetic pigments, must be more resistant to oxidative stress than mitochondrion, where ubiquinone (the quinone moiety of MitoQ) serves as an electron transport chain component. Therefore, plastoquinone should be more suitable for the protection of membrane components against oxidation than ubiquinone, which is consistent with our data on the antioxidant activity of SkQ1 and MitoQ.

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