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Efficiency of mitochondrial uncoupling by modified butyltriphenylphosphonium cations and fatty acids correlates with lipophilicity of cations: Protonophoric vs leakage mechanisms

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ABSTRACT

In order to determine the share of protonophoric activity in the uncoupling action of lipophilic cations a number of analogues of butyltriphenylphosphonium with substitutions in phenyl rings (C_4TPP-X) were studied on isolated rat liver mitochondria and model lipid membranes. An increase in the rate of respiration and a decrease in the membrane potential of isolated mitochondria were observed for all the studied cations, the efficiency of these processes was significantly enhanced in the presence of fatty acids and correlated with the octanol-water partition coefficient of the cations. The ability of C_4TPP-X cations to induce proton transport across the lipid membrane of liposomes loaded with a pH-sensitive fluorescent dye increased also with their lipophilicity and depended on the presence of palmitic acid in the liposome membrane. Of all the cations, only butyl[tri(3,5-dimethylphenyl)]phosphonium (C_4TPP-diMe) was able to induce proton transport by the mechanism of formation of a cation-fatty acid ion pair on planar bilayer lipid membranes and liposomes. The rate of oxygen consumption by mitochondria in the presence of C_4TPP-diMe increased to the maximum values corresponding to conventional uncouplers; for all other cations the maximum uncoupling rates were significantly lower. We assume that the studied cations of the C_4TPP-X series, with the exception of C_4TPP-diMe at low concentrations, cause nonspecific leak of ions through lipid model and biological membranes which is significantly enhanced in the presence of fatty acids.

1. Introduction

Lipophilic cations have been widely used for decades to determine the membrane potential of prokaryotic and eukaryotic cells, as well as eukaryotic sub-cellular organelles in vitro [1,2]. Since a significant electrical potential difference with a “minus” sign in the matrix is created on the inner membrane of mitochondria during their energization, lipophilic cations, such as alkyl triphenylphosphonium, alkyl rhodamine B, and others, are well accumulated in the matrix. In recent years, such cations have also acted as a vector of mitochondrial targeting in the search of new mitochondria-protecting compounds [3–5]. One of the first such compound was a conjugate of triphenylphosphonium (TPP) with the antioxidant ubiquinone, MitoQ [6]. Another conjugate of TPP and plastoquinone (SkQ1) was also shown to accumulate in mitochondria and exhibit protective effects on several physiological models [7]. SkQ1 and MitoQ have also been found to have an uncoupling effect on mitochondria [8,9] due to the fact that they are able to form complexes with fatty acids present in natural membranes. Since it was previously shown that the formation of reactive oxygen species in mitochondria largely depends on the potential difference across the inner mitochondrial membrane ($\Delta\Psi$) [10], it is possible that the antioxidant effect of mitochondria-targeted compounds is partially determined by their uncoupling effect.

The uncoupling of oxidative phosphorylation by MitoQ or SkQ1, as well as dodecyltriphenylphosphonium (C_{12}TPP), can occur due to the protonophoric action of the lipophilic cation - fatty acid ion pair. This

Abbreviations: BLM, bilayer lipid membrane; DPhytanylPC, 1,2-di-O-phytanyl-sn-glycero-3-phosphocholine; TPP, tetraphenylphosphonium bromide; C_4TPP, butyltriphenylphosphonium bromide; C_4TPP-diMe, *n*-butyl[tri(3,5-dimethylphenyl)]phosphonium bromide; C_4TPP-Cl, *n*-butyl[tri(*p*-chlorophenyl)]phosphonium bromide; C_4TPP-F, *n*-butyl[tri(*p*-fluorophenyl)] phosphonium bromide; C_4TPP-Me, *n*-butyl[tri(*p*-tolyl)]phosphonium bromide; C_4TPP-OMe, *n*-butyl[tri(*p*-methoxyphenyl)]phosphonium bromide.

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mechanism suggests that the neutral complex of the cationic compound and the anionic form of the fatty acid performs a flip-flop from the side of the mitochondrial matrix to the side of the intermembrane space. Further, the ion pair dissociates, the fatty acid is protonated, and the protonophoric cycle ends with a flip-flop of the lipophilic cation and the neutral form of the acid to the side of the matrix. As a result of the cyclic movement of the cation and fatty acid, the proton is transported from the intermembrane space to the matrix, thereby reducing ΔμH+ on the inner mitochondrial membrane [8]. It is assumed that this mechanism is self-limiting, since as ΔΨ falls, the accumulation of lipophilic cations in mitochondria should decrease (mild uncoupling). With the on-going studies of the effect of mitochondria-targeted compounds and triphenylphosphonium alkyls (C_nTPP) on cells and their organelles, more and more information began to appear about their uncoupling effect, which is not related to the above mechanism [11–14]. Apparently, with an increase in the concentration of lipophilic cations, an increase in the nonspecific conductivity of the lipid membrane occurs, which can be caused by the surface-active properties of these compounds. Conventional detergents such as SDS, Triton-X100 and CTAB have previously been shown to increase the ionic permeability of lipid membranes at concentrations orders of magnitude lower than their critical micelle concentrations [15]. It has also been long known that detergents are able to stimulate mitochondrial respiration and give rise to collapse of their membrane potential [16–18].

With an increase in the length of the alkyl, the ability of C_nTPP to accelerate the respiration of isolated rat liver mitochondria [19] and to have a negative effect on the mitochondrial membrane potential and activity of the respiratory chain of intact C2C12 cells [12] increased. In the case of structural modifications of C_nTPP in phenyl rings, which lead to a decrease in the electron density at the phosphorus atom, the uncoupling activity of the lipophilic cation was suppressed and the dissipation of the mitochondrial potential on C2C12 mouse myoblasts was prevented [20]. Despite the growing number of studies on the uncoupling and toxic properties of lipophilic cations and their conjugates, there is no understanding in the literature about the mechanism of this uncoupling i.e. protonophoric or detergent-like. Since in the first case the transport of protons implies the presence of a slow stage of the flip-flop of the cationic compound through the lipid membrane, we assumed that mild uncoupling should be more pronounced in the case of lipophilic cations with a high flip-flop rate constant. In the case of conventional uncouplers, which are hydrophobic weak acids, the magnitude of the proton current through the lipid membrane is proportional to the anion mobility in the membrane and to the partition coefficient between the membrane and water [21].

We have previously shown that analogs of dodecyltriphenylphosphonium (C_{12}TPP-X) with structural modifications in the phenyl rings, differ in the flip-flop rate constants through the hydrophobic part of the membrane by several orders of magnitude [22]. However, it has been shown that the lengths of the alkyl linker of the MitoQ determine their extents of adsorption to de-energized mitochondria [23]. Therefore, it can be assumed that the binding of the most hydrophobic analogs of C_{12}TPP-X will be determined to a small extent by the potential of mitochondria, which means that the self-limiting principle of the uncoupling action of such cations can be limited or absent. At the same time, significant differences were found in the permeability through lipid bilayer membranes for a number of butyltriphenylphosphonium analogues with structural modifications in the phenyl rings (C_4TPP-X) [24], which accumulate in mitochondria in a voltage-dependent manner [24,25]. Having set out to determine the main factors affecting the uncoupling of mitochondrial oxidation from ATP production under the action of lipophilic cations, we studied the action of the C_4TPP-X series (Fig. 1A) on isolated rat liver mitochondria, liposomes loaded with a fluorescent pH-sensitive probe pyranine, and also on planar bilayer lipid membranes (BLM), focusing on the role of fatty acids.

2. Materials and methods

2.1. Materials

Triphenylphosphine, tris(4-chlorophenyl)phosphine, tris(4-fluorophenyl)phosphine, tri(*p*-tolyl)phosphine, tris(4-methoxyphenyl) phosphine, tris(3,5-dimethyl)phosphine and tricyclohexylphosphine were purchased from Sigma-Aldrich (USA), 1-bromobutane from Alfa Aesar (Germany) and were used without further purification. *n*-butyl [triphenyl]phosphonium (C_4TPP), *n*-butyl[tri(*p*-chlorophenyl)]phosphonium (C_4TPP-Cl), *n*-butyl[tri(*p*-fluorophenyl)] phosphonium (C_4TPP-F), *n*-butyl[tri(*p*-tolyl)]phosphonium (C_4TPP-Me), *n*-butyl[tri(*p*-methoxyphenyl)] phosphonium (C_4TPP-OMe) and *n*-butyl[tri(3,5-dimethylphenyl)]phosphonium (C_4TPP-diMe) bromides were

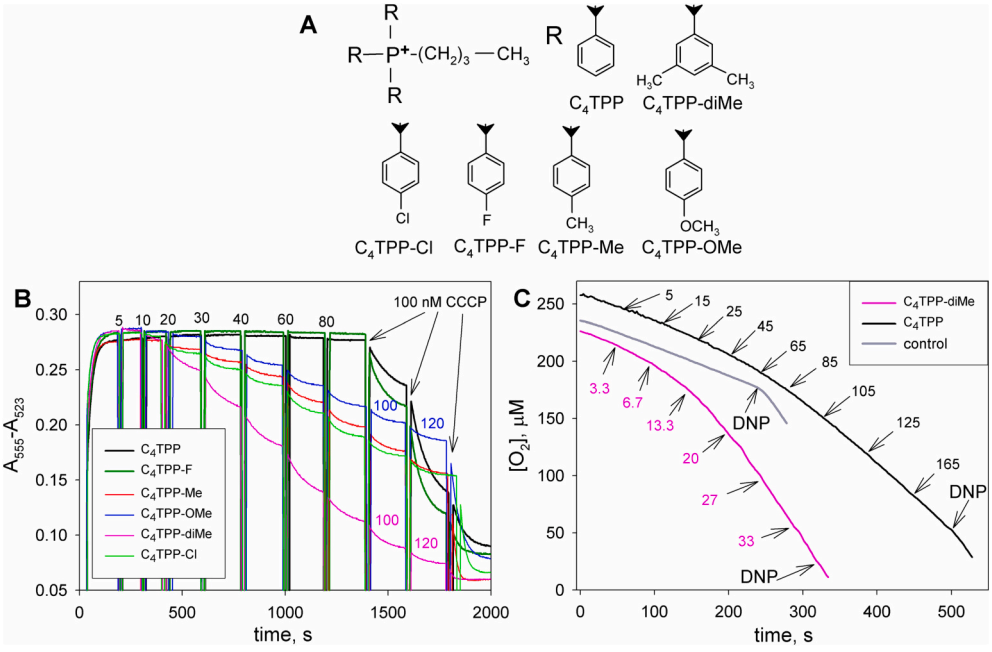

Fig. 1. A Chemical structure of butyltriphenylphosphonium and its analogues. B Influence of C_4TPP-X cations on mitochondrial potential estimated using safranine after adding at increasing concentration (C_4TPP – black curve, C_4TPP-F – dark green curve, C_4TPP-Me – red curve, C_4TPP-OMe – blue curve, C_4TPP-diMe – pink curve, C_4TPP-Cl – green curve). The numbers in the figure indicate the final concentration of the lipophilic cations in μM. C Representative polarographic data on oxygen consumption by mitochondria after adding C_4TPP-diMe (pink curve), C_4TPP (black curve) or without adding (grey curve). The numbers in the figure indicate the final concentrations of cations in micromoles. 40 μM DNP was added at the end of the experiment. Succinate (5 mM) was used as a respiratory substrate in the presence of rotenone (2 μM) and oligomycin (1 μg/ml). The incubation medium contained 250 mM sucrose, 5 mM MOPS-KOH, 1 mM KH_2PO_4, 1 mM EGTA, pH = 7.4. The concentration of mitochondrial protein was 0.6–0.8 mg/ml at panel B and about 1 mg/ml at panel C.

synthesized according to earlier described methods [19,24].

1,2-di-O-phytanyl-sn-glycero-3-phosphocholine (DPhytanylPC), 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (POPG) and cholesterol were purchased from Avanti Polar Lipids (USA), palmitic acid (PA), carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) and 2,4-dinitrophenol (DNP) were purchased from Sigma-Aldrich (USA).

2.2. Isolation of rat liver mitochondria

Rat liver mitochondria were isolated by differential centrifugation [26] in a medium containing 250 mM sucrose, 5 mM MOPS, 1 mM EGTA, and 0.5 mg/ml bovine serum albumin (BSA); pH 7.4. The final wash was in medium without BSA. Final suspension contained 90–100 mg/ml of mitochondrial protein/ml, as determined by the biuret method. Handling of animals and experimental procedures were conducted in accordance with international guidelines for animal care and use and were approved by the Institutional Ethics Committee of A.N. Belozersky Institute of Physico-Chemical Biology at Moscow State University.

2.3. Mitochondrial respiration

Respiration of isolated mitochondria was measured using a standard polarographic technique with a Clark-type oxygen electrode (Strathkelvin Instruments, UK) at 25 °C using 782 system software. The incubation medium contained 250 mM sucrose, 5 mM MOPS, 1 mM EGTA, pH 7.4. The concentration of total mitochondrial protein was about 1.0 mg/ml. Oxygen uptake was expressed as nmol/min/mg protein.

2.4. Membrane potential measurement in isolated mitochondria

ΔΨ was estimated using safranin O dye [27]. The difference in absorbance at 555 nm and 523 nm was recorded with an Aminco DW-2000 spectrophotometer in a dual wavelength mode. The following incubation medium was used: 250 mM sucrose, 1 mM EGTA, 5 mM MOPS-KOH (pH 7.4), 15 μM safranin O. The mitochondrial protein content was 0.6–0.8 mg protein/ml.

2.5. Mitochondrial swelling

The swelling of mitochondria was assessed by recording the change in optical density of the mitochondrial suspension at a wavelength of 540 nm using a Specord 50 spectrophotometer (Germany). The incubation medium contained 10 mM Hepes, 130 mM KCl, 0.2 mM EDTA, 1.5 mM succinate, pH = 7.4. The concentration of mitochondrial protein in the cuvette was about ~0.2 mg/ml.

2.6. Measurement of proton transport in pyranine-loaded liposomes

The pH of liposomes was assayed by a slightly modified procedure [28]. To prepare pyranine-loaded liposomes, the lipid mixture from POPC, POPG and cholesterol (5.4, 1.5 and 3.1 mg, respectively) or POPC, POPG, cholesterol and PA (5.0, 1.3, 2.4 and 1.3 mg, respectively) in a chloroform suspension was dried in a round-bottom flask under a stream of nitrogen. The lipid was then resuspended in 1 ml buffer (100 mM KCl, 20 mM MES, 20 mM MOPS, 20 mM Tricine titrated with KOH to pH 6.0) containing 0.5 mM pyranine. The suspension was vortexed and freeze-thawed three times. Unilamellar liposomes were prepared by extrusion through 0.1 μm pores (Nucleopore polycarbonate membranes) using an Avanti Mini-Extruder. The untrapped pyranine was removed by passage through a Sephadex G-50 coarse column equilibrated with the same buffer solution. The liposomes were diluted in the buffer without a pH gradient (pH = 6.0 inside and outside) and final lipid concentration in the cuvette was about 20 μg/ml. pH gradient on liposomal membrane was created by the addition of a small aliquot of KOH adjusting pH to 8.0. The proton transport was initiated by the addition of C_4TPP-X lipophilic cations. To measure the pH dissipation rate in liposomes, the suspension was supplemented with 2 mM *p*-xylene-*bis*-pyridinium bromide to quench the fluorescence of leaked pyranine. The internal pH was estimated from the intensity of fluorescence measured at 505 nm upon excitation at 455 nm, as the fluorescence emission of liposome-loaded pyranine monotonously increases upon excitation at 455 nm over a pH range of 6.0 to 7.9 [28]. The fluorescence was monitored on a Panorama Fluorat 02 spectrofluorimeter (Lumex, Saint-Petersburg). At the end of each recording, 1 μM X537A (Lasalocid A) was added to dissipate the remaining pH gradient. To prevent the formation of H^+-diffusion potential, the experiments were carried in the presence of 5 nM valinomycin. To decrease the leakage of H^+ from liposomes, the experiments were performed at 15 °C.

The extent of the proton transport was calculated as $(F_t - F_0)/(F_{100}-F_0)$, where F_0 and F_t represent the initial fluorescence before C_4TPP-X adding and the fluorescence at time *t*, and F_{100} is the fluorescence after complete dissipation of the pH gradient by addition of the X537A.

2.7. Formation of planar bilayer lipid membrane

Planar bilayer lipid membranes were formed using the Mueller-Rudin technique [29] from a 2 % solution of lipid in decane. The membranes were spread from a lipid solution across a circular aperture (800 μm) in a polytetrafluorethylene septum, which separated two aqueous phases of a PTFE chamber. Membrane thinning was observed optically.

2.8. Electric current measurement on BLM

The electric currents (*I*) were recorded under voltage-clamp conditions. Voltages were applied to BLMs with Ag-AgCl electrodes connected via agar bridges. The currents measured by means of a patch-clamp amplifier (OES-2, OPUS, Moscow) were digitized using an NI-DAQmx (National Instruments) and analyzed with a personal computer with the use of WinWCP Strathclyde Electrophysiology Software designed by J. Dempster (University of Strathclyde). In the current relaxation experiments the voltage was switched from zero to some particular value of V at $t = 0$ and the current across the membrane ($I(t)$) started to decrease from the initial level $I(0)$ to steady-state level $I(\infty)$. When a stable membrane was formed, we recorded capacitance response of the unmodified membrane (control record of the current after voltage-jump).

Lipophilic cations were added from stock solutions in ethanol or water to the bathing solutions at both sides of the BLM and incubated for at least 5 min with constant stirring. The record of the current in the presence of a lipophilic ion was measured without stirring to minimize noise and was analyzed after subtraction of the control record. In most of the experiments, the solution contained 100 mM KCl, 10 mM Mes, 10 mM β-Ala. All experiments were carried out at room temperature (23–25 °C). Statistics is given as Mean ± StdDev for at least three experiments, unless otherwise specified.

3. Results

3.1. Uncoupling effect of butyltriphenylphosphonium and analogues with modifications in the phenyl rings on mitochondria

The ability of lipophilic cations of the C_4TPP-X series (Fig. 1A) to decrease the membrane potential of mitochondria and increase the rate of oxygen consumption was studied on isolated rat liver mitochondria (Fig. 1B, C). The most active was C_4TPP-diMe, which markedly increased the rate of respiration at a concentration of 10 μM and decreased the membrane potential at a concentration of 20 μM. Compounds C_4TPP-Me, C_4TPP-OMe and C_4TPP-Cl at the same concentrations

led to a significantly lower decrease in ΔΨ, while C_4TPP and C_4TPP-F at concentrations of 80 μM did not affect the membrane potential (Fig. 1B). Fig. 1C shows an example of the kinetic curve of oxygen consumption by mitochondria upon additions of increasing concentrations of C_4TPP and C_4TPP-diMe. At a concentration of 33 μM C_4TPP-diMe, the maximum increase in the rate of mitochondrial respiration was observed; further addition of 40 μM 2,4-dinitrophenol (DNP) at the end of the experiment did not change the rate of oxygen consumption. At the same time, C_4TPP stimulated mitochondrial respiration at significantly higher concentrations, and the subsequent addition of DNP led to an increase in the respiration rate to maximum values. On average, the rate of maximum respiration after the addition of 40 μM DNP was $v_{max} = 82 \pm 7$ nmole O_2/(min × mg protein) (Mean ± StDDev).

It was previously shown that the uncoupling effect of lipophilic cations with a triphenylphosphonium group with a longer alkyl (dodecyltriphenylphosphonium) and a conjugate with plastoquinone (SkQ1) is caused by cyclic proton transfer through the inner mitochondrial membrane by an ion pair - a penetrating cation and an endogenous fatty acid anion [8]. Triphenylphosphonium alkyls (C_nTPP) with $n = 4$–10 also accelerated the mitochondrial respiration in the presence of fatty acids [19]. Uncoupling of mitochondrial oxidation and phosphorylation by free fatty acids was described nearly 70 years ago by Pressman and Lardy in 1956 [30]. Therefore, it can be assumed that the mechanism of uncoupling of mitochondrial respiration in the case of C_4TPP-X cations with substituents in the phenyl group is also determined by the presence of fatty acids in mitochondria. When measuring the membrane potential of mitochondria in the presence of palmitic acid, we found a more pronounced decrease in the membrane potential under the action of 10 μM C_4TPP-diMe (pink curve), 20 μM C_4TPP-Me (red curve), 20 μM C_4TPP-OMe (blue curve), 20 μM C_4TPP-Cl (green curve) compared to fatty acid action (grey curve) (Fig. 2A). The C_4TPP and C_4TPP-F cations had to be added at higher concentrations in order to induce a cumulative effect with the fatty acid (Fig. S1). Fig. 2B shows that the addition of 10 μM palmitic acid in the presence of 10–20 μM C_4TPP-diMe leads to a complete drop in the membrane potential of the mitochondria. The addition of bovine serum albumin (BSA) after a decrease in the membrane potential led to a return of ΔΨ to the initial level. The rate of oxygen uptake by mitochondria under the action of various concentrations of C_4TPP-OMe decreased also with the addition of BSA even in the absence of exogenous fatty acids (Fig. 2C). BSA is known to have a high binding constant for long-chain fatty acids [31], so its action confirms the requirement of endogenous fatty acids for the uncoupling action of C_4TPP-X.

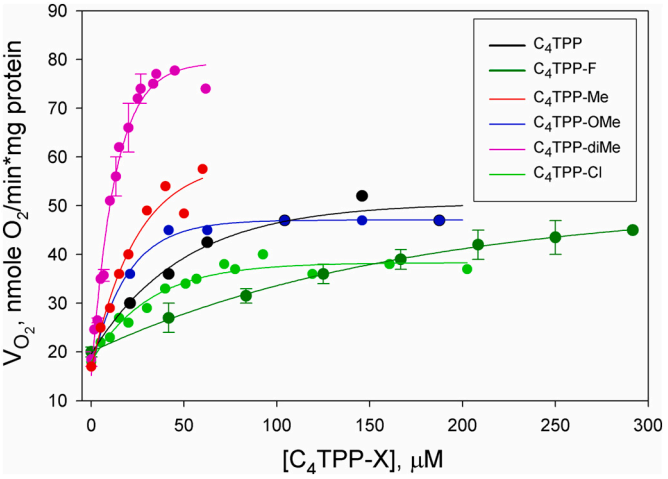

Fig. 3. Dependence of mitochondrial respiration rate in rat liver mitochondria on concentration of lipophilic C_4TPP-X cations (C_4TPP - black circle, C_4TPP-F - dark green circle, C_4TPP-Cl - green circle, C_4TPP-OMe - blue circle, C_4TPP-Me - red circle, C_4TPP-diMe - pink circle). Succinate (5 mM) was used as a respiratory substrate in the presence of rotenone (2 μM) and oligomycin (1 μg/ml). The incubation medium contained 250 mM sucrose, 5 mM MOPS-KOH, 1 mM KH_2PO_4, 1 mM EGTA, pH = 7.4. The concentration of mitochondrial protein was about 1 mg/ml. Fitting curves are obtained according to the equation $v = c - \Delta v_{max} \times 2^{-[C4TPP-X]/(C1/2)}$, the parameter values for each cation are given in Table 1.

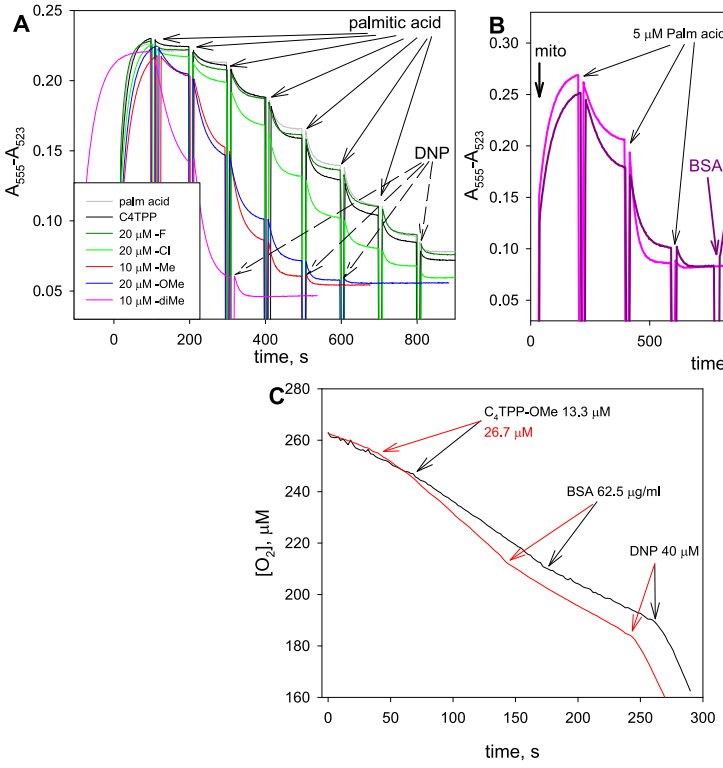

Fig. 2. A Effect of cations of the C_4TPP-X series on the decrease in mitochondrial potential under the action of palmitic acid (each addition of 5 μM). C_4TPP cations (C_4TPP - 20 μM, black curve; C_4TPP-F - 20 μM, dark green curve; C_4TPP-Cl - 20 μM, green curve; C_4TPP-OMe - 20 μM, blue curve; C_4TPP-Me - 10 μM, red curve, and C_4TPP-diMe - 10 μM, pink curve) were added to the measurement medium prior to the addition of mitochondria. B Restoration of mitochondrial potential induced by the addition of 0.1 mg/ml BSA after a decrease in ΔΨ under the action of 15 μM palmitic acid and 20 μM C_4TPP-diMe. C Effect of BSA on the rate of oxygen consumption by mitochondria in the presence of C_4TPP-OMe (13.3 μM, black curve, and 26.7 μM, red curve). The experiments were done under conditions noted in the legend to Fig. 1.

Fig. 3 shows the concentration dependences of the respiratory rate of isolated rat liver mitochondria in the presence of C_4TPP-X cations. The results are based on three independent experiments. C_4TPP-diMe accelerated mitochondrial respiration at the lowest concentrations among all the compounds. The maximum respiration rates in its presence reached the same values as in the case of DNP. Other cations increased the respiration rate at higher concentrations, and the maximum rate was much less than v_{max}. In each experiment, we approximated the dependence of the respiration rate on the concentration of the cation by a power function $\nu = c - \Delta\nu_{\max} \times 2^{-[C4TPP-X]/(C1/2)}$ and, based on the results of all experiments, determined the values of the parameters that are given in Table 1. c is the maximum respiration rate after addition of a saturating concentration of C_4TPP-X, Δv_{max} is the difference between the maximum and the initial respiration rate for each analogue, $C_{1/2}$ is the concentration of half increase in respiration rate.

3.2. Lipophilic C_4TPP-X cations stimulate proton transport on pyranine-loaded liposomes. Study of the mechanism of transport and the effect of fatty acids

We studied the ability of C_4TPP-X cations to transport protons across lipid membranes on liposomes loaded with a pH-sensitive fluorescent probe pyranine at pH gradient on membranes of liposomes (Fig. 4). In the case of the addition of palmitic acid to the lipid composition during the preparation of liposomes, 20 μM C_4TPP-diMe led to a significant release of protons from liposomes. At this concentration, the effect of C_4TPP-Cl and C_4TPP-Me was also noticeable (Fig. 4A). The measurements were carried out in the presence of the pyranine quencher p-Xylene-bis(N-pyridinium bromide) (DPX) in an external solution, which makes it possible to register the release of pyranine from liposomes if the level of final fluorescence decreases (compared with the control experiment) after addition of the non-electrogenic K+/H+ exchanger X537A causing complete pH equilibration. The cations at a concentration of 20 μM were unable to cause a change in the level of the final fluorescence of liposomes (Fig. S2, A) meaning that they did not compromise the membrane integrity. At a concentration of 150 μM all the studied cations initiated proton release from liposomes to varying degrees (Fig. 4B), C_4TPP-diMe and C_4TPP-Cl being able to quench partially the fluorescence of pyranine. To calculate the average proton efflux values under the action of C_4TPP-X cations, the initial fluorescence records (Fig. S2, A) were presented as a portion of the maximum change in fluorescence (Fig. 4A), as described in Materials and Methods. The percentage proton yields (Mean ± StDDev) at 500 s after the addition of lipophilic cations are shown in Fig. 4C for the concentrations of 20 and 60 μM.

Similar measurements were made on liposomes without the addition of palmitate to the liposomes (Fig. 4, D and E). The protonophoric activity of C_4TPP-diMe in the absence of fatty acids was significantly suppressed. The proton transport rate enhanced with increasing concentration of C_4TPP-diMe, at a concentration of 150 μM fluorescence was quenched after equilibration of pH inside and outside the liposomes with X537A (Fig. 4,E). Other cations from the C_4TPP-X series, with the exception of C_4TPP-Me, did not show a noticeable protonophoric effect under these conditions. The addition of 150 μM C_4TPP-Cl caused a decrease in the final level of fluorescence after X537A addition, although the initial proton transport was negligible.

Table 1
Average values of the maximum respiration rate (c), half-increase concentrations of the respiration rate ($C_{1/2}$), the difference between the maximum and the initial respiration rate (Δv_{max}) and octanol-water partition coefficient (determined in [24]) for C_4TPP-X cations.

	$C_{1/2}$, μM	Δv_{max}, nmole O_2/(min×mg protein)	c, nmole O_2/(min×mg protein)	$log\ (P_{ow})$
C_4TPP	45.0 ± 6.0	37.7 ± 7.2	54.4 ± 7.2	1.42
C_4TPP-F	120.6 ± 19.8	30.7 ± 2.6	50.5 ± 2.1	1.57
C_4TPP-Cl	15.1 ± 1.9	23.9 ± 5.0	41.2 ± 3.7	2.28
C_4TPP-OMe	13.9 ± 1.0	27.5 ± 2.3	42.4 ± 4.0	2.0
C_4TPP-Me	19.5 ± 5.0	31.4 ± 9.6	45.7 ± 9.1	2.37
C_4TPP-diMe	8.7 ± 1.0	71.3 ± 9.0	86.2 ± 5.0	2.98

The ability of C_4TPP-diMe to transport protons across lipid membranes without prior addition of a fatty acid to the membrane-forming lipid mixture indicates the presence of fatty acids in the lipid membrane due to the processes of spontaneous lipid hydrolysis. Previously, it was shown on planar bilayer lipid membranes made from lipids with ester bonds the establishment of a proton gradient on the lipid membrane led to the formation of an electrical potential difference in the presence of SkQ1 [32]. Such a process was not observed in BLM made from diphytanylphosphatidylcholine, which contains ether bonds instead of ester bonds excluding the appearance of fatty acids in the membrane.

The cyclic movement of a protonophore in the lipid membrane resulting in a proton transport is accompanied by a stage of a transfer of an electrical charge. Thus, proton transport to the outside of liposomes is quickly blocked by the generated diffusion potential on the membrane. Therefore, in the absence of valinomycin (an electrogenic K+ carrier), the fast dissipation of the pH gradient is blocked [28,33]. The stage of electrogenic transport is also present during the transport of a proton by an ion pair of cation-fatty acid [8]. Therefore the protonophoric action of lipophilic cations and fatty acids the kinetics of pyranine fluorescence inside liposomes must significantly depend on the presence of valinomycin. We tested the effect of valinomycin in case of C_4TPP-diMe, C_4TPP-Me and C_4TPP-Cl cations. Fig. S2, B shows that in case of C_4TPP-diMe valinomycin stimulated the proton transport (at a concentration of 20 μM) while the effects of the other cations were insensitive to valinomycin suggesting the induction of proton transport via a leakage mechanism (fig. S2, C and D). These results suggest that the mechanism of the protonophore cycle of the ion pair: fatty acid - cation is realized only for C_4TPP-diMe at low concentrations.

We further studied the ability of C_4TPP-X cations to induce leakage (increase the permeability for large molecules) by measurements of the release of pyranine from liposomes or the entry of the DPX. To do this, a decrease in pyranine fluorescence was recorded after the addition of C_4TPP-X in the absence of a pH gradient (at pH = 8.0) (fig. S3). The most significant decrease in fluorescence was caused by C_4TPP-Cl. C_4TPP-F as well as C_4TPP had a minimal effect.

3.3. Study of the protonophoric action of the C_4TPP-X cation and a fatty acid on a planar bilayer lipid membrane

To accelerate the translocation of lipophilic cations and to increase the transport of protons through a planar bilayer lipid membrane we made BLM from a lipid with ether bonds, namely diphytanyl phosphatidylcholine (DPhytanylPC). It was previously shown that the lack of fatty acid carbonyl groups in the ether lipid decreased the dipole potential of a BLM by >100 mV [32,34,35], and increased tenfold the rate constants of the flip-flop of penetrating cations through lipid membranes [36,37]. On a BLM formed from DPhytanylPC and palmitic acid (9:1, w:w), the current relaxation kinetics were measured after applying a voltage of V = 50 mV in the presence of 0.1 μM C_4TPP-diMe at different pH (Fig. 5A). The experimental current traces were fitted by the equation $I(t) = I_\infty + (I_0 - I_\infty)\cdot exp(-t/\tau)$ (I_0 is initial current, I_∞ is steady-state current) at $t \leq 2$ s. At longer times, there was also a slow decrease in the current due to diffusion polarization in the near-membrane unstirred layer [24]. Fig. 5A shows that upon an increase in pH from 4.8 to 8.8 the initial and stationary currents increased significantly and the characteristic current relaxation time (τ) decreased. In contrast to that the kinetics of current in the presence of 0.1 μM

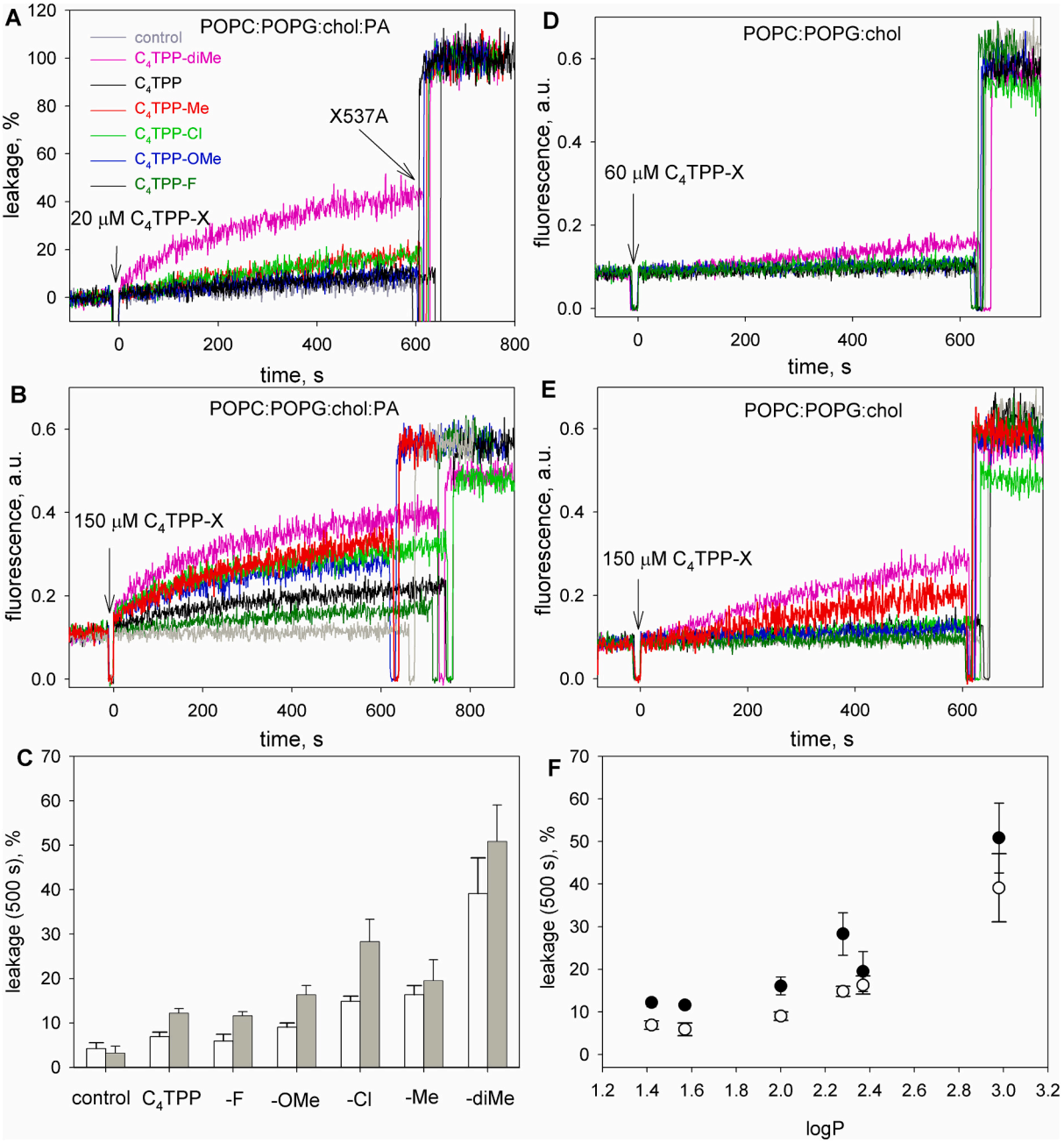

Fig. 4. Dissipation of the pH gradient by C_4TPP-X cations on membranes of pyranine-loaded liposomes formed from POPC, POPG, cholesterol and palmitic acid (panels A and B) or without palmitic acid (panels D and E). The addition of C_4TPP-X is indicated by an arrow, the concentrations were 20 μM (panel A), 150 μM (panel B), 60 μM (panel D) and 150 μM (panel E). The kinetics of pyranine fluorescence inside liposomes are shown for each cation in different colors: C_4TPP – black curve, C_4TPP-F – dark green curve, C_4TPP-Cl – green curve, C_4TPP-OMe – blue curve, C_4TPP-Me - red curve, C_4TPP-diMe – pink curve and control with adding ethanol – grey curve. Panel A shows normalized curves obtained according to the procedure described in Materials and Methods. After $t = 600$ s, the lasalocide A was added to the cuvette to equilibrate the pH inside and outside the liposomes. Relative magnitudes of change in fluorescence at 500 s after addition of lipophilic cations at 20 μM (light bars) and 60 μM (grey bars) (panel C) and dependence of magnitudes of change in fluorescence at 500 s on logP_{ow} (panel F) are presented for liposomes with the addition of palmitic acid. The lipid concentration in the cuvette was about 20 μg/ml. Liposomes were produced from the mixture of lipids POPC: POPG:chol:PA (5:1.3:2.4:1.3, w/w) (panel A, B, C, F) or POPC:POPG:chol (5.4:1.5:3.1, w/w).

C_4TPP-diMe at pH = 4.8, 7.8, and 8.8 for BLM formed from DPhytanylPC without the addition of palmitic acid were almost identical to each other (inset to Fig. 5A presents). This result proves that the pH dependence of the current relaxation kinetics in the presence of C_4TPP-diMe is determined by the fatty acids in the membrane-forming solution and their ability to be protonated-deprotonated. The dependences of the characteristic relaxation time of the current after application of 50 mV (τ_{50mV}) on pH are shown in Fig. 5B for BLM formed from DPhytanylPC (open circles) and DPhytanylPC: palm acid (filled circles). In the latter case, the data fit well with the sigmoid function $\tau(pH) = \tau_{alk} + \frac{a}{1+10^{\wedge}((pH-pK_a)/b)}$, where $\tau_{alk} = 0.12$ s is a limiting value of τ at alkaline pH, $pK_a = 7.47$ is a dissociation constant of palmitic acid at the lipid membrane interface, $a = 0.243$ s, $b = 1.5$. The value of pKa is close to that found in [38]. The acceleration of the current relaxation kinetics with an increase in the proportion of protonated weak acids with a decrease in pH was previously shown for uncouplers CCCP [39] and FCCP [40].

An increase in the stationary BLM current upon alkalization of an

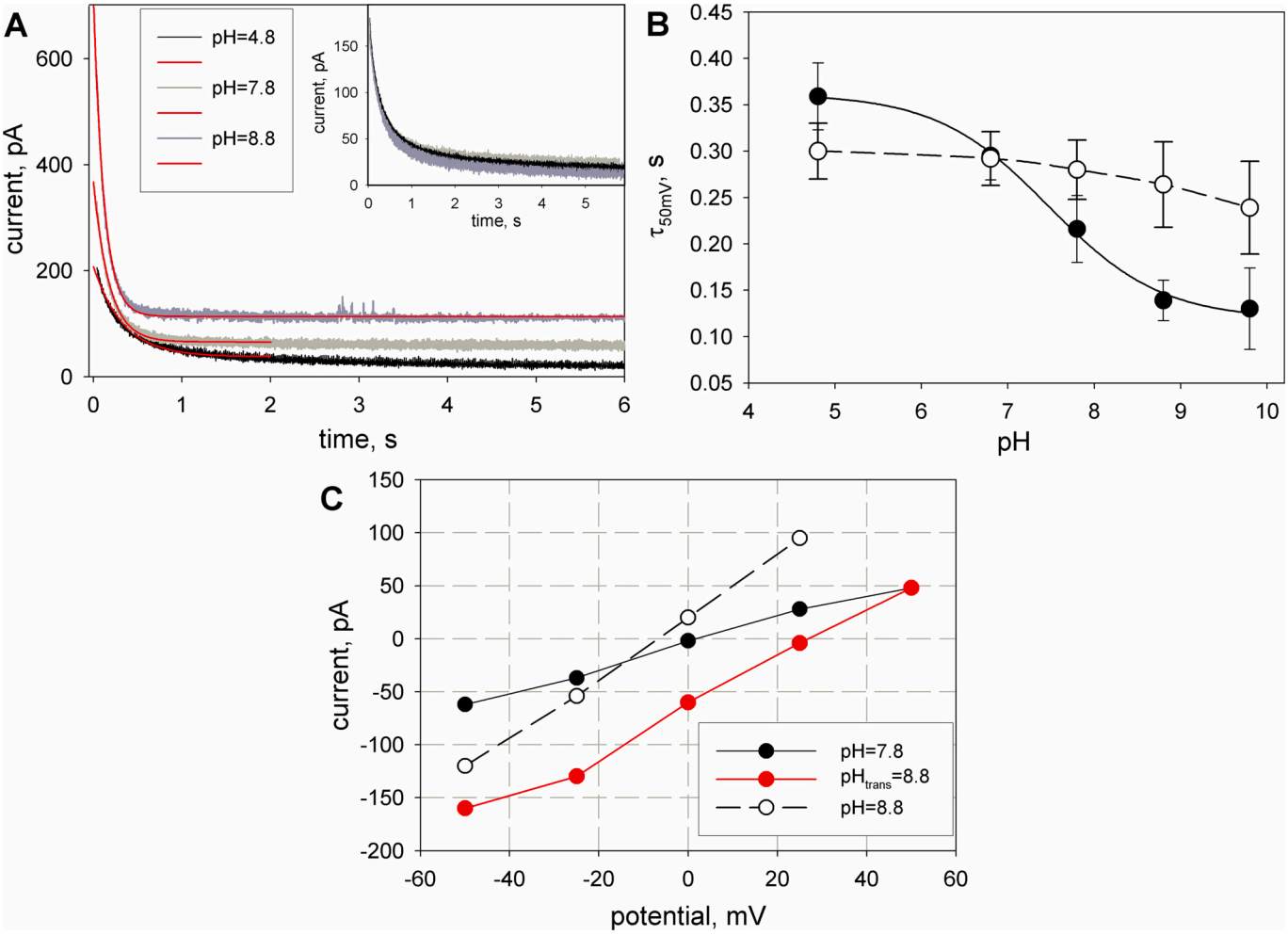

Fig. 5. A Relaxation kinetics of the BLM current in the presence of 0.1 μM C_4TPP-diMe after applying a voltage of 50 mV at time $t = 0$ s at different pH of the buffer solution (pH = 4.8, black curve; pH = 7.8, light grey curve; pH = 8.8, dark grey curve). Measurement medium: 10 mM Mes, Tris, β-Alanine, 100 mM KCl. The lipid solution contained DPhytanylPC: palmitic acid (9:1, w:w). The inset shows the kinetics for BLM formed from DPhytanylPC. B Dependences of the characteristic relaxation time of the C_4TPP-diMe current upon application of 50 mV on the pH of the medium in the presence (full circles) or in the absence of palmitic acid in BLM (open circles). C Voltage dependences of the stationary BLM current for 0.1 μM C_4TPP-diMe at symmetrical pH values on both sides of the membrane (pH = 7.8, full circles; pH = 8.8, empty circles) and at pH gradient on the lipid membrane (pH_{cis}:pH_{trans} = 7.8:8.8, red circles) for BLM containing palmitic acid.

aqueous solution in the presence of C_4TPP-diMe (Fig. 5A) is related to the appearance of a proton current. To confirm this we measured the current-voltage characteristic in the presence of 0.1 μM C_4TPP-diMe under symmetrical conditions (at pH = 7.8, Fig. 5C), and after pH increase to 8.8 on one side of the membrane. The observed shift in the current-voltage dependence was about ΔV_{zero} = 30 MB with plus sign at the alkaline side. Returning to symmetrical conditions with pH = 8.8 on both sides of the membrane resulted in a return to the symmetrical current-voltage curve with zero ΔV_{zero} with a steeper slope corresponding to higher membrane conductivity (Fig. 5C). The ΔV_{zero} values were only a half of the theoretical value estimated by the Nernst equation (58 mV at ΔpH = 1).

We studied also the dependence of the current relaxation kinetics after applying a voltage of V = 50 mV on pH in the presence of 0.5 μM C_4TPP-Me (Fig. S4 A) using BLMs formed from DPhytanylPC and palmitic acid. At pH = 4.8 the current with a weak decay was recorded which could not be described by monoexponential kinetics even at short times, and this result is consistent with previously obtained data on BLM from DPhytanylPC [24]. However, at pH = 7.8 an increase in the current values was observed, and at pH = 8.8 and 9.8, the initial current decay kinetics can be fitted by a monoexponential function. The pH dependence of the current kinetics for C_4TPP-Me can be explained by its insufficient binding. At low pH and a small surface potential of the membrane, the lipophilicity of the cation is insufficient for strong adsorption of the cation on the surface of the lipid membrane leading to poor current decay which is characteristic of the cation flip-flop stage between two monolayers. At pH > pKa the palmitic acid contributes to the negative surface potential of the membrane due to deprotonated form of the fatty acids leading to a significant amount of adsorbed cations and the pronounced flip-flop stage. The voltage dependence of the stationary current shifted by approximately 30–35 mV when creating ΔpH = 1 on the BLM (Fig. S4, B) confirming the possibility of proton transfer by the complex of C_4TPP cation and palmitic acid on the lipid membranes formed from ether lipid. In experiments with 20 μM C_4TPP a stationary current was recorded through the BLM which slightly increased when the buffer solution was alkalinized. However no shift in the current-voltage characteristic was observed upon when creating a pH gradient on both sides of the membrane (pH_1 = 7.8, pH_2 = 8.8, Fig. S5).

3.4. *Cations of the C_4TPP-X series cause swelling of rat liver mitochondria*

It was shown previously that tetraphenylphosphonium, C_{12}TPP and SkQ1 cause swelling of isolated mitochondria in a potassium medium at certain concentrations [11,14], while C_{12}TPP and MitoQ induced mitochondrial swelling in living proximal tubular cells [13]. We found

that several C_4TPP-X cations at a concentration of 200 μM also induce the swelling of isolated rat liver mitochondria in a medium containing 130 mM KCl (Fig. 6). C_4TPP-diMe had the strongest effect (Fig. 6, pink curve) measured by a decrease in the optical density of the mitochondrial suspension. The effect of C_4TPP-diMe reduced substantially in a sucrose medium (pink dotted curve). C_4TPP-Me, C_4TPP-Cl and C_4TPP-OMe induced mitochondrial swelling to a lesser extent (red, green, and blue curves, respectively) than C_4TPP-diMe. The effect of C_4TPP and C_4TPP-F at 200 μM was minimal. The addition of 1.5 mM succinate or 3 μM rotenone did not affect the optical density kinetics after the addition of lipophilic cations (Fig. S6). Increasing the concentration of C_4TPP-F to 0.5 and 1 mM caused a slight decrease in optical density (Fig. S6). The studied cations caused a significantly slower swelling of the mitochondria than after the induction of the permeability transition (in the presence of phosphate and calcium, Fig. S6).

3.5. Induction of the BLM current fluctuations by C_4TPP-X cations

It was shown that conventional detergents can induce the fluctuations of the BLM current [41] stimulating the ionic permeability of lipid membranes [15]. The ability of lipophilic cations of the C_4TPP-X series at concentrations of tens of micromoles to exert a detergent-like effect on lipid membranes was found on BLM formed from diphytanoylphosphatidylcholine using C_4TPP-Me, C_4TPP-Cl, C_4TPP-diMe and C_4TPP-OMe. The permeability of C_4TPP-X cations through such a lipid membrane is significantly reduced (compared to DPhytanylPC) due to a higher jump in the dipole potential of the membrane [32,37]. When the cations were symmetrically added to both sides of the membrane at the concentration of 40 μM the conductivity of the membrane increased and the observed current fluctuations were indiscrete (Fig. S7 A, C, E, G). If palmitic acid was present in the BLM, the membrane conductivity was even higher (Fig. S7 B, D, F, H), and the current fluctuations were observed to approximately the same magnitude. C_4TPP and C_4TPP-F at the concentration of 40 μM did not induce current fluctuations and insignificantly increase the membrane conductivity (Fig.S8).

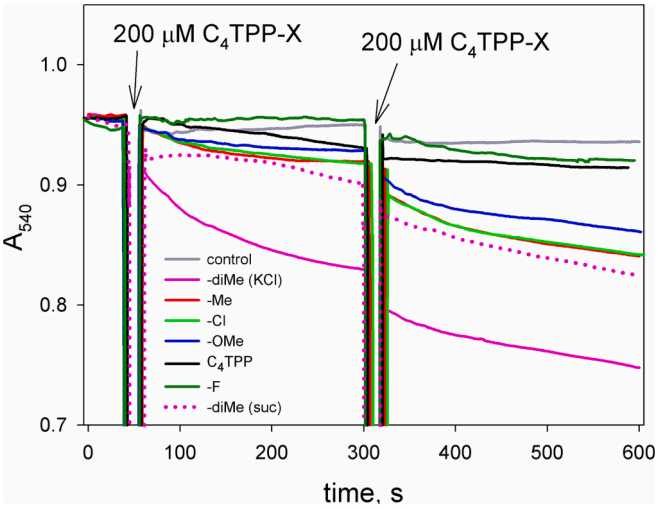

Fig. 6. Kinetics of changes in light scattering of the suspension of rat liver mitochondria after addition of 200 μM C_4TPP-X (pointed by arrows) in the medium with KCl (5 μl of ethanol – grey curve; C_4TPP-diMe – pink curve; C_4TPP-Me – red curve; C_4TPP-Cl – light green; C_4TPP-OMe – blue curve; C_4TPP – black curve; C_4TPP-F – dark green curve). The incubation medium contained 10 mM Hepes, 130 mM KCl, 0.2 mM EDTA, 1.5 mM succinate, pH = 7.4. Pink dashed curve obtained in the presence of C_4TPP-diMe in sucrose medium (250 mM sucrose, 20 mM MOPS, 0.2 mM EDTA, 1.5 mM succinate, pH = 7.4). The concentration of mitochondrial protein in the cuvette was about ~0.2 mg/ml.

4. Discussion

The mechanism of the protonophoric action of uncouplers mostly represented by weak acids has been carefully studied previously in many laboratories [39,40,42–44]. The effectiveness of such compounds in the induction of a proton flux through the lipid membrane is proportional to the mobility of the charged form in the membrane and their partition coefficient [21]. The transport of protons mediated by the cyclic movement of the lipophilic cation and fatty acid in the lipid membrane should also be limited by the cation flip-flop stage because ions permeate through the hydrophobic layer of the membrane much more slowly than neutral molecules [40,45]. We have previously shown that the permeability of C_4TPP-X cations differs by six orders of magnitude [24], so one could expect the same significant differences in the efficiency of their protonophoric action on liposomes and isolated mitochondria. However, the effective concentrations of cations in experiments on mitochondria and liposomes varied by no >15 times according to the results presented in Fig. 3 and Fig. 4A, B.

When analyzing the concentration dependences of the mitochondrial respiration rate in the presence of C_4TPP-X cations (Fig. 3) the most noticeable differences are in the concentrations of cations corresponding to the half acceleration of respiration ($C_{1/2}$) and in the maximum respiration rates (Table 1). Namely, Δv_{max} for C_4TPP-diMe is approximately 2 times bigger than for any other cation and reaches the maximum respiration rate in the presence of DNP. Importantly it is generally accepted that the maximum respiration rate is a function of the respiratory chain itself and is essentially independent of the uncoupler used [46]. In contrast to that the respiration rates induced by detergents never reaches maximum values as in the case of conventional uncouplers [16,17,47]. The reason for this phenomenon is unclear but one can consider the ability of detergents to inhibit the enzymes of mitochondrial respiratory chain and/or efflux of cytochrome *c* from the intermembrane space [48]. It can be assumed that such differences in maximum respiration rates reflect a difference in the mechanism of action of C_4TPP-diMe and other cations. This hypothesis is confirmed by the results obtained on the BLM where it was found that only C_4TPP-diMe and C_4TPP-Me of all the studied cations had a protonophoric effect in the presence of a fatty acid in the lipid membrane resulting in a high value of the stationary current (I_∞). I_∞ was approximately 50 and 20 pA, at pH = 7.8 and V = 50 mV at 0.1 μM C_4TPP-diMe and at 0.5 μM C_4TPP-Me, respectively (Fig. 5 and Fig. S4). Thus, in the case of a single mechanism of mitochondrial uncoupling by an ion pair of a lipophilic cation and a fatty acid, the effective concentrations of C_4TPP-diMe and C_4TPP-Me would have to differ by >10 times while they differed only 2-fold in the experiment (Table 1).

It can be assumed that the transport of protons through the mitochondrial membrane under the action of C_4TPP-X cations (except for low concentrations of C_4TPP-diMe) occurs by the mechanism of leakage of protons and other inorganic ions (such as potassium and chloride). The input of this mechanism should increase with the adsorption of C_4TPP-X cations on the mitochondrial membrane. Previously we determined the partition coefficient in octanol – water (P_{ow}) and the permeability values of C_4TPP-X through lipid membranes [24]. It can be seen from Fig. 7A that the reciprocal concentration of the half acceleration of oxygen consumption ($1/C_{1/2}$) proportionally increased with the increase in P_{ow} according to the equation: $1/C_{1/2} \sim P_{ow}^n$, where $n = 0.42$. The similar dependence on the permeability ($1/C_{1/2} \sim Permeability^n$, where $n = 0.1$, Fig. 7B) points to a dependence of lesser extent. At low concentrations (3 μM) C_4TPP-X cations are approximately equally accumulated in energized mitochondria, with the exception of C_4TPP-F and C_4TPP-Cl, for which the degree of accumulation was somewhat lower [24]. However, at high concentrations of alkyltriphenylphosphonium analogs (0.5 mM), the degree of accumulation of the cations in mitochondria correlates with P_{ow} [49]. We showed (Fig. 6) that at high concentrations of the cations not only inorganic ions but also larger molecules permeated into mitochondria resulting in their

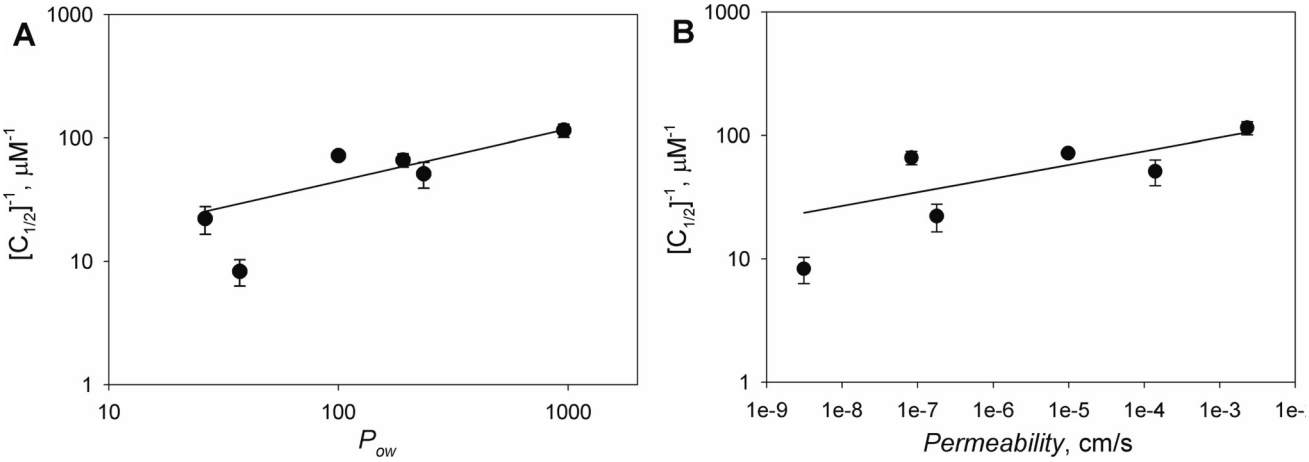

Fig. 7. The reciprocal concentration of the half acceleration of oxygen consumption by mitochondria ($1/C_{1/2}$) plotted against the octanol−water partition coefficient $logP_{ow}$ (A) and experimental permeability (B) of the lipophilic cations.

swelling in potassium chloride and even in sucrose medium.

Experiments on liposomes loaded with pyranine points also on the different mechanisms of the induction of the transport of protons by C_4TPP-X cations (Fig. 4). As wrote above protonophoric mechanism assumes a rate-limiting electrogenic step which can be identified in the system by the significant differences in proton release rates upon the addition of valinomycin. The absence of the effect of valinomycin points to the absence of the generation of the electrical potential on the membrane of liposomes suggesting the induction of nonspecific permeability characteristic for detergents. In our experiments, the addition of 20 μM C_4TPP-diMe in the presence of palmitic acid and valinomycin in the lipid membrane led to a significant proton release from liposomes and this process was almost completely blocked in the absence of valinomycin (Fig. S2, B). Other cations of the C_4TPP-X series either had no noticeable effect at such concentrations or the proton release was much smaller (Fig. 4A). 60 μM C_4TPP-Me or C_4TPP-Cl induced a significant proton leak that was independent of the presence of valinomycin (Fig. S2, C and D). When C_4TPP-diMe was added at the concentrations of 60 μM and higher proton transport was almost independent of the addition of valinomycin (data not shown), which indicates the detergent effect of high concentrations of C_4TPP-diMe. With a further increase in the concentrations up to 150 μM all cations increased the proton release rate (Fig. 4B) and a significant reduction of the final level of fluorescence is seen in the presence C_4TPP-diMe and C_4TPP-Cl. This effect indicates the induction of the leak of larger ions through the liposomal membrane such as pyranine and/or DPX.

Thus in experiments on lipid vesicles one can consider three distinct transport mechanisms changing from low to high concentrations of the lipophilic cation. 1) At small concentrations of C_4TPP-diMe the protonophoric cycling mechanism predominates involving an ion pair of the cation and a fatty acid (a scheme in Fig. 8, panel A). In the absence of palmitic acid in the lipid membrane the protonophoric action of C_4TPP-diMe is significantly reduced. This mechanism fails to operate for other cations studied. 2) In the range of the cation concentrations of 60 μM and

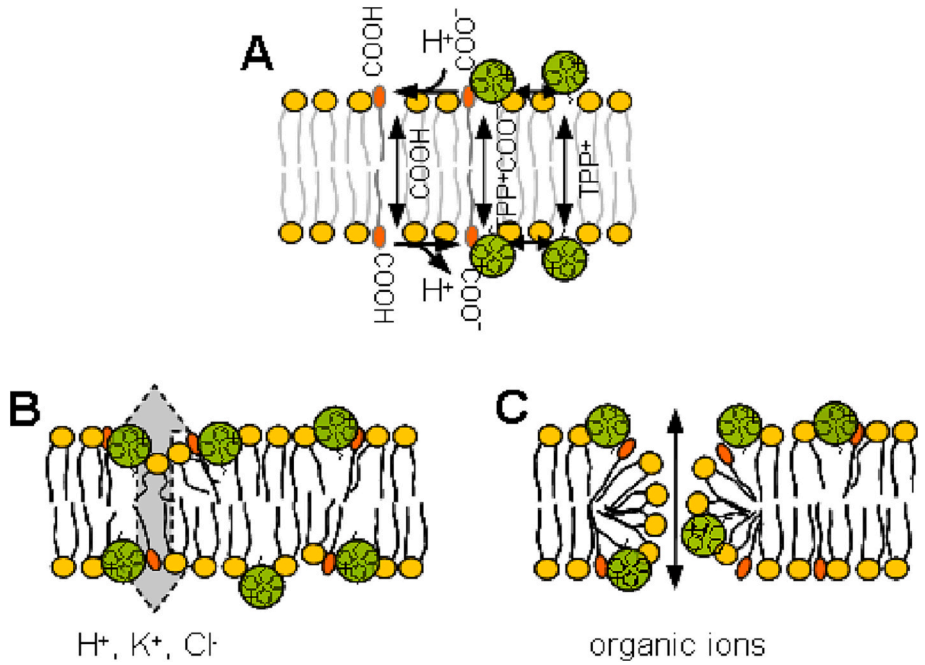

Fig. 8. Scheme illustrating mechanism of protonophoric activity (A) of fatty acid mediated by lipophilic cation C_4TPP-X, inducing lipid membrane leakage for inorganic ions at high density of cations and fatty acids in the lipid membrane (B), and the formation of defects (C) in lipid bilayer integrity by C_4TPP-X and fatty acids, enabling leakage of organic compounds.

above proton transport occurs via a mechanism formation of small membrane defects which is characteristic for all the cations and is much more pronounced in the presence of fatty acids in the lipid membrane (Fig. 4, Fig. S2, Scheme 8B). Apparently, for a noticeable perturbation of the membrane a certain concentration of the lipophilic cation is required on the surface of the lipid membrane enabling the formation of their complexes with fatty acids. The defects formed are capable of facilitating the passive diffusion of protons and small inorganic ions (Scheme 8, panel B). The effectiveness of the membrane perturbation increases with increasing $logP_{ow}$ of the lipophilic cation (Fig. 4F). 3) In the range of the concentrations of the lipophilic cations above 150 μM larger molecules leak across the lipid membrane (fig. S3, Scheme 8, panel C). This leakage can be a result of the local changes in membrane curvature by the ionic complex of the phosphonium cation and the fatty acid and formation of transient channels in the lipid membrane with the walls lined up with the head groups of the lipids. The relevance of lipid curvature and the shape of mitochondrial cristae in the respiratory process is actively discussed in the literature [50,51]. As follows from the results in model membrane systems and in vitro the presence of fatty acids is essential for the effectiveness of the protonophore and leakage mechanism of the lipophilic cations. Surprisingly, the switch from one mechanism to the other occurs in a rather narrow range of the cation concentrations. For example in case of C_4TPP-diMe cation the concentrations differs by only 7 times.

Studies of the effect of a homologous series of anionic alkyl sulfates on energy linked mitochondrial membrane functions showed that these detergents uncoupled phosphorylation from electron transport and that their relative potencies correlated quantitatively with their lipophilicities [52]. Cationic detergents caused the collapse of the mitochondrial membrane potential due to elevation of the permeability of the mitochondrial membrane to the ions, the effect increased with increasing alkyl length of the compounds [18,48]. It is known that triphenylphosphonium alkyls which are most often used to create mitochondria-targeted compounds can accelerate respiration, reduce the membrane potential of mitochondria, and even lead to swelling of mitochondria [11–13]. Ong and coauthors showed that TPP salts have significant impact on the mitochondrial membrane potential of HeLa cells [53]. The effects appeared to be correlated with the lipophilicity of the nine salts, with no significant difference between alkyl chain extension and aryl methylations. It has recently been suggested that a decreased electron density on the phosphorus atom can abrogate uncoupling activity as compared to the parent triphenylphosphonium (TPP) [20]. Moreover Kulkarni and coworkers have found that modulation of Hückel charge on the TPP phosphorus atom has a greater impact on uncoupling potency than alteration in lipophilicity. However our results do not correlate with the values of Hückel charges on the phosphorus atom for the modified in *para* position triphenylphosphonium cations which were calculated in [20]. The value of the charge was 0.326 for C_4TPP, 0.328 for C_4TPP-Cl, 0.328 for C_4TPP-F, 0.299 for C_4TPP-OMe, and 0.31 for C_4TPP-Me. For example at the same calculated values of the Hückel charge for C_4TPP-F and C_4TPP-Cl their effective concentrations differ by a factor of 6 or more in experiments on mitochondria and liposomes (Table1, Fig. 4C, Fig. S3).

It is known that alkyl triphenylphosphonium cations with linear hydrocarbon chain inhibited the growth of the Gram-positive bacterium *Bacillus subtilis* wherein the inhibitory effect was upregulated with growing lipophilicity [19]. It has been shown recently for various types of bacteria that the lipophilicity of 2-(2-hydroxyaryl)alkenylphosphonium salts play a decisive role in their antibacterial efficacy [54] and the mechanism of this action is apparently due to their protonophoric or detergent-like action on lipid membranes [55]. Our results combined with the literature data make it possible to determine the strategy for choosing the mitochondria-targeted or bacteria-targeted lipophilic cation upon the synthesis of new compounds. Namely more hydrophobic analogs of cations should be used to create effective antibacterial drugs. In case of the synthesis of drugs for the targeted delivery of biologically important molecules to mitochondria the more hydrophilic analogs of cations should cause a lesser destabilizing effect on the lipid structures of cells and therefore should be less toxic.

5. Conclusion

We studied the uncoupling and protonophoric abilities of lipophilic cations of the C_4TPP-X series using isolated rat liver mitochondria and model lipid membranes. All lipophilic cations of this series cause an acceleration of mitochondrial respiration, decrease in membrane potential and the leakage of protons from liposomes with a pre-formed pH gradient on the liposomal membrane. The extent of these effects depends significantly on the presence of fatty acids. We found that the uncoupling abilities of these cations (with the exception of C_4TPP-diMe at low concentrations) are explained by their ability to perturb the general permeability of the membranes similar to the action of detergents, and their effectiveness correlates well with the octanol-water partition coefficient. We suggest that in order to avoid the detergent action of mitochondria targeted conjugates, the most hydrophilic penetrating cations should be chosen so that their impact on the uncoupling of oxidative phosphorylation would be minimal while their accumulation in mitochondria can be maintained. The most promising in this case may be fluorinated derivatives in phenyl residues of CnTPP cations.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbamem.2023.184183.

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