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Minireview

A critical appraisal of the mitochondrial coenzyme Q pool

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Abstract The function of the coenzyme Q (CoQ) pool in the inner mitochondrial membrane is reviewed in view of recent findings suggesting a supramolecular organization of the mitochondrial respiratory complexes. In spite of the structural evidence for preferential aggregations of the inner membrane components, most kinetic evidence is in favor of a dispersed organization based on random collisions of the small connecting redox components, in particular CoQ, with the individual complexes. The shape of the CoQ molecule in the pool, suggested to be a folded one, is in agreement with its very rapid lateral diffusion mobility in the membrane midplane. Since the structural evidence in favor of specific supercomplexes is rather strong, it cannot be excluded that electron transfer may follow either pool behavior or preferential channeling depending on the physiological conditions. Another function ascribed to the CoQ pool is the antioxidant action of the reduced CoQ molecules; although it cannot be excluded that protein-bound ubisemiquinones may be a source of oxygen radicals, particularly at the level of complex III, the available evidence suggests that the mitochondrial pool only behaves as an antioxidant under physiological conditions.

Key words: Mitochondrion; Respiratory complex; Coenzyme Q; Antioxidant

1. Introduction

The observation that coenzyme Q (CoQ) in the inner mitochondrial membrane is in stoichiometric excess over the other components [1] has emphasized its substrate-like role, while its utter hydrophobicity has left little doubt about its localization in the lipid bilayer of the membrane. Morphological studies of the inner membrane [2] and kinetic studies of respiration [3] have put forward the idea of a homogeneous CoQ pool dissolved in the membrane lipid phase. Such belief has prompted a plethora of biophysical investigations of the state and localization of CoQ in model lipid bilayers (cf. [4]). Recently we have provided new biophysical evidence pertaining to this issue, which I consider of interest in view of the functional features of the CoQ pool.

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2. A folded conformation for CoQ

The natural CoQ is 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone, where the polyprenylated side chain is 6–10 units long. Within mammals, only CoQ_9 and CoQ_{10} are found, with CoQ_9 only distributed among rodents. CoQ exists in three redox states, fully oxidized, semiquinone, and fully reduced: nevertheless, the existence of different possible levels of protonation increases the possible redox forms of the quinone ring [5].

Due to its extreme hydrophobicity, natural CoQ can be present in three physical states only: forming micellar aggregates, dissolved in lipid bilayers, and bound to proteins. The former state is very important working with CoQ in cell-free systems [6], however in the living cell CoQ should be distributed among the other two states.

It has been assumed for a long time that the shape of the CoQ molecule is linear, with some possibility of rotation allowed for the long isoprenoid tail. Bending of the molecule is required in a model proposed by us [7], on the basis of previous evidence and of theoretical considerations, and confirmed by linear dichroism studies [8] of the location of CoQ_{10} in the hydrophobic midplane of the lipid bilayer, with the polar head oscillating about the third isoprene unit between the midplane (wholly linear shape) and the polar heads of the phospholipids (maximal bending of 90°). The model allows for movement of the redox center of CoQ, that is required for interaction with other redox centers in the mitochondrial complexes. For example, the Rieske iron–sulfur center of complex III, that is the first electron acceptor from ubiquinol, is situated at the level of the hydrophilic heads of the phospholipids [9]. The model also allows for the reduced form, in which the benzoquinone ring is more polar, to be preferentially located at the polar surface of the membrane [10].

In contrast to these predictions, a study of molecular dynamics computer simulation of CoQ homologues in vacuum starting from different initial configurations has shown that the conformation with lowest energy level is a folded one, where the polar head is in tight contact with the last isoprenoid unit of the hydrophobic tail [11]. Within the series of homologues, the cutoff for the folded conformation is four isoprenoid units. The folded conformation was found for both oxidized and reduced quinones, however only small energy differences were found between oxidized and reduced ubiquinones in the folded conformation. The difference is not the same for the various quinones: the different behavior would be the result of geometric differences imposed by the

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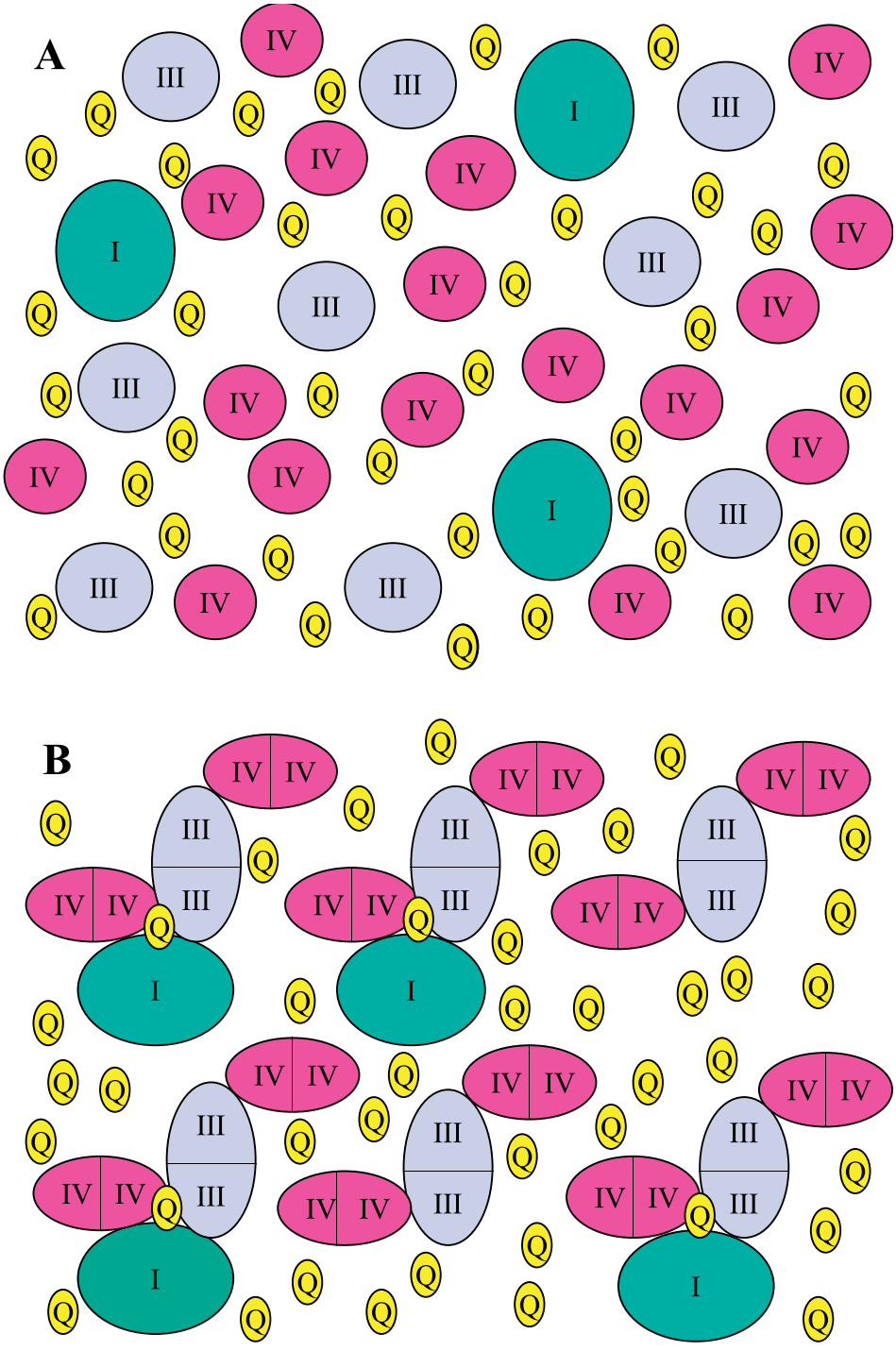

Fig. 1. Two models of the organization of the mitochondrial respiratory chain. In (A) a random distribution of mitochondrial complexes is envisaged, according to the postulates of the random collision model [2]. In (B) the presence of supramolecular specific aggregates is depicted, according to the respirasome concept (modified from [24]). For simplicity, only complexes I, III, and IV and CoQ are indicated in both models.

folding of isoprenoid units of slightly different length. For example the enthalpy difference between oxidized and reduced form is much higher for CoQ_{10} than for CoQ_9, indicating that the former has a stronger tendency to become oxidized, i.e. a lower midpoint redox potential (unpublished observations from our laboratory).

Although the molecular modeling has been performed in vacuum, we have reason to believe that the folded conformations also apply to the CoQ homologues in natural membranes; this idea is supported by the experimental demonstration by magnetic resonance techniques that ubisemiquinones are folded in organic solvents [12].

There are important implications of a folded structure. First, the similar size of short and long homologues would explain the similar high rates of lateral diffusion found in our laboratory for all quinone homologues [6,11]. In addition,

protein-binding during electron transfer may require unfolding, contributing to the high activation energy and low collision efficiency observed for electron transfer (e.g. [13,14]).

3. The CoQ pool in mitochondria

Two opposite models have alternated for the organization of the mitochondrial respiratory chain. The original solid-state model of Chance [15] was substituted by the model of enzyme complexes individually dissolved in the lipid bilayer, based on the discovery of the complexes in Green's laboratory (e.g. [16]) and advanced in a systematic way by Hackenbrock [2,17] in his random diffusion model: the first postulate of this model is that electron transfer depends on random collisions between complexes and small diffusing molecules (CoQ and cytochrome *c*); in addition it was also postulated that CoQ diffusion is rate-limiting for electron transfer.

Is there a CoQ pool? Certainly some CoQ is protein-bound: but how much? If we consider bound CoQ as stoichiometric with one site in the complexes that have been shown to contain bound CoQ (I, II, III) [18–20], in beef heart mitochondria we come up to no more than 0.35 nmol/mg protein, that would increase to ca. 0.5 nmol assuming more than one site to be fully occupied in complex I and complex III. Since the total CoQ content is higher than 3 nmol/mg [1,14], we must assume that most CoQ ($>84\%$) is free in the bilayer. This calculation is in agreement with the direct measurements of CoQ bound to mitochondrial proteins after extraction in detergent micelles [21].

In spite of the favor of the CoQ pool idea, the concept of a solid-state organization was never abandoned, since preferential associations were occasionally found between specific complexes, starting from the pioneering studies of Hatefi [16]. In 1987 Ozawa [22] advanced the concept of the supramolecule, an assembly of electron transfer complexes and H+-pump ATPase, with a molecular weight of $1.8–1.9\times10^6$. Recently Schägger [23,24] using digitonin solubilization and Blue Native PAGE has produced new evidence of preferential associations between mitochondrial complexes, in particular a complex I monomer with a complex III dimer, and suggested a model of the respiratory chain (the respirasome) based on direct electron channeling between complexes and not on random collisions. In this view, the CoQ pool dissolved in the lipid bilayer may be in contact with the CoQ within the supercomplexes, however its function might be no more than a reservoir of CoQ molecules. The two extreme models are depicted in Fig. 1.

A critical appraisal of the above model requires its reconciliation with previous kinetic and morphological evidence that was in favor of a random distribution of the complexes.

Morphological evidence by freeze fracture electron microscopy [2,25] always showed the majority of the intramembrane particles to have a random distribution, with little evidence for organized aggregations; the size of the particles does not seem to be compatible with that of large supercomplexes.

Both the manipulations required for freeze fracture electron microscopy and the isolation of protein complexes in digitonin may suffer from artifacts, albeit in opposite directions; thus, the kinetic evidence in intact mitochondrial membranes may become decisive to understand the role of the CoQ pool. To this purpose it is puzzling that, in the past, preferential association was proposed rather for complexes II–III than for complexes I–III, based on the calorimetric properties of the reconstituted succinate cytochrome *c* reductase [26] and on the isolation and properties of a well defined succinate cytochrome *c* reductase that could be resolved into a succinate CoQ reductase and an ubiquinol cytochrome *c* reductase [27].

The pool equation of Kröger and Klingenberg [3] relates the total rate of electron transfer through the CoQ pool (V_{obs}) to both the rate of CoQ reduction (V_{red}) and of ubiquinol oxidation (V_{ox}) in a hyperbolic fashion:

$$V_{obs} = V_{ox}\cdot V_{red}/(V_{ox} + V_{red})$$

A large body of experimental evidence has validated the pool equation in a variety of mitochondrial systems [3,14,28]. In bovine heart submitochondrial particles the observed rate of electron transfer between complex I and complex III is the same as that calculated from the pool equation [14] (Table 1). In other systems the rate of complex I is underestimated, so that the pool equation is not directly applicable [29]. One exception appears to be represented by yeast mitochondria, where the pool behavior on antimycin A titration was observed only in the presence of chaotropic agents [30].

The substrate-like nature of CoQ is also shown by the fact that it exhibits saturation kinetics, not only when a short homologue is used as a substrate for an individual enzyme, but also when the natural CoQ_{10} is titrated in integrated respiration (e.g. NADH cytochrome *c* reductase) [31–33]. Another puzzling observation to this purpose is that the K_m for CoQ_{10} of NADH cytochrome *c* reductase is much higher than that of succinate cytochrome *c* reductase [33]: the latter is of the same order of magnitude as the concentration of respiratory enzymes, a possible suggestion in favor of a stoichiometric association of complex II with complex III, that is however not experimentally found in the Blue Native PAGE investigations [24].

The pool equation is only valid if CoQ behaves as a homogeneous diffusible pool between all reducing enzymes (V_{red}) and all oxidizing enzymes (V_{ox}) [28]: is this compatible with the existence of preferential associations? Stoichiometric channeling of CoQ between complex I and complex III [22–24] would exclude the bulk of the CoQ pool from kinetic determination and would therefore be incompatible with the pool

Table 1
NADH oxidation activities in bovine heart submitochondrial particles (data from [14])

Activity	Calculation	μmol/min/mg protein
NADH–CoQ	Experimental (NADH–CoQ_1) (V_{red})	1.08
Ubiquinol–O_2	Experimental (reduced CoQ_2–O_2) (V_{ox})	1.05
NADH–O_2	Experimental	0.58
NADH–O_2	Calculated (pool equation) (V_{obs})	0.53
NADH–O_2	Calculated (stoichiometric)	1.05

Calculation of NADH oxidase as stoichiometric is made on the basis of the lower rate between V_{red} and V_{ox}.

behavior. Thus, in the presence of preferential associations, the pool equation would be experimentally validated only if the rate of association/dissociation of the complexes was faster than the rate of electron transfer between complexes and CoQ molecules in the pool. An alternative possibility would be that, within a supercomplex, CoQ reduced by one enzyme has anyway to dissociate in the pool in order to meet any other supercomplex, including the same one in a different site, for being oxidized: this explanation, however, frankly appears hard to support.

Most kinetic features of electron transfer available now are compatible with a random organization, in which CoQ behaves as a substrate-like diffusible molecule. Within this view, we have produced a large body of biophysical and kinetic evidence that CoQ diffusion is not rate-limiting for electron transfer [6,7].

Nevertheless, the evidence produced for the existence of supercomplexes is difficult to reject on structural terms. If supercomplexes exist structurally, it should make little sense if they had no function.

Of course much work has to be made to discriminate between the kinetic evidence (CoQ pool) and the structural evidence (supercomplexes). However, a simple-minded compromise can be advanced as a working hypothesis that both models are true. How can that be? The structural evidence of Schägger shows that the supercomplexes are labile, for instance an aliquot of complex III appears to be dissociated from the supercomplex [23]. The authors ascribe the existence of dissociated complexes to the effect of the solubilizing agent [24]. Why not postulate that the supercomplexes physiologically exist in equilibrium with the isolated complexes? Thus electron transfer would either follow a specific channeling or be governed by random collisions depending on the conditions of the system. The kinetic behavior obeying to the pool equation was only studied in submitochondrial particles, in which electron transfer is a simplified system of three consecutive enzymes (complexes I, III, and IV). It would be of interest to investigate the kinetics of electron transfer in more physiological systems, such as in intact mitochondria respiring under state III and state IV conditions with natural substrates.

Discrimination between the two models is amenable to kinetic testing: besides a deep scrutiny of pool behavior, flux control analysis [34] represents a powerful method to this purpose. If a metabolic pathway is composed of distinct enzymes, the extent to which each enzyme is rate-limiting may be different, and the sum of all flux coefficients for the different enzymes should be one. On the other hand, in a supercomplex, the metabolic pathway would behave as a single enzyme, and inhibition of anyone of the respiratory complexes would elicit the same flux control. In particular, in a system in which the respiratory chain is totally dissociated from other components of the oxidative phosphorylation machinery (carriers, ATP synthase, membrane potential), such as open non-phosphorylating submitochondrial particles from rat liver, the existence of a supercomplex would elicit a flux control coefficient near unity at anyone of the respiratory complexes. We have preliminary data that aerobic NADH oxidation in such particles exhibits a control coefficient between 0.7 and 1 after titration with either rotenone (complex I) and mucidin (complex III).

4. The CoQ pool: pro-oxidant or antioxidant?

A role of the bulk of CoQ in the mitochondrial membrane (as well as in other membranes) is to serve as an antioxidant in its reduced form [35]. The first hints on an antioxidant action of CoQ were provided by the classical studies of Mellors and Tappel [36] and Takeshige et al. [37] in mitochondria. Since then, overwhelming evidence has accumulated that reduced CoQ is an antioxidant in a variety of conditions, from model lipid bilayers to mitochondrial and other cellular membranes, and to physiological systems in vivo ([38] and references therein).

Thus, since the CoQ pool in mitochondria is partly reduced under steady-state conditions of electron transfer, $CoQH_2$ may serve as an antioxidant under oxidative stress conditions. The observation that CoQ_{10} has a stronger antioxidant activity than CoQ_9 [39] is hard to explain on a mere difference of hydrophobicity, but may find a plausible explanation in our observation by molecular modeling that the midpoint potential of CoQ_{10} is lower than that of CoQ_9 (see above).

The antioxidant action of CoQ has been challenged on the basis that mitochondria produce reactive oxygen species (ROS) under a variety of conditions, and that ubisemiquinone was considered the main source of oxygen radicals [40].

Within complex III, for a number of reasons, ubisemiquinone (SQ) at center o was assumed as the most plausible candidate for univalent oxygen reduction, however autoxidation of SQ bound in complex III to lead to superoxide requires the availability of protons [41]; according to Nohl et al. [42] CoQ may be transformed from a safe electron carrier to a superoxide generator when protons are allowed to penetrate the inner membrane, as in toluene-treated mitochondria. Although SQ is autoxidizable in ethanol under suitable conditions [5], no direct demonstration is available for the role of SQ in reacting with oxygen at center o: the notion that ubiquinol at center o delivers both electrons simultaneously to Rieske's iron–sulfur cluster and to cytochrome b_{566} [20] would argue against significant accumulation of SQ to be able to reduce oxygen, even in the presence of antimycin. Indeed, SQ at center o has never been directly demonstrated (cf. [9]).

Addition of NADH to mucidin-inhibited SMP promoted superoxide formation [43], that was enhanced to similar extents by complex I inhibitors belonging to all three classes described by Degli Esposti [44] among quinone antagonists and acting at three different sites in the hydrophobic core of the complex, and by combinations thereof. The fact that a combination of inhibitors, acting on three quinone-binding sites of the complex, enhances superoxide formation suggests that the site of oxygen reduction lies upstream the quinone-binding sites of the complex. In agreement with this interpretation, studies in CoQ-depleted and reconstituted mitochondria [43] indicated that endogenous CoQ is not required for superoxide generation. It is worth noting that reconstituted mitochondria, containing a large excess of CoQ_{10}, produce the same amount of superoxide as CoQ-depleted mitochondria, indicating that endogenous CoQ_{10} is not a source of ROS.

Ubisemiquinone is relatively stable only when protein-bound [20], therefore the CoQ pool in the lipid bilayer should be no source of ROS. Indeed, with concern to the pro-oxidant/antioxidant role of CoQ, there seems to be an inherent

misunderstanding. Even allowing that SQ is the pro-oxidant species in antimycin-treated mitochondria, this species is a sort of enzyme cofactor and its amount can be at most stoichiometric with the enzyme content and cannot be changed by extensive decreases or increases of the ubiquinone pool, that behaves as a substrate in large excess over the respiratory chain content. Exogenously administered CoQ has always been found to behave as an antioxidant in vivo (cf. [38,45]): thus, the pro-oxidant species deriving from its antioxidant action [46] would not seem to be operative in vivo, at least in mitochondria. This cannot be excluded for other biomembranes that have no recycling system for CoQ metabolites [47].

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References

[1] Capaldi, R.A. (1982) Biochim. Biophys. Acta 694, 291–306.
[2] Hackenbrock, C.R., Chazotte, B. and Gupte, S.S. (1986) J. Bioenerg. Biomembr. 18, 331–368.
[3] Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 34, 358–368.
[4] Lenaz, G. (2001) in: Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V.E. and Quinn, P.J., Eds.), pp. 5–27, CRC Press, Boca Raton, FL.
[5] Kozlov, A.V., Nohl, H. and Gille, L. (1998) Bioorg. Chem. 26, 334–344.
[6] Fato, R., Battino, M., Degli Esposti, M., Parenti Castelli, G. and Lenaz, G. (1986) Biochemistry 25, 3378–3390.
[7] Lenaz, G. (1988) J. Membr. Biol. 104, 193–209.
[8] Samorì, B., Lenaz, G., Battino, M., Marconi, G. and Domini, I. (1992) J. Membr. Biol. 128, 193–203.
[9] Xia, D., Yu, C.A., Kim, H., Xia, J.Z., Kachurin, A.M., Zhang, L., Yu, L. and Deisenhofer, J. (1997) Science 277, 60–66.
[10] Lenaz, G., Samorì, B., Fato, R., Battino, M., Parenti Castelli, G. and Domini, I. (1992) Biochem. Cell. Biol. 70, 504–514.
[11] Di Bernardo, S., Fato, R., Casadio, R., Fariselli, P. and Lenaz, G. (1998) FEBS Lett. 426, 77–80.
[12] Joela, H., Kasa, S., Lehtovuori, P. and Bech, M. (1997) Acta Chem. Scand. 51, 233–241.
[13] Fato, R., Cavazzoni, M., Castelluccio, C., Parenti Castelli, G., Palmer, G., Degli Esposti, M. and Lenaz, G. (1993) Biochem. J. 290, 225–236.
[14] Fato, R., Estornell, E., Di Bernardo, S., Pallotti, F., Parenti Castelli, G. and Lenaz, G. (1996) Biochemistry 35, 2705–2716.
[15] Chance, B. and Williams, G.R. (1955) Nature 176, 250–254.
[16] Hatefi, Y., Haavik, A.G. and Rieske, J.S. (1962) J. Biol. Chem. 237, 1676–1680.
[17] Gupte, S., Wu, E.S., Hoechli, L., Hoechli, M., Jacobson, K., Sowers, A.E. and Hackenbrock, C.R. (1984) Proc. Natl. Acad. Sci. USA 81, 2606–2610.
[18] Vinogradov, A.D., Sled, V.D., Burbaev, D.S., Grivennikova, V.G., Moroz, I.A. and Ohnishi, T. (1995) FEBS Lett. 370, 83–87.
[19] Lee, G.Y., He, D.Y., Yu, L. and Yu, C.A. (1995) J. Biol. Chem. 270, 6193–6198.
[20] Trumpower, B.L. (1981) J. Bioenerg. Biomembr. 13, 1–24.
[21] Lass, A. and Sohal, R.S. (1999) Free Radic. Biol. Med. 27, 220–226.
[22] Ozawa, T., Nishikimi, M., Suzuki, H., Tanaka, M. and Shimomura, Y. (1987) in: Bioenergetics: Structure and Function of Energy-Transducing Systems (Ozawa, T. and Papa, S., Eds.), pp. 101–119, Japan Sci. Soc. Press, Tokyo.
[23] Schägger, H. and Pfeiffer, K. (2000) EMBO J. 19, 1777–1783.
[24] Schägger, H. and Pfeiffer, K. (2001) J. Biol. Chem. 276, 37861–37867.
[25] Schneider, H., Lemasters, J.J. and Hackenbrock, C.R. (1982) J. Biol. Chem. 257, 10789–10793.
[26] Gwak, S.H., Yu, L. and Yu, C.A. (1986) Biochemistry 25, 7675–7682.
[27] Yu, L. and Yu, C.A. (1982) J. Biol. Chem. 257, 2016–2021.
[28] Gutman, M. (1985) in: Coenzyme Q (Lenaz, G., Ed.), pp. 215–234, Wiley, Chichester.
[29] Genova, M.L., Castelluccio, C., Fato, R., Parenti Castelli, G., Merlo Pich, M., Formiggini, G., Bovina, C., Marchetti, M. and Lenaz, G. (1995) Biochem. J. 311, 105–109.
[30] Boumans, H., Grivell, L.A. and Berden, J. (1998) J. Biol. Chem. 273, 4872–4877.
[31] Ragan, C.I. and Cottingham, I.R. (1985) Biochim. Biophys. Acta 811, 13–31.
[32] Lenaz, G. and Fato, R. (1986) J. Bioenerg. Biomembr. 18, 369–401.
[33] Estornell, E., Fato, R., Castelluccio, C., Cavazzoni, M., Parenti Castelli, G. and Lenaz, G. (1992) FEBS Lett. 311, 107–109.
[34] Kacser, A. and Burns, J.A. (1979) Biochem. Soc. Trans. 7, 1149–1160.
[35] Ernster, L. and Dallner, G. (1995) Biochim. Biophys. Acta 1271, 195–204.
[36] Mellors, A. and Tappel, A.L. (1966) J. Biol. Chem. 241, 4353–4356.
[37] Takeshige, K., Takayanagi, K. and Minakami, S. (1980) in: Biomedical and Clinical Aspects of Coenzyme Q (Yamamura, Y., Folkers, K. and Ito, Y., Eds.), Vol. 2, pp. 15–26, Elsevier, Amsterdam.
[38] Kagan, V.E., Fabisiak, J.P. and Tyurina, Y.Y. (2001) in: Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V.E. and Quinn, P.J., Eds.), pp. 117–129, CRC Press, Boca Raton, FL.
[39] Lass, A., Agarwal, S. and Sohal, R.S. (1997) J. Biol. Chem. 272, 19199–19204.
[40] Turrens, J.F., Alexandre, A. and Lehninger, A.L. (1985) Arch. Biochem. Biophys. 237, 408–414.
[41] Nohl, H. and Stolze, K. (1992) Free Radic. Res. Commun. 16, 409–419.
[42] Nohl, H., Gille, L., Schönheit, K. and Liu, Y. (1996) Free Radic. Biol. Med. 20, 207–213.
[43] Genova, M.L., Ventura, B., Giuliano, G., Bovina, C., Formiggini, G., Parenti Castelli, G. and Lenaz, G. (2001) FEBS Lett. 505, 364–368.
[44] Degli Esposti, M. (1998) Biochim. Biophys. Acta 1364, 222–235.
[45] Beyer, R.E., Segura-Aguilar, J., Di Bernardo, S., Cavazzoni, M., Fato, R., Fiorentini, D., Galli, M.C., Setti, M., Landi, L. and Lenaz, G. (1996) Proc. Natl. Acad. Sci. USA 93, 2528–2532.
[46] Nohl, H., Gille, L. and Kozlov, A.V. (1998) Free Radic. Biol. Med. 6, 666–675.
[47] Nohl, H., Gille, L., Kozlov, A.V. and Staniek, K. (2001) in: Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V.E. and Quinn, P.J., Eds.), pp. 163–180, CRC Press, Boca Raton, FL.