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Derivatives of the cationic plant alkaloids berberine and palmatine amplify protonophorous activity of fatty acids in model membranes and mitochondria

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ABSTRACT

Previously it has been shown by our group that berberine and palmatine, penetrating cations of plant origin, when conjugated with plastoquinone (SkQBerb and SkQPalm), can accumulate in isolated mitochondria or in mitochondria of living cells and effectively protect them from oxidative damage. In the present work, we demonstrate that SkQBerb, SkQPalm, and their analogs lacking the plastoquinone moiety (C_{10}Berb and C_{10}Palm) operate as mitochondria-targeted compounds facilitating protonophorous effect of free fatty acids. These compounds induce proton transport mediated by small concentrations of added fatty acids both in planar and liposomal model lipid membranes. In mitochondria, such an effect can be carried out by endogenous fatty acids and the adenine nucleotide translocase.

1. Introduction

In the preceding paper (Trendeleva et al., 2012), it was shown that screening of positive charge by bulky residues and/or delocalization of this charge are obligatory requirements for fast permeation of lipophilic cations through model and mitochondrial membranes. This analysis was done using synthetic compounds. In 2001, in our group it was found that there are natural delocalized cations easily penetrating through model and natural membranes, namely the plant alkaloids berberine and palmatine (Severina et al., 2001).

Berberine and palmatine are isoquinoline alkaloids which were found in plants of the *Berberidaceae* family. Extracts containing these alkaloids have been used in traditional Chinese medicine for many centuries. As early as in 1964, Biriuzova et al. (1964) reported that berberine accumulates in isolated yeast mitochondria. Later (Borodina and Zelenin, 1977), berberine accumulation was documented in mitochondria of a living cell culture. In 2011, we synthesized nonylplastoquinone derivatives of berberine and palmatine (SkQBerb and SkQPalm) and showed that their nanomolar concentrations protected cells against oxidative stress. Accumulation of these compounds in isolated mitochondria and in mitochondria of cells was found to be $\Delta\Psi$-dependent (Lyamzaev et al., 2011).

Studies of mitochondria-targeted antioxidants had led to the discovery of a strong increase in proton conductance of model and natural membranes by phosphonium conjugates of plastoquinone (Antonenko et al., 2011; Severin et al., 2010). These conjugates were found to catalyze proton transfer across BLM and mitochondrial membrane resulting in uncoupling of oxidative phosphorylation in isolated mitochondria and in intact cells. The effect depended on the presence of free fatty acids (FFA). A similar effect of tetraphenylphosphonium but at very much higher concentrations was found earlier by Schönfeld (1992). It was suggested that penetrating cations accelerated transmembrane transfer of FFA anions, facilitating protonophorous FFA cycling (Severin et al., 2010).

Abbreviations: $\Delta\Psi$, transmembrane electric potential difference; ANT, adenine nucleotide translocase; BLM, bilayer planar phospholipid membrane; BSA, bovine serum albumin; C_{10}Berb, 13-(decyloxycarbonylmethyl)berberine; C_{10}Palm, 13-(decyloxycarbonylmethyl) palmatine; C_{12}TPP, dodecyltriphenylphosphonium; CATR, carboxyatractyloside; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; DPhPC, diphytanoyl phosphatidylcholine; DiS-C3-(5), 3,3′-dipropylthiadicarbocyanine iodide; FFA, free fatty acid; FCCP, carbonyl cyanide *p*-(trifluromethoxy)phenylhydrazone; ROS, reactive oxygen species; SkQ1, 10-(6-plastoquinonyl)decyltriphenylphosphonium; SkQBerb, 13-[9-(6-plastoquinonyl)nonyloxycarbonylmethyl]berberine; SkQPalm, 13-[9-(6-plastoquinonyl) nonyloxycarbonylmethyl] palmatine; SkQR1, 10-(6-plastoquinonyl)decylrhodamine-19.

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The data presented below demonstrate that conjugates of berberine and palmatine induce proton transport mediated by FFA both in model lipid membranes and in mitochondria. In the latter case, this process was found to be facilitated by the translocase of adenine nucleotides (ANT).

2. Materials and methods

2.1. Reagents

Synthesis of the berberine and palmatine conjugates (Fig. 1) was described in detail elsewhere (Lyamzaev et al., 2011). Phosphonium-containing compounds SkQ1 and C_{12}TPP were synthesized as described by Antonenko et al. (2008). DiS-C3-(5) was purchased from Molecular Probes, diphytanoylphosphatidylcholine (DPhPC) was from Avanti Polar Lipids, and safranine O was from Serva. Other chemicals were from Sigma.

2.2. Planar bilayers

A BLM was formed from 2%-decane solution of diphytanoylphosphatidylcholine (DPhPC) on a 0.6-mm aperture in a Teflon septum separating the experimental chamber into two compartments (total volume, 3 ml). Where indicated, the decane solution of DPhPC was supplemented with palmitate (1 mg per 20 mg DPhPC). Electrical parameters were measured with AgCl electrodes using a Keithley 6517 amplifier (Cleveland, OH) (Severina, 1982). The incubation mixture contained 50 mM Tris–HCl and 50 mM KCl, pH 7.0.

2.3. Liposomes

Liposomes were prepared by evaporation under a stream of nitrogen of a diphytanoylphosphatidylcholine solution in chloroform, followed by hydration with buffer solution containing 150 mM KCl and 2 mM Tris, pH 7.4. The mixture was vortexed, passed through a cycle of freezing and thawing, and extruded through 0.1-μm pores in a Nucleopore polycarbonate film, using an Avanti Mini-Extruder. Membrane potential on the liposome membrane was generated by K^+ efflux and measured using fluorescence of DiS-C3-(5) as a probe. The K^+-loaded liposomes were treated with valinomycin in buffer containing 150 mM NaCl and 2 mM Tris, pH 7.4 as described by Konishi et al. (1986). The fluorescence was monitored at 690 nm (excitation at 650 nm). Experiments were carried out using a mixture that contained liposomes (0.03 mg/ml), 1 μM DiS-C3-(5), and 4 nM valinomycin. Gramicidin A (0.2 μg/ml) was added at the end of the experiments to determine the zero level of the membrane potential.

2.4. Isolation of mitochondria

Rat heart mitochondria were isolated as described by Palmer et al. (1977) and suspended in medium containing 250 mM sucrose, 10 mM MOPS KOH (pH 7.4), 1 mM EGTA, and 0.1% of defatted BSA.

The yeast *Yarrowia lipolytica* was cultivated with aeration in semi-synthetic succinate-containing medium at 28 °C and harvested at the late exponential phase. Mitochondria from *Y. lipolytica* cells were isolated as described earlier (Kovaleva et al., 2009). These mitochondria have high respiratory control ranging from 4 to 6 upon oxidation of NAD-dependent substrates.

2.5. Mitochondrial respiration

Oxygen consumption by mitochondria was measured amperometrically with a Clark-type oxygen electrode at 25 °C. The incubation medium for rat heart mitochondria (0.1 mg protein/ml) contained 250 mM sucrose,10 mM HEPES, 1 mM EGTA, 2 mM $MgCl_2$, 2 mM KH_2PO_4, 5 mM succinate, 2 μM rotenone, and 0.1% BSA (pH 7.4). It should be stressed that a small amount of BSA was added to increase the respiratory control. However, an increase in the BSA concentration abolished uncoupling by berberine derivatives just as by C_{12}TPP (Trendeleva et al., 2012).

The basal incubation medium for yeast mitochondria (0.5 mg protein/ml) contained 0.6 M mannitol, 2 mM Tris-phosphate, 20 mM Tris-pyruvate, and 5 mM malate, pH 7.4.

2.6. Mitochondrial membrane potential

Mitochondrial transmembrane potential ($\Delta\Psi$) was measured with a Beckman DU-650 spectrophotometer at the wavelength pair 511–533 or 555–523 nm with safranine O as a $\Delta\psi$-related probe at 25 °C. The incubation medium for rat heart mitochondria (0.7 mg protein/ml) contained 250 mM sucrose,10 mM MOPS-KOH, 0.1 mM EGTA, 5 mM succinate, 2 μM rotenone, and 15 μM Safranin O, pH 7.4. The membrane potential of yeast mitochondria was measured in the same medium.

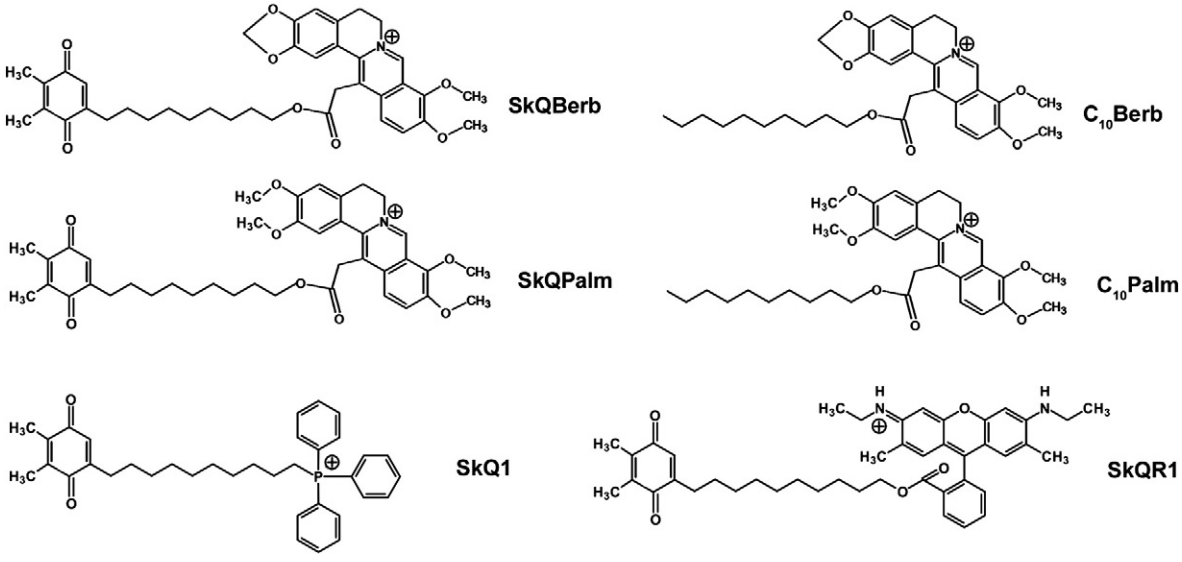

Fig. 1. Chemical structures of SkQBerb, SkQPalm, C_{10}Berb, C_{10}Palm, SkQ1, and SkQR1.