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Molecular Mechanisms of Transformation of SkQ Mitotropic Quinones and the Search for New Approaches to Creation of Selective Free Radical Traps

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Abstract—Features of the mechanism of action of positively charged benzoquinone derivatives (SkQ), which are the analogs of coenzyme Q (I), plastoquinone (II), and tocopherol (III), are discussed. It is usually considered that the main target of these compounds is mitochondria, where they accumulate due to the positive charge of the molecule. In the present work, it is shown with model systems that the reduced forms of compounds (I-III) under certain conditions can transform into electrically neutral cyclic zwitterions, which theoretically can escape from the matrix of energized mitochondria against the concentration gradient. A weak uncoupling effect of molecules I-III has been found on mitochondria. Its existence is in agreement with the abovementioned transformation of positively charged hydroquinones of type Ia-IIIa into electrically neutral molecules. The data obtained with model systems suggest that the target of SkQ hydroquinones as free radical traps may be not only mitochondria but also biochemical systems of the cytoplasm. Due to the presence of a large number of reactive oxygen species (ROS)-dependent signal systems in a cell, the functioning of cytoplasmic systems might be disturbed under the action of antioxidants. The problem of selective effect of antioxidants is discussed in detail in the present work, and a functional diagram of selective decrease of the "background level" of ROS based on differences in the intensity of background and "signal" ROS fluxes is considered.

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Key words: free radical traps, mitochondria, ROS, SkQ, MitoQ, uncoupling

At present, some laboratories carry out intensive studies of the novel class of antioxidants, the primary target of which is supposed to be mitochondria [1-3]. The structure of these compounds contains a positively charged triphenylphosphonium (TPP^+) group (see Scheme 1), which determines the ability of quinones I-III to selectively accumulate in the mitochondrial matrix when driven by the electric field, where they are effectively reduced by the enzymes of the mitochondrial respiratory system. Previously, Murphy showed that compound I that he had synthesized (Scheme 1) could be reduced by purified complex II of the mitochondrial respiratory chain with the formation of the respective hydroquinone being practically not oxidized by complex III [4, 5]. The properties of MitoQ (I) were described in detail in work [2]. This compound does not restore the functioning of the respiratory chain of the mutant yeast strain deficient in coenzyme Q (CoQ) [4, 5]. Later we revealed that MitoQ in intact mitochondria could be effectively reduced by DT-diaphorase and oxidized by complex III [6]. These data do not contradict Murphy's works because CoQ was not present in the systems in his experiments. Thus, comparison of our results with the data of works [4, 5] shows that $MitoQH_2$ can be effectively oxidized in mitochondria by ubiquinone. Our conclusion that MitoQ interacts with CoQ in mitochondria is in agreement with the data of

Abbreviations: CoQ, coenzyme Q; DQ, duroquinone; MitoQ, 2,3-dimethoxy-5-methyl-1,4-quinone-6-decylbenzene triphenylphosphonium chloride; $MitoQH_2$, 2,3-dimethoxy-5-methyl-1,4-diol-6-decylbenzene triphenylphosphonium chloride; ROS, reactive oxygen species; SkQ1, 2,3-dimethyl-1,4-quinone-5-decylbenzene triphenylphosphonium chloride; $SkQ1H_2$, 2,3-dimethyl-1,4-diol-5-decylbenzene triphenylphosphonium chloride; SkQ3, 2,3,5-dimethyl-1,4-quinone-6-decylbenzene triphenylphosphonium chloride; $SkQ3H_2$, 2,3,5-dimethyl-1,4-diol-6-decylbenzene triphenylphosphonium chloride; TPP^+, triphenylphosphonium cation.

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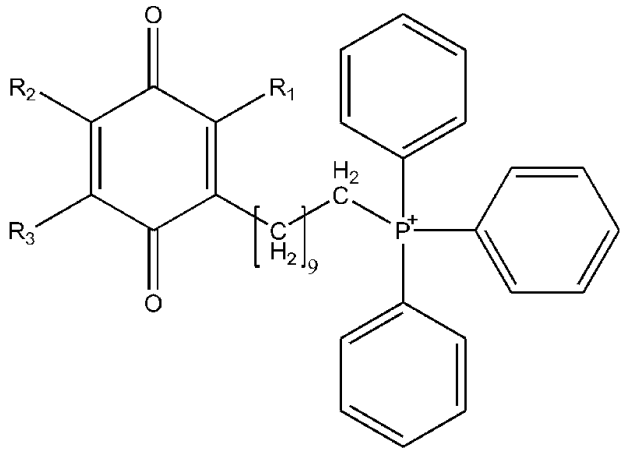


Structure of studied substances. I, MitoQ ($R_1 = CH_3$, $R_2 = OCH_3$, $R_3 = OCH_3$); II, SkQ1 ($R_1 = H$, $R_2 = CH_3$, $R_3 = CH_3$); III, SkQ3 ($R_1 = CH_3$, $R_2 = CH_3$, $R_3 = CH_3$); IV is duroquinone (tetramethylbenzoquinone); Ia-IVa are hydroquinones, reduction products of compounds I-IV

Scheme 1

work [7], where another product of DT-diaphorase – durohydroquinone (IVa) – like quinone (Ia), is oxidized in mitochondria by ubiquinone only.

In the second half of the previous century, antioxidants were widely used as free radical traps for prevention and therapy of radiation sickness under conditions of intensive generation of abnormally large quantities of reactive oxygen species (ROS) in cells. It was the goal in general to find the most efficient "traps" for free radicals. Such a strategy was quite justified for acute radiation sickness. However, in the last 10-20 years it has become evident that ROS are toxic only in abnormally high concentrations appearing in pathologies. Quickly accumulating data provide evidence that normally ROS are regulators of basic cell metabolism. In signaling systems, these compounds act as secondary messengers regulating tens of the most important biochemical processes. At the same time, it has been established that active oxygen forms regulate processes of basic cell metabolism [8, 9]. They are involved in the control of genome functioning; they activate mitosis [10], apoptosis [11], and transcription factors; in particular, H_2O_2 has been shown to activate the synthesis of 115 proteins in yeasts [12]. In bacteria, H_2O_2 participates in activation of factor Oxy-R controlling the synthesis of an antioxidant system [13]; the activation of nuclear transcription factor NF-κB [8] and heat shock factor HSf [14] has been observed in eucaryotes. Also, ROS are involved in systems of transduction of external signals directed from cell surface to the nucleus. About 100 such systems of tyrosine phosphatases have been revealed in the human genome. Their sequences are homologous in a conservative cysteine-containing region, which is apparently the target of ROS signals [15, 16].

Due to the finding of new ROS functions in biology, it is evident that highly efficient "traps" for these compounds may significantly change the normal functioning of ROS-dependent regulatory systems. Therefore, in parallel with the strategy of maximal inhibition of free-radical processes (which is acceptable only for very acute pathologies), it is necessary to search for methods of regulation of ROS level in the cell, i.e. create selectively acting antioxidants. One of the possibilities for solving this problem lies in a feature of the functioning of natural ROS-dependent signal systems. This feature is that the intensity of ROS signal in the regulatory system, by definition, must significantly exceed the background ROS level. This difference must be high enough to exceed the values of frequently occurring "background" fluctuations of the level of active oxygen forms. Analysis of the literature [17-20] has shown that this difference underlies the natural, so-called peroxide reductase, trap of "background" hydrogen peroxide [21]. This trap does not have sufficient power for decreasing below the critical level or suppressing the intensive short "signal" discharge of H_2O_2. However, this quickly operating but low-power system is capable of arbitrarily long degradation of ROS formed in the course of low intensity background processes. A number of ROS-dependent controlling systems have been described, where ROS (H_2O_2) signals are induced on the plasma membrane on binding of an agonist. Such agonists can be growth factors, i.e. TGF [17] and epidermal growth factor [22], some mitogens [23], and interleukins [24]. After attachment of an agonist, H_2O_2 is synthesized with the involvement of GTP-dependent NADPH-oxidases (NOX). However, it is interesting to note that NOX is activated by the same group of Rac-GTPases [25-29] that activates intensive ROS discharge by immune cells.

The goal of the present work was to show in model experiments the possibility in principle for compounds I-III (Ia-IIIa) to play the role of antioxidants not only in mitochondria but also in the cytoplasm of the cell; to specify particular approaches for creation of systems for selective suppression of background ROS level in the cell that do not disturb the functioning of ROS-dependent signal systems.

Specific tasks:

– to show the possibility of transformation of hydroquinones Ia-IIIa into cyclic electrically neutral zwitterions, which can escape from the matrix of energized mitochondria (in the presence of electric potential on the membrane);

– using the model of isolated mitochondria, to show the uncoupling effect of SkQ quinols (Ia-IIIa) as evidence of the ability of these compounds to escape from the matrix of energized mitochondria.

The study of the properties of SkQ hydroquinones and respective semiquinones in model systems has shown that these compounds have the properties necessary for

functioning under certain conditions as selective regulators of the background ROS level in the cytoplasm.

MATERIALS AND METHODS

Mitochondria were isolated from outbred white rat liver by differential centrifugation according to the standard procedure [30]. The experiments were carried out in isotonic conditions. The isolation medium contained mannitol, 210 mM; sucrose, 40 mM; Hepes-Tris, 10 mM, pH 7.5; EDTA, 0.5 mM; BSA, 0.5 mg/ml. For removal of BSA and EDTA from the isolation medium, the mitochondria were twice washed with a solution containing mannitol, 210 mM; sucrose, 40 mM; Hepes-Tris, 10 mM, pH 7.5. The resultant mitochondrial suspension was stored on ice. Protein concentration was measured by the biuret method [31].

Mitochondrial respiration rate was measured by the polarographic method using a Clark oxygen electrode on an Oroborus Oxygraph 2K polarograph (Austria). The incubation medium contained KCl, 110 mM; KH_2PO_4, 20 mM; Hepes-Tris, 10 mM, pH 7.5. The ADP/O coefficient was determined from the polarographic curve by the method of B. Chance.

Mass spectra of matrix-activated laser desorption/ionization (MALDI) [32] were recorded using a Bruker AutoFlex II reflectron time-of-flight mass spectrometer (Germany) and a 337-nm nitrogen laser emitting 1-nsec pulses. Trans-2-[3-(*tert*-butylphenol)-2-methyl-propenylidene]-malononitrile (DCTB, ⩾99%; Fluka, Switzerland) was used as the matrix. Samples were prepared by the "dried drop" method: hydroquinone chlorides were taken as solutions in chemically pure isopropyl alcohol, and the matrix was taken as a solution in chemically pure toluene. The molar ratio of matrix/analyte in applied samples was ⩾ 1000 : 1.

The p*K* values of hydroquinones Ia-IVa were determined by pH titration using a Cary spectrophotometer (Varian, USA). The UV spectra were recorded in the range of pH values 6.00-12.00 under anaerobic conditions. The titration curves for compounds Ia-IVa were plotted using the extinction values close to the λ_{max} (Table 1). The absence of oxidized form was monitored on the absorption band of quinones in the UV spectrum of each sample. The pH titration curves were calculated using ACD/Labs software. The experiments were carried out in media with low and high ionic strength containing 10 and 30 mM of phosphate buffer, respectively. The pH was measured in each sample before and after registration of the spectrum.

Reagents used: KCl, KOH, and KH_2PO_4 (Helicon, Russia); EGTA and EDTA (AppliChem, Germany); HCl (Kaustik, Russia); BSA, rotenone, myxothiazol, and HEPES (Sigma, USA); sucrose (Merck, USA); ADP (Calbiochem, Germany). Compounds I-III, as well as $MitoQH_2$, $SkQ1H_2$, and $SkQ3H_2$, were synthesized and purified in our laboratory. Concentrations in stock solutions in the experiments with mitochondria were no more than 10^{-4} M; in the polarographic cell, quinone concentrations were varied from 50 nM to 2 μM. The concentrations of inhibitors in the experiments were as follows: rotenone, up to 0.5 μM; myxothiazol, up to 0.5 μM; ADP, 500 μM.

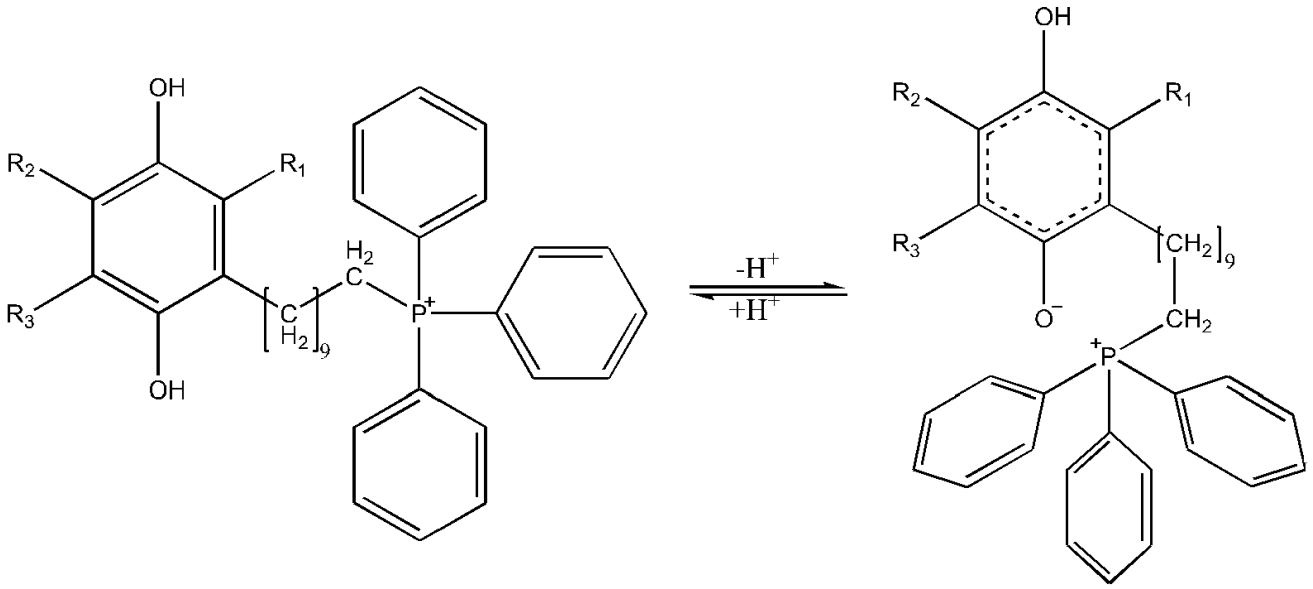
RESULTS

Increase in dissociation constant of hydroquinones Ia-IIIa on cyclization in aqueous solutions. Intramolecular convergence of the positive charge of the phosphonium radical and the OH-group of the hydroquinone fragment in bifunctional compounds Ia-IIIa must intensify dissociation of the O–H bond in this group. Therefore, the formation of a neutral intramolecular zwitterion (see Scheme 2) in such system must be coupled with a decrease in p*K* of the hydroquinones; hence, the effect of p*K* decrease in hydroquinones Ia-IIIa with the spatially distant positively charged group can be used as a criterion of cyclic zwitterion formation.

The p*K* values were determined by spectrophotometric pH titration. The p*K* values were compared to the p*K* of durohydroquinone (IVa), which, in contrast to Ia-IIIa, does not form a cyclic structure. As an example, Fig. 1 shows the spectra of the monoanion hydroquinone IIa (SkQ1) (curve *a*) and durohydroquinone IVa (curve *b*).

Table 1. The λ_{max} values (nm) of UV spectra of quinones I-III, hydroquinones Ia-IIIa, and their anions

Quinones and derivatives	Quinone	Quinol	Monoanion	Dianion	Semiquinone
DQ	265, 270	283.5	298	312	416, 440
Ia, MitoQ	276	295	298	305	420, 445
IIa, SkQ1	–	287	303	315	413, 437
IIIa, SkQ3	267	284	293	311	416, 442



Reaction of electrically neutral cyclic zwitterion formation

Scheme 2

The λ_{max} values of the bands of all hydroquinones, as well as their mono- and dianions, are given in Table 1. The p*K* values were measured in solutions containing sodium phosphate, 10 mM; the results are presented in Fig. 2 (a-d). One can see that the p*K* values of compounds Ia-IIIa are 1-2 units lower than the p*K* values of durohydroquinone IVa. In accordance with this, the effect is the evidence of transformation of hydroquinones under the experimental conditions into cyclic zwitterions IIa and IIIa.

Effect of ionic strength of Ia-IIIa solutions on stability of intramolecular cyclic ion pair. As is known, increasing solution ionic strength weakens electrostatic interactions. Therefore, it can be suggested that ion pairs must not be formed at increased ionic strength of Ia-IIIa solutions. In this case, an abrupt increase in p*K* of hydroquinones must be observed due to decrease of the intramolecular interaction of hydroquinone anion with phosphonium cation. This effect, indeed, could be observed in the experiments with hydroquinones Ia-IIIa, when phosphate concentration in the medium was increased from 10 to 30 mM. For the studied compounds, the p*K* values ranged from 11 to 11.5. This effect was observed especially well for hydroquinone IIa (Fig. 3, curves *a* and *b*).

Study of steric limitations in transformation of hydroquinones Ia-IIIa into cyclic zwitterions. The chlorides of hydroquinones Ia-IIIa were subjected to MALDI spectrometry before the beginning of basic studies. The purpose of these experiments was to show the absence of steric exclusions preventing transformation of the anions of hydroquinones Ia-IIIa into cyclic zwitterions.

The MALDI spectra of the chlorides of hydroquinones Ia-IIIa (for each substance) were shown to contain four groups of derivatives with the same molecular skeleton, which are presented in Table 2. The structures in groups 1 and 4 were determined using Occam's razor and the assumption that the weakest O–H bonds in the group of hydroquinones and the $P^+\cdots Cl^-$ bonds enter into the reaction in the first place. According to Table 2, the compounds in groups 1 and 4 contain no chlorine atomic

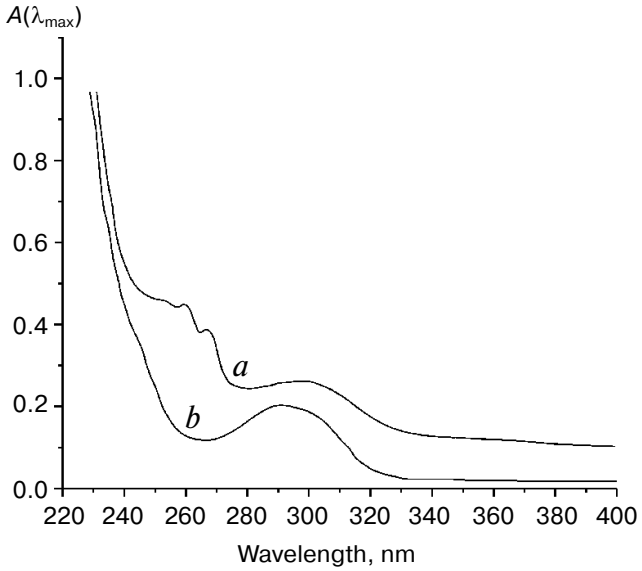


Fig. 1. Absorption spectra of monoanions: SkQ1 (*a*) and durohydroquinone (*b*).

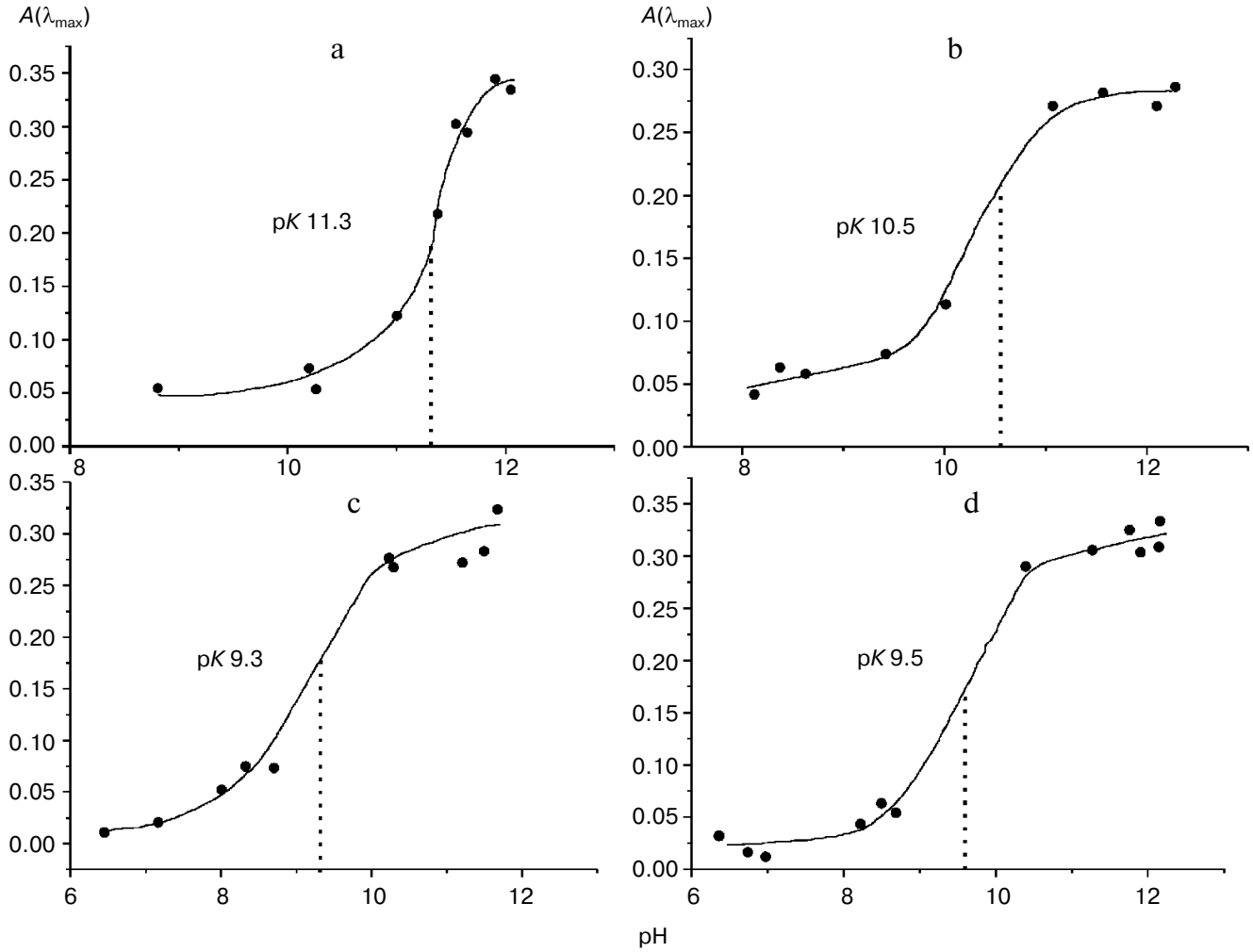


Fig. 2. Curves of pH titration of SkQ hydroquinones (Ia-IIIa) and durohydroquinone (IVa): a) durohydroquinone (IVa), p*K* 11.30; b) MitoQ (Ia), p*K* 10.59; c) SkQ1 (IIa), p*K* 9.30; d) SkQ3 (IIIa), p*K* 9.50. Medium: sodium phosphate, 10 mM.

mass and have a positive charge. The molecular masses of compounds in groups 4 and 1 correspond to the masses of hydroquinones (Ia-IIIa) and quinones (I-III), respectively, which are obviously formed through isomerization of the hydroquinone biradical. The fractions of cations (groups 1 and 4), where phosphonium ion is screened by chloride, have no charge and are not visible in the spectra. On the other hand, neutral monoanion radicals (Table 2, group 2) are visible in the spectra only as chlorides, which impart an extra negative charge to the molecules. Theoretically, it seemed possible to reveal in the MALDI spectra the negatively charged cyclic zwitterions formed by dianions of hydroquinones Ia-IIIa where, in contrast to the compounds of group 2, the positive charge of the phosphonium cation is screened by one of the negative charges of hydroquinone dianion; in such molecules, the mass of chloride must be absent. However, we failed to reliably establish the formation of negatively charged molecules with the masses corresponding to such structures. This is most likely associated with the extremely low probability of formation of hydroquinone dianions under conditions of the MALDI experiment.

At the same time, the experiment revealed the presence of negatively charged compounds which, as expected, contained no chloride mass (Table 2 (group 3) and Fig. 4). However, the molecular mass of these substances was less by two but not three units compared to the masses of the respective hydroquinones. This indicates that the supposed cyclic zwitterion must be formed not by hydroquinone dianion, but by semiquinone dianion (see group 3). Scheme 3, where the observed zwitterion is formed on cyclization of tautomeric forms (b and c) emerging from the anion radical (a), proved to be fruitful. This scheme made it possible to explain the formation of compounds of both group 3 and group 2 from a single viewpoint (see Table 2) based on the effect of tautomerism of the respective semiquinones (a-d). In accordance with this scheme, the compounds of groups 2 and 3 (Table 2) are apparent-

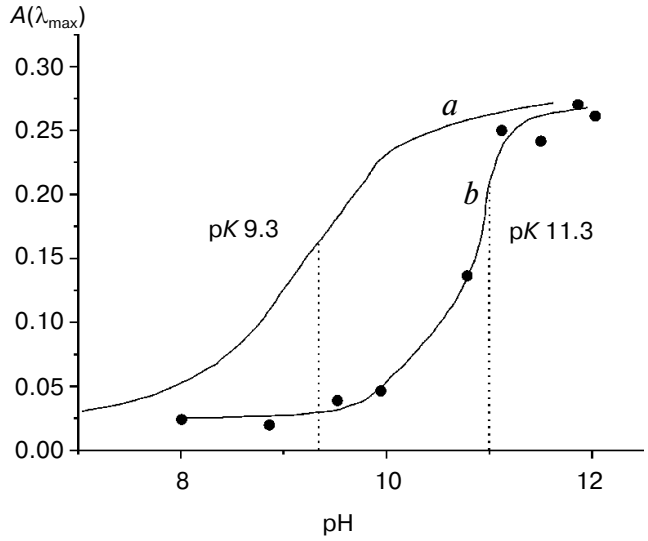


Fig. 3. Effect of drastic p*K* increase in SkQ1 (IIa) hydroquinone at increased solution ionic strength: *a*) curve of hydroquinone (IIa) titration in medium with 10 mM sodium phosphate (transferred from Fig. 2c); *b*) in medium with 30 mM sodium phosphate. The p*K* shift on curve *b* indicates the formation of an open SkQ1 monoanion (IIa) (with p*K* close to the p*K* value of durohydroquinone (IVa)).

ly formed in the course of competition between chloride and dianion-radical (Scheme 3d) for the interaction with phosphonium cation.

Also, one can see that Scheme 3 is based on tautomerization of semiquinones and explains the pathway of cyclic dianion-radical formation (Table 2, group 3). At the same time, the scheme follows the general pattern of formation of compounds revealed under experimental conditions and given in Table 1. The process passes in accordance with the accepted model, which assumes the preferred breakage of the least stable bonds ($P^+\cdots Cl^-$ and -O–H) in the studied molecules. The breakage of these bonds results in formation of cation molecules (Table 2, groups 1 and 4). The compounds of groups 2 and 3 are formed, according to Scheme 3, as secondary products of transformation of the cation (a), which also appears during the breakage of $P^+\cdots Cl^-$ and -O–H bonds.

Effect of quinones I-III on mitochondrial respiration and phosphorylation. Previously we described the effect of stimulation of mitochondrial respiration under the influence of micromolar concentrations of quinone I (MitoQ) [6]; in the present work, this effect has been confirmed for the whole group of quinones I-III.

Two pathways of activation of mitochondrial respiration by these compounds have been revealed. The first is associated with the effect of nonspecific pore opening; it is observed during malate oxidation by liver mitochondria under the influence of quinones I and III in conditions when the incubation medium contains no EDTA or cyclosporin (Fig. 5, c and d, curves *1*). The decrease in respiration rate with time in this case is determined by the escape of pyridine nucleotides from the matrix through the open pore. The addition of pore formation blocker, cyclosporin, eliminates this effect. In this case an uncoupling effect is realized that is probably associated with a proton cycle; the rate of uncoupled respiration does not vary with time (Fig. 5, c and d, curves *2*). These figures also show that uncoupling in both cases was incomplete, because the phosphorylating function of mitochondria was partially preserved. It is important to note that partial uncoupling in itself reduces ROS generation in mitochondria [33].

Figure 5a shows that quinone III (SkQ3) stimulates mitochondrial respiration under both malate and succinate oxidation. The comparison of titration curves of mitochondrial respiration in Fig. 5 (a and b) demonstrates that quinone II (SkQ1) is less active as an uncoupler compared with SkQ3. These findings are in agreement with the proposed mechanism of uncoupling effect of $SkQH_2$ hydroquinones – their ability to convert to electrically neutral forms (see Scheme 2 and "Discussion").

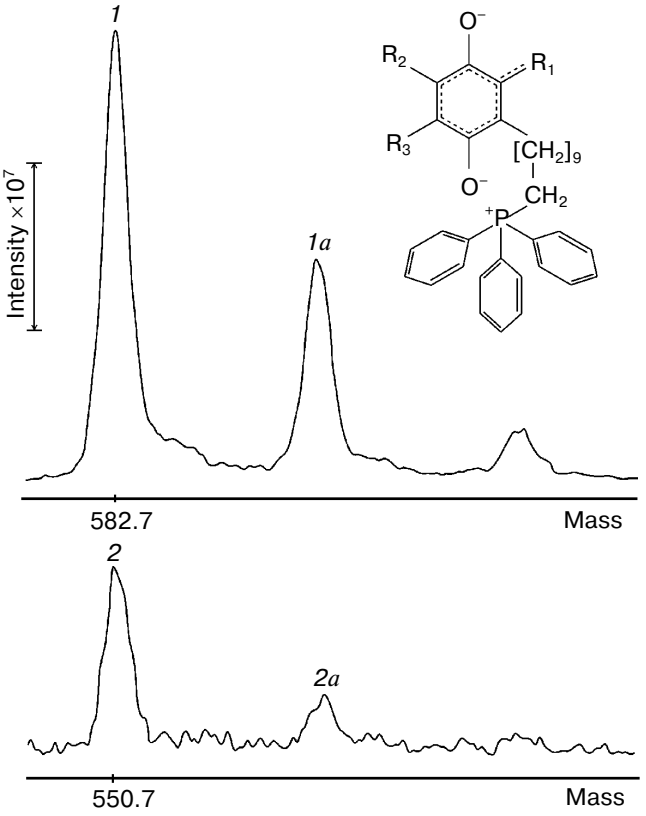
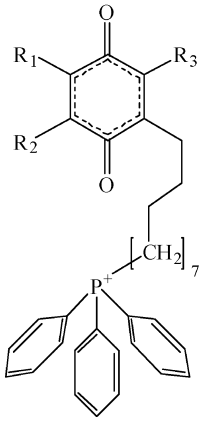
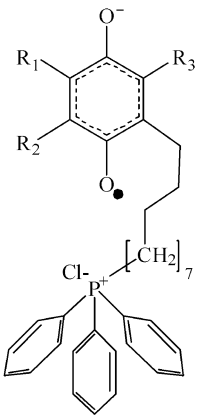
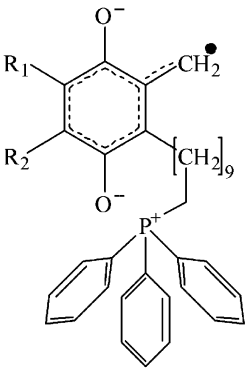
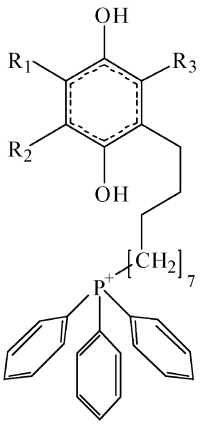


Fig. 4. MALDI spectra of compounds with attributed structure of cyclic configurations of zwitterions formed from hydroquinones Ia and IIIa. Spectrum *1*, MitoQ derivative, m = 582.7; spectrum *2*, SkQ3 derivative, m = 550.7. Peaks *1a* and *2a*, masses of corresponding isotopes.

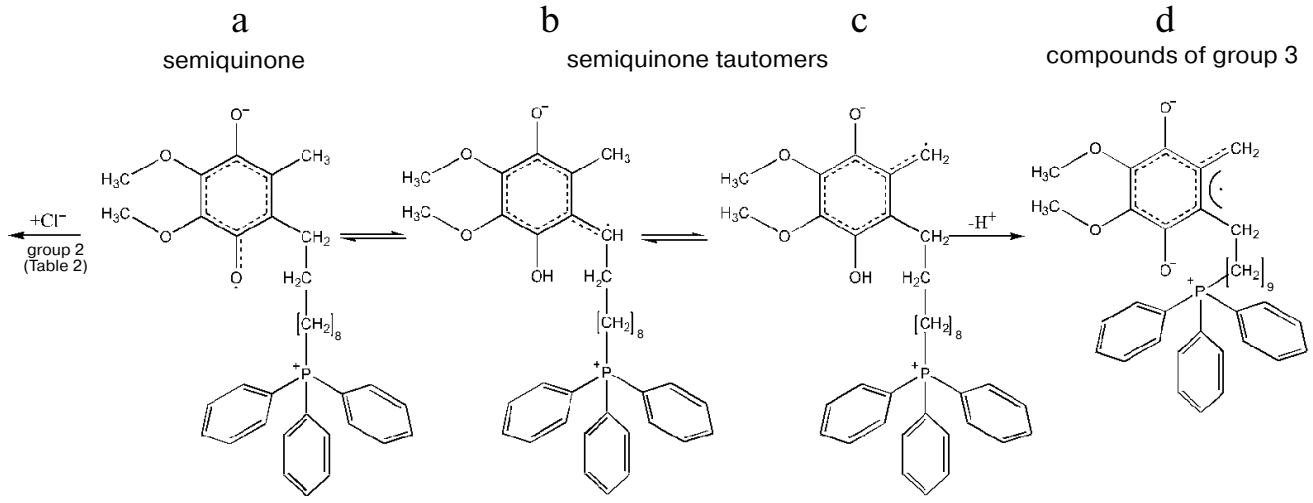
Table 2. Supposed structures of the compounds revealed in the MALDI spectra of hydroquinones Ia-IIIa

Group No.		1	2	3***	4
General structure of skeleton of decomposition products					
Masses and intensities of bands*	I	583.3 ($0.4\cdot10^9$)	618.3 ($0.51\cdot10^8$)	582.7 ($2.74\cdot10^7$)	585.3 ($6.0\cdot10^8$)
	II	537.3 ($0.48\cdot10^9$)	572.3 ($0.98\cdot10^8$)	537.3 (not determined)**	539.3 ($6.0\cdot10^8$)
	III	551.3 ($1\cdot10^9$)	586.3 ($1.1\cdot10^8$)	550.7 ($1.25\cdot10^7$)	553.3 ($5.4\cdot10^8$)
Sign of charge		+	–	–	+

* Values of the intensities of spectral bands are given in parentheses.
** Peaks of matrix merge with peaks of dianion-radical $SkQ1H_2$ (II).
*** The structures are constructed on the basis of Occam's razor. In group No. 3, the structure relates to compounds Ia and IIIa.

DISCUSSION

The analysis of MALDI spectra of hydroquinones Ia-IIIa for the whole set of revealed compounds (Table 1 and Scheme 3) allowed us to present the formation of all products as a result of single-type reactions of breakage/formation of the least stable polar O–H bonds and dipoles $P^+\cdots Cl^-$ in Ia-IIIa phosphonium chlorides. It turned out that the general nature of these processes involves the formation of cyclic zwitterion-radical (Table 2, group 3) as a



Formation of compounds of groups 2 and 3 (Table 2) from semiquinone (a)

Scheme 3

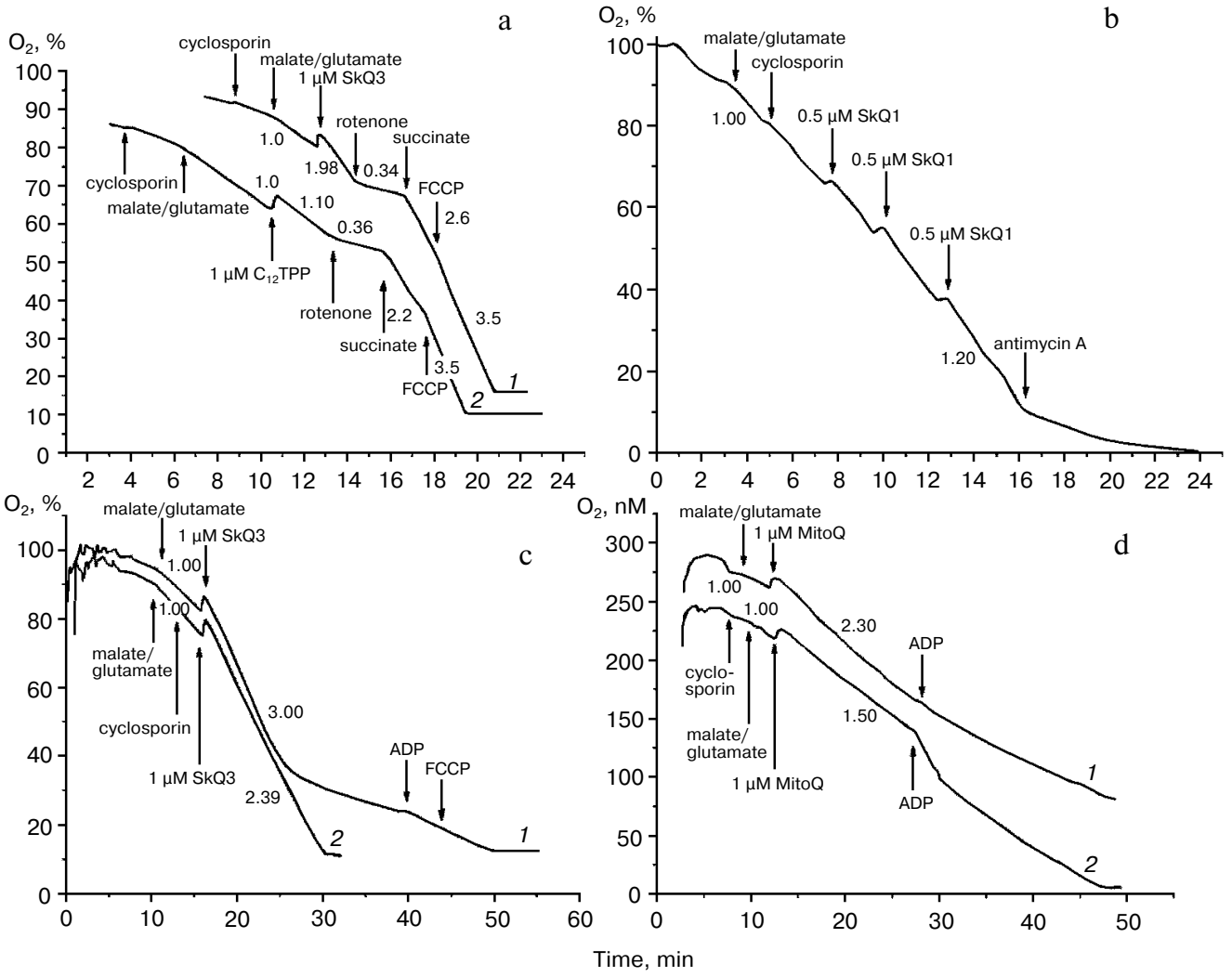


Fig. 5. Effect of stimulation of mitochondrial respiration by SkQ quinones (I-III). a) Uncoupling effect of quinone III; acceleration of respiration of (liver) mitochondria by quinone III (SkQ3): *1*) experiment; *2*) control. b) Uncoupling effect of quinone II (SkQ1); acceleration of mitochondrial respiration by quinone II (SkQ1). c, d) Curves: *1*) effect of pore opening under the influence of quinones III and I, respectively (medium containing no EDTA and cyclosporin); *2*) effect of partial uncoupling of mitochondria under the influence of quinones III and I (cyclosporin-containing medium). Formation of cyclic form of SkQ having uncoupling properties (see Schemes 2 and 4).

potential reaction of competitive binding or substitution of chloride at phosphonium cation by hydroquinone dianion radical. Exploratory research of the MALDI spectra aimed at analysis of the possibility of transformation of anions of Ia-IIIa hydroquinones into cyclic zwitterions has shown that steric exclusions for the cyclization reactions are clearly absent under conditions of the high-temperature experiment. This was a stimulus for the beginning of systematic studies of the transformation in aqueous media of SkQ hydroquinones into electrically neutral cyclic forms which can act as uncouplers (see Scheme 2).

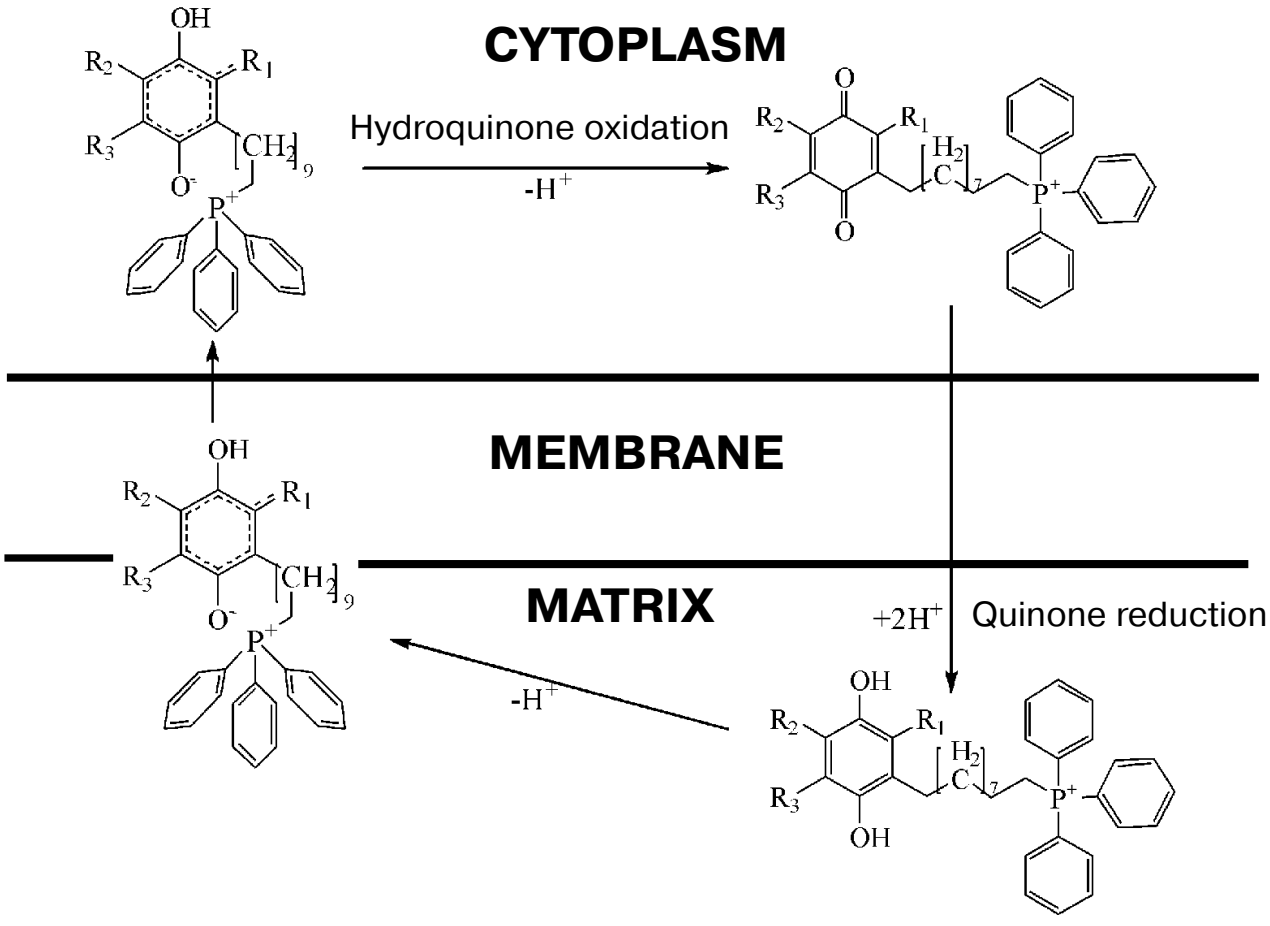
The existence of strong interaction between the negative charge of the hydroquinone anion and phosphonium cation in Ia-IIIa molecules in weakly alkaline aqueous solutions of these compounds was unambiguously proved by the strong shift (decrease) in the pK of these phosphonium derivatives compared with durohydroquinone IVa (Fig. 2, a-d) having no phosphonium group in its structure. The electrostatic nature of this interaction was confirmed in the experiments with solutions of high and low ionic strength (Fig. 3). It was shown that increasing solution ionic strength really results in disappearance of intramolecular electrostatic interaction between the charges of hydroquinone anion (Ia-IIIa) and phosphonium cation, and the pK of these compounds becomes practically the same as the pK of duroquinone.

It should be specially noted that the pK values of hydroquinones IIa and IIIa significantly decreased (by two units) during the formation of cyclic configuration compared with the pK of durohydroquinone. Our experi-

ments have proved that the observed pK decrease in hydroquinones is not associated with the induction effect of phosphonium cation in the molecule but is determined by the possibility of its spatial approach to the OH-group of the hydroquinone, and only if cyclization is permitted, the system shifts to the formation of cyclic zwitterions of hydroquinones with lower pK values.

These data show that the phosphonium cation, increasing the affinity of quinones I-III to mitochondria, after their reduction in mitochondria increases the constant of dissociation of the formed hydroquinones and thus enhances the probability of transformation of the hydroquinone anions into electrically neutral zwitterions. At the same time, the probability of escape of these compounds from the matrix into the cytoplasm increases as well. Touching upon the question of cyclization of zwitterions in the membrane system of mitochondria, it is necessary to emphasize that SkQ compounds have very high affinity to lipids and to the mitochondrial membrane in particular. In this connection, from thermodynamic considerations it follows that under conditions of binding with the membrane there must be a drastic additional stabilization of the intramolecular ion pair, hydroquinone anion/phosphonium cation, similar to stabilization of the complex of potassium ion/valinomycin at the phase boundary. The latter example of complex stabilization on binding with the membrane is very comprehensively described in work [34]. In paper [35], Murphy with his coauthors demonstrated an effect of stabilization of the ion pair, phosphonium ion and dinitrophenol anion, on the ion transfer from water to hydrophobic solvent.

When discussing the findings, it is important to stress that in the cases when hydroquinones formed in mitochondria are not oxidized in the extramitochondrial space (Scheme 4) they, being aromatic acids, must function as classical uncouplers, i.e. transmembrane hydrogen ion carriers. Hence, two consequences ensue: first, the revealed effect of uncoupling of mitochondria under the influence of quinones I-III correlates with the theoretically predicted ability of hydroquinones to convert into electrically neutral cyclic forms and, second, in this case the minor discharge of potential, "mild uncoupling" according to Skulachev [33], must decrease ROS generation in the mitochondria.



Redox cycle of SkQ compounds between matrix and cytoplasm

Scheme 4

The uncoupling effect of Ia-IIIa compounds was described previously by the example of compound I [6] and has been confirmed in the present work for the whole group of these substances. The uncoupling effect on its own is relatively low (Fig. 5, a-d); it is displayed in stimulation of respiration and partial suppression of phosphorylation. However, phosphorylation is partially preserved, suggesting the maintenance of sufficiently high potential on the mitochondrial membrane. At the same time, the discovery of the uncoupling effect of hydroquinones is in agreement with Scheme 2, i.e. it can be considered as a consequence of formation of a neutral form of hydroquinone.

It should be noted that the uncoupling scheme suggesting the formation of cyclic zwitterion does not exhaust all possible ways of positive charge neutralization in the molecules of compounds Ia-IIIa. Therefore, one cannot exclude the existence of alternative pathways of stimulation of respiration under the influence of compounds I-III (Ia-IIIa). In this connection, let us once again point to the fact that the penetrating electrically neutral form of hydroquinones Ia-IIIa appears due to the effect revealed in this work: the increase in dissociation constant on cyclization of these molecules (Fig. 2). This effect is actually determined by competitive "displacement" of hydrogen cation by phosphonium cation in the course of cyclic zwitterion formation (see Scheme 2). However, the electrically neutral penetrating configurations of compounds Ia-IIIa must be formed without cyclization, i.e. at single-electron oxidation of these compounds resulting in formation of a semiquinone, which is a much stronger acid than hydroquinone. As an example, note that the semiquinone of CoQ has pK 6.0 [36].

The fundamental possibility of escape from the mitotropic traps of free radicals, Ia-IIIa hydroquinones and semiquinones, from the mitochondrial matrix into the cytoplasm, which has been considered in detail above, poses a problem of considering as targets of these compounds not only mitochondrial but also cytoplasmic cell systems. In this connection, one should mention the specific property of bifunctional antioxidants of this class, which determines their potential ability for selective suppression of background "parasitic" processes of ROS generation.

Scheme 4 reflects an important feature of Ia-IIIa hydroquinones compared with the common radical traps, which relates this group of antioxidants to the cytoplasmic natural peroxyreductase system. The natural antioxidant system, which selectively suppresses "background" ROS accumulation in a cell, functions due to the radical trap regeneration system. After oxidation in the extramitochondrial space, Ia-IIIa hydroquinones are able to regenerate (re-reduce) in the mitochondria and escape into the cytoplasm again. That is why they are not used up like usual antioxidants and can function at low concentrations.

The second peculiarity of hydroquinones Ia-IIIa is also associated with the fact that, due to cyclic re-reduction, they are not used up and their concentrations in the system must not significantly vary with time. Therefore, selectivity of the action of such antioxidants in the simplest case can be achieved owing to the fact that it is possible to find for them exact concentrations not varying with time at which their regeneration rates are comparable to the rates of background processes. However, at the same time regeneration rate must be much lower than the rate of ROS "discharge" during the work of signal systems. It should also be mentioned that the activity of ROS-dependent regulatory systems also can be controlled by gradual increase in the concentration of such antioxidants in the cell.

However, it is important to emphasize that hydroquinones, according to work [37], as lipid peroxidation blockers, are more active as antioxidants by three orders of magnitude compared with quinones. Therefore, formation of hydroquinones in the mitochondrial matrix not only provides the transfer of SkQ compounds to the cytoplasm but also drastically increases their antioxidant activity.

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