Biochimica et Biophysica Acta 1797 (2010) 878–889

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Prevention of cardiolipin oxidation and fatty acid cycling as two antioxidant mechanisms of cationic derivatives of plastoquinone (SkQs)

Vladimir P. Skulachev [a,b,*], Yury N. Antonenko [a], Dmitry A. Cherepanov [c], Boris V. Chernyak [a], Denis S. Izyumov [a], Ludmila S. Khailova [a], Sergey S. Klishin [a], Galina A. Korshunova [a], Konstantin G. Lyamzaev [a], Olga Yu. Pletjushkina [a], Vitaly A. Roginsky [d], Tatiana I. Rokitskaya [a], Fedor F. Severin [a], Inna I. Severina [a,e], Ruben A. Simonyan [a], Maxim V. Skulachev [a,f], Natalia V. Sumbatyan [g], Evgeniya I. Sukhanova [h], Vadim N. Tashlitsky [g], Tatyana A. Trendeleva [h], Mikhail Yu. Vyssokikh [a], Renata A. Zvyagilskaya [h]

[a] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Vorobyevy Gory 1, Moscow 119991, Russia
[b] Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Vorobyevy Gory 1, Moscow 119991, Russia
[c] Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Leninsky Av. 31, Moscow 119991, Russia
[d] Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Kosygina St. 4, Moscow 117977, Russia
[e] Biological Faculty, Lomonosov Moscow State University, Vorobyevy Gory 1, Moscow 119991, Russia
[f] Institute of Mitoengineering, Lomonosov Moscow State University, Vorobyevy Gory, Moscow, 119991, Russia
[g] Chemical Faculty, Lomonosov Moscow State University, Vorobyevy Gory 1, Moscow 119991, Russia
[h] Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky Av., 33, Moscow 119071, Russia

ARTICLE INFO

Article history:
Received 13 January 2010
Received in revised form 12 March 2010
Accepted 13 March 2010
Available online 20 March 2010

Keywords:
Mitochondria
Reactive oxygen species
SkQ
Antioxidant
Cardiolipin
Fatty acid cycling
Mild uncoupling

ABSTRACT

The present state of the art in studies on the mechanisms of antioxidant activities of mitochondria-targeted cationic plastoquinone derivatives (SkQs) is reviewed. Our experiments showed that these compounds can operate as antioxidants in two quite different ways, i.e. (i) by preventing peroxidation of cardiolipin [Antonenko et al., *Biochemistry (Moscow)* 73 (2008) 1273–1287] and (ii) by fatty acid cycling resulting in mild uncoupling that inhibits the formation of reactive oxygen species (ROS) in mitochondrial State 4 [Severin et al. *Proc. Natl. Acad. Sci. USA* 107 (2009), 663–668]. The quinol and cationic moieties of SkQ are involved in cases (i) and (ii), respectively. In case (i) $SkQH_2$ interrupts propagation of chain reactions involved in peroxidation of unsaturated fatty acid residues in cardiolipin, the formed $SkQ^{\bullet -}$ being reduced back to $SkQH_2$ by heme b_H of complex III in an antimycin-sensitive way. Molecular dynamics simulation showed that there are two stable conformations of SkQ1 with the quinol residue localized near peroxyl radicals at C_9 or C_{13} of the linoleate residue in cardiolipin. In mechanism (ii), fatty acid cycling mediated by the cationic SkQ moiety is involved. It consists of (a) transmembrane movement of the fatty acid anion/SkQ cation pair and (b) back flows of free SkQ cation and protonated fatty acid. The cycling results in a protonophorous effect that was demonstrated in planar phospholipid membranes and liposomes. In mitochondria, the cycling gives rise to mild uncoupling, thereby decreasing membrane potential and ROS generation coupled to reverse electron transport in the respiratory chain. In yeast cells, dodecyltriphenylphosphonium (C_{12}TPP), the cationic part of SkQ1, induces uncoupling that is mitochondria-targeted since C_{12}TPP is specifically accumulated in mitochondria and increases the H^+ conductance of their inner membrane. The conductance of the outer cell membrane is not affected by C_{12}TPP.

Abbreviations: $\Delta\psi$, transmembrane electric potential; AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride; BLM, planar bilayer phospholipid membrane; Cart, carboxyatractyloside; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; CTMA, cetyltrimethylammonium; C_{10}TPP, decyltriphenylphosphonium; C_{12}R1, decylrhodamine 19; C_{12}R4, decylrhodamine B; C_{12}TPP, dodecyltriphenylphosphonium; DCF, 2′,7′-dichlorodihydrofluorescein diacetate; DMQ, 3-demethoxy ubiquinonyl decyltriphenylphosphonium; DPQ, decylplastoquinone; FCCP, carbonylcyanide p-trifluoromethoxyphenylhydrazone; MDA, malondialdehyde; MitoQ, ubiquinonyl decyltriphenylphosphonium; ML, methyl linoleate; NAC, N-acetyl cysteine; ROS, reactive oxygen species; SkQ, a compound composed of plastoquinone or methylplastoquinone and decyl (or amyl) triphenylphosphonium, Rhodamine 19, or Rhodamine B; SkQ1, plastoquinonyl decyltriphenylphosphonium; SkQ3, 5-methylplastoquinonyl decyltriphenylphosphonium; SkQ5, plastoquinonyl amyltriphenylphosphonium; SkQR1, plastoquinonyl decylrhodamine 19; SkQR4, plastoquinonyl decylrhodamine B; TMRM, tetramethylrhodamine methyl ester; TPP, tetraphenylphosphonium

* Corresponding author. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Vorobyevy Gory 1, 119991 Moscow, Russia. Tel.: +7 4959395530; fax: +7 4959390338.
E-mail address: skulach@belozersky.msu.ru (V.P. Skulachev).

0005-2728/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2010.03.015

1. Introduction

In 2003 we started an ambitious project with the goal of creating a small molecule that decelerates the senescence of organisms. Mitochondria-targeted antioxidants (cationic derivatives of plastoquinone) were chosen for this purpose. The investigation was based on the discovery of mitochondria-penetrating ions by groups of Dr. E.A. Liberman and of one of the authors (V.P.S.) in 1969–1970. Penetrating ions are hydrophobic molecules that can easily penetrate through membranes in spite of the presence of an ionized atom in their structures [1–4]. An example is the alkyltriphenylphosphonium ion. In this cation, the positive charge on phosphorus is strongly delocalized over the three phenyl residues. For this reason, water dipoles cannot be held on the cation and do not form an aqueous shell preventing penetration of the cation through the hydrophobic membrane core. In the same studies, it was found that the mitochondrial interior is the only intracellular compartment negatively charged relative to its surrounding (i.e. to the cytosol) [4,5]. Therefore, on entering the cell, penetrating cations will be specifically concentrated within mitochondria. This concentrating effect should be described by the Nernst equation, being 1000 fold if the mitochondrial $\Delta\psi$ is 180 mV [5].

Following this hypothesis, we suggested that penetrating cations can be used as "molecular electric locomotives" to target uncharged substances attached to these cations specifically to mitochondria [5,6].

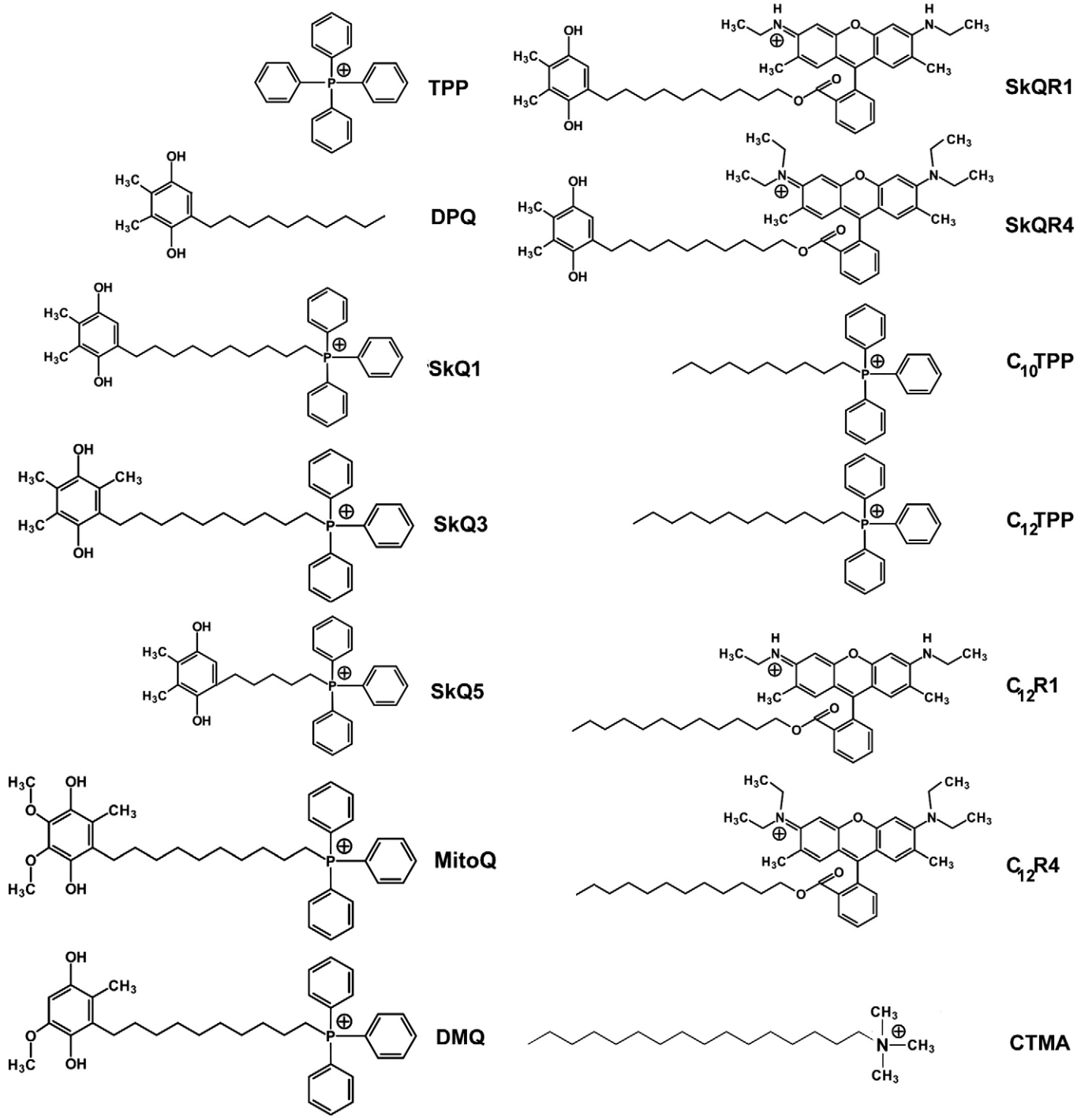

Fig. 1. Formulas of the studied penetrating cations. The quinone moieties are shown in their reduced forms.