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Assessing the effect of the lipid environment on the redox potentials of the coenzymes Q_{10} and Q_4 using lipid monolayers made of DOPC, DMPC, TMCL, TOCL, and natural cardiolipin (nCL) on mercury

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ABSTRACT

Adhesion-spreading of DOPC, DMPC, TOCL, TMCL and nCL liposomes containing coenzymes Q_{10} and Q_4 on mercury was used to prepare lipid monolayers incorporating these coenzymes. The CoQ_{10} and CoQ_4 electrochemistry was interrogated in these films adjacent to aqueous solutions of different pH to understand the effect of the embedded lipids on the redox properties of CoQ_{10} and CoQ_4.

1. Introduction

Coenzyme Q_{10} (CoQ_{10}), also referred to as ubiquinone-10, is almost ubiquitous in the membranes of eukaryotic cells, and plays there an essential role as electron and proton shuttling molecule of the respiratory chain. Coenzyme Q_4 (CoQ_4) is an intermediate of the biochemical Q_{10} synthesis and has also been found in living systems. For the function of CoQ_{10}, it is essential that its redox potential is so carefully tuned that it is reduced by the NADH-dehydrogenase complex and able to oxidize cytochrome. Certainly, the redox potential of CoQ_{10} is mainly fixed by the structure of the molecule, but – as always – an effect of the chemical environment of the molecule is expected. CoQ_{10} is a freely diffusing molecule in the lipid membranes. Therefore, the effect of the nature of the membrane constituting lipids on its redox potential is of interest. Here we report a new experimental approach to assess the redox potential of CoQ_{10} and CoQ_4 in lipid monolayer models. From previous studies it is well known that liposomes disintegrate on the surface of mercury and form islands of monolayers in an adhesion-spreading process [1–3]. When the liposome disintegration is continued over longer times, the mercury can be completely covered by a monolayer. Here we show that using liposomes with admixtures of CoQ_{10} and CoQ_4 allows to cover a mercury drop electrode with a lipid monolayer spiked with CoQ_{10} and CoQ_4. The chemical nature of the lipids affects the redox properties of these coenzymes. The lipid monolayers were made of the two phosphatidylcholines DOPC and DMPC, the two synthetic cardiolipins TMCL and TOCL, and natural cardiolipin (nCL) from bovine heart. The cardiolipins are major constituents of the inner mitochondrial membranes housing CoQ_{10}.

2. Experimental

Liposomal suspensions of the two synthetic phosphocholines DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), and DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine), and the two synthetic cardiolipins TMCL (1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (sodium salt)), and TOCL (1′,3′-bis[1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (sodium Salt)), as well as one natural cardiolipin (nCL, bovine heart (sodium salt)) (Avanti Polar Lipids, USA) were prepared according to a modified rapid evaporation method suggested by Moscho [4]. After dissolving the lipids in organic solvents, the required amount of CoQ10 (≥ 98%, Sigma-Aldrich) or CoQ4 (≥ 90%, Sigma-Aldrich) was added from a chloroform stock solution (1 mg/ml). Final concentrations of 300 μM lipids and 3 μM ubiquinones were obtained by adding 20 ml of phosphate buffered saline solution (0.01 M phosphate, 0.0027 M KCl, 0.137 M NaCl, pH 7.4, Sigma-Aldrich) as background electrolyte.

Electrochemical measurements were performed with an Autolab PGSTAT12 (Eco Chemie, Netherlands) connected to a PC in conjunction with the electrode stand VA 663 (Metrohm, Switzerland). A multimode electrode in HMDE mode (drop size 2, ca. 0.28 mm^2 surface area) served as working electrode, a platinum rod and an Ag|AgCl (3 M KCl, E = 0.207 V vs. SHE) were used as auxiliary and reference electrode, respectively. The redox systems were studied with cyclic voltammetry using a scan rate of 0.05 V s^{-1} and a step potential of 0.00412 V. The pH of the suspensions was adjusted by adding small amounts of NaOH and HCl. Phosphate buffered saline solution at pH 7.4 was used to have

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a model system mimicking physiological conditions. Therefore other electrolyte types were not tested in this study. Below pH 6 the liposomes are not stable.

2.1. Preparation of lipid monolayers on the hanging mercury drop electrode

The liposome suspension in the aqueous electrolyte was deaerated with nitrogen, which was washed by passing it through two alkaline pyrogallol solutions and distilled water, for at least 30 min. Then a new mercury drop was formed and then the solution was stirred for at least 15 min (if not otherwise given). During that time span, the formation of the coenzyme-spiked monolayer could be followed by the increase of the electrochemical quinone system, and the final stable monolayer was indicated by a constant CV. When the liposome suspension was exchanged for a pure aqueous electrolyte solution after monolayer formation, the CV remained unaffected. This proves that the signals are generated by the monolayer and are not influenced by the suspended liposomes.

3. Results and discussion

In Fig. 1 the CVs (2nd to 10th cycle) of CoQ_{10} in monolayers of DOPC, DMPC, TMCL, TOCL, and nCL are depicted (pH of aqueous electrolyte 7.4). The pH-dependence of peak potentials and peak separations is generally in accordance with the behaviour of CoQ_{10} in DOPC monolayers as described by Gordillo and Schiffrin [5]. These authors have prepared CoQ_{10}-spiked DOPC monolayers on a mercury drop electrode by transferring the mercury drop across a self-assembled DOPC monolayer at the water|air interface. The procedure which we are reporting here, i.e., the formation of the coenzyme-spiked monolayer by spreading of liposomes, allowed a much faster experimenting and an expansion to monolayers of natural membrane constituents (see further down). The CVs indicate that the nature of the monolayers affects the kinetics of the quinone redox system. The smallest peak separation is observed for DMPC and the largest for DOPC. Considering the chemical structure in terms of number and length of alkyl chain, and degree of saturation, lipids with higher similarity of structure, e.g. TOCL (18:1 cis-Δ^9) and nCL (90% 18:2 cis-$\Delta^{9,12}$), seem to have a more similar electrochemistry of the coenzymes in regard to peak separation and mid-peak potential. According to some studies, CoQ_{10} is housed in lipid mono- and bilayers with the poly-isoprene chain perpendicular to the lipid chains and most probably in a curved conformation ("pseudo-ring") [6,7]. The quinone head group is probably near the polar head groups of the lipids, i.e., near to the aqueous phase of the cells.

In Fig. 2 the CV mid-peak potentials of CoQ_{10} and CoQ_4 in the five monolayers of the lipids on mercury are given on a potential scale for pH 7.4 and 9.0. We are aware of the problems associated with taking the mid-peak potentials as the formal potentials, especially in view of the complex reaction scheme (see [5]) of ubiquinones. Nevertheless the mid peak potentials can be used to discuss the effect of the lipid nature on the redox properties of the ubiquinones in the monolayers. Clearly, the mid-peak potentials, even when taken as good approximates of the formal potential, cannot be taken as the redox potentials of the ubiquinones under biological conditions in bilayer membranes. However,

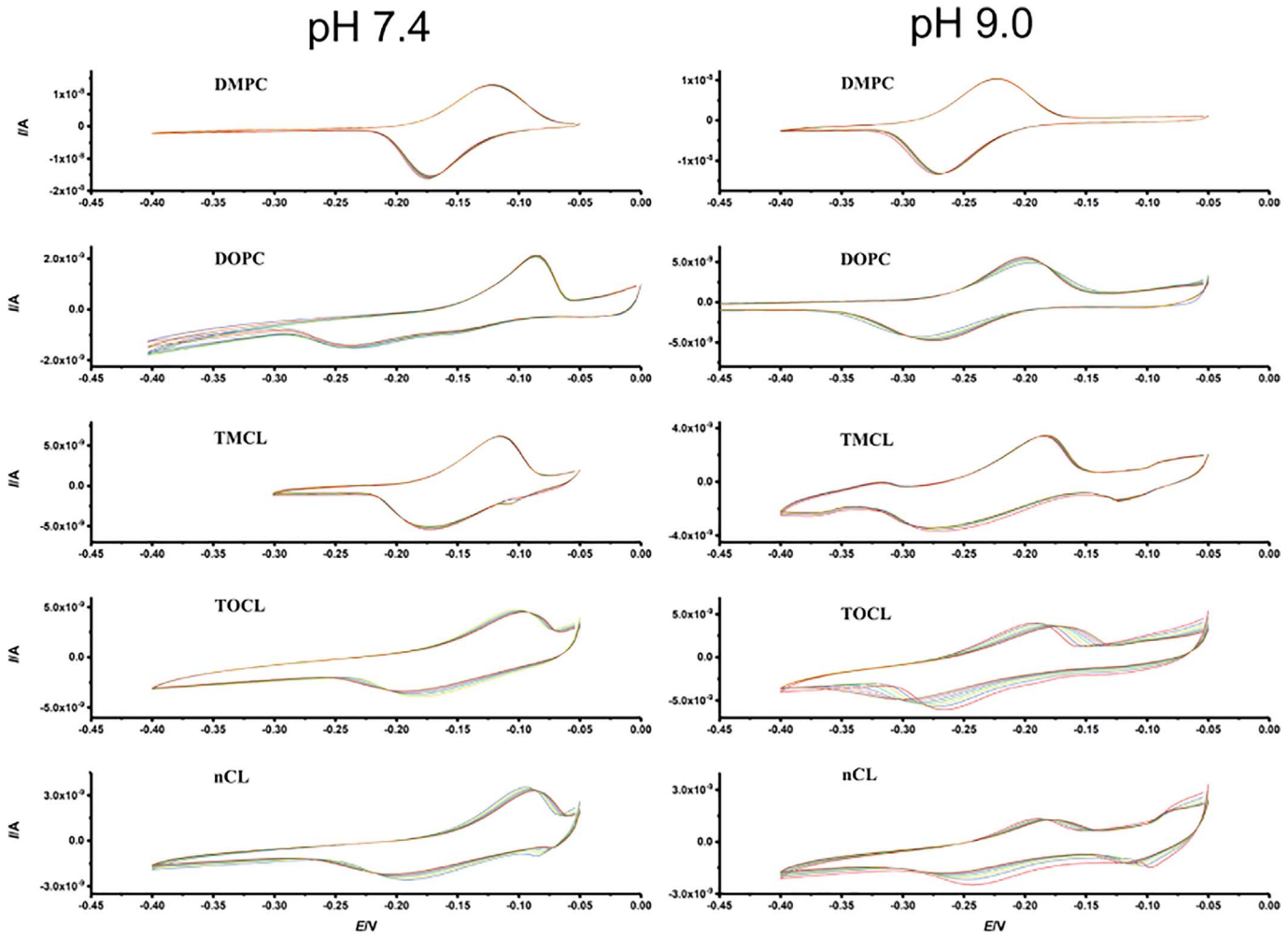

Fig. 1. Cyclic voltammograms of CoQ_{10} redox system incorporated into lipid monolayers at pH 7.4 (left) and 9.0 (right). CVs were recorded after 15 min of adhesion-spreading of CoQ_{10} spiked liposomes of different lipids on a mercury electrode. Scan rate: 0.05 V s^{-1}.