PLASTO- AND UBIQUINONE AS TRANSLOCATORS OF ELECTRONS AND PROTONS THROUGH MEMBRANES

A facilitating role of the isoprenoid side chain

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1. Introduction

Plastoquinone and ubiquinone were implied in the mechanism of proton translocation as soon as the concept of oriented loops of electron transport had been developed [1,2]. The former functions in photosynthetic electron transport of chloroplasts (see also [3–6], the latter in electron transport of mitochondria (see [7]) and bacteria (see [8]). In spite of published results [9,10], which show that ubiquinone is able to carry reducing equivalents through lipid barriers, the translocating role of isoprenoid quinones per se has been questioned by the argument, that tumbling through the lipid bilayer might be restriced by the side chain ([6,11], see [5] for a review). The fact that the isoprenoid chain, if in the stretched all-*trans* form, almost exactly doubles the length of a lipid acyl-chain might indeed reflect the anchoring in the membrane.

To clarify the discussion we have investigated the ability of quinones, with differing length of the side chain, to carry electrons and protons to ferricyanide trapped in liposomes, the model system introduced by Hinkle [12].

2. Methods

Liposomes were preparated from soy bean lecithin (Sigma) as described previously [13]. Quinones were incorporated by dissolving them together with lecithin in chloroform and evaporating the mixture to dryness under a stream of N_2, before adding 0.2 M ferricyanide, buffered at pH 8.0 with 20 mM Tricine–NaOH. The sonication mixture contained in 5 ml ferricyanide solution 200 mg lecithin and 1 μmol quinone. Sonication was performed in a small beaker with a Branson sonicator using the microtip for 30 min at full output. By cooling the beaker in ice water the temperature was maintained below 25°C. Subsequently liposomes were separated from external ferricyanide on a small column of Sephadex G-50 equilibrated with 0.3 M NaCl, 50 mM KCl and 20 mM Tricine–NaOH, pH 8.0. Lipid content of the liposome fractions was estimated by comparing the turbidity with that of not fractionated liposomes.

Reduction of ferricyanide and fluorescence quenching of 9-amino-acridine was measured as described [13,14], except for the fact that dithionite instead of ascorbate was used as external reducing agent. The conditions of the assays are given in the legends. Additions during measurements were performed via a plunger, furnished with a back-pushing spring, on top of the cuvette, accessible for a microsyringe from outside the sample compartment. The minimal mixing time achieved was about 1 s.

3. Results and discussion

Table 1 and fig.1A. clearly show that benzoquinones with long isoprenoid chains (PQ_9 and UQ_{10}) are more efficient than UQ_1, and much more than TMQ, in transporting reducing equivalents through the liposomal membrane to internal ferricyanide. Dithionite alone is also able to cross the membrane at a slow rate, as seen with liposomes without quinone (control). It was

Table 1
Reduction half times of ferricyanide trapped in quinone-containing liposomes

Additions	Control	Half-time (min) TMQ	PQ_9	UQ_1	UQ_{10}
–	28	6	< 0.1	1	< 0.1
Val + Nig	17	1	< 0.1	0.2	< 0.1

The assay mixture contained in a final volume of 3 ml: 0.3 M NaCl, 50 mM KCl, 20 mM Tricine–NaOH, pH 8.0 and 10 mg lecithin in the form of liposomes which were prepared as described under Methods. The preparation contained 5×10^{-8} mol/quinone/mg lecithin, corresponding closely to the quinone/lipid ratio of chloroplasts or mitochondria. The control contained no quinone. Where indicated valinomycin plus nigericin, 3 μg each, were included as uncoupling system. The reaction was started by addition of 3 μmol dithionite from an anaerobic stock solution. Excess oxygen had been removed by bubbling nitrogen through the assay mixture before the liposomes were added. The reaction was carried out at room temperature. The total change in A_{420nm} was about 0.6 μmol which corresponds to 1.8 μmol ferricyanide in the cuvette. TMQ, PQ and UQ stand for trimethyl-benzoquinone, plastoquinone and ubiquinone, respectively

not possible with our set up to estimate initial rates of reduction accurately, partly because of slow mixing and recording, partly because of superposition by absorption changes not resulting from reduction of ferricyanide. The latter are caused in part by the reduction of the quinones. However, even the control liposomes show a small bleaching at 420 nm in response to the addition of dithionite, tested with a preparation lacking ferricyanide. In the absence of ionophores the course of reduction often exhibits more than one phase even after the rapid initial part. For these reasons only the half time for ferricyanide reduction is given in table 1. All reduction rates, also that with dithionite alone (control), are enhanced by addition of valinomycin and nigericin, which should abolish any electrochemical proton potential formed across the membrane during the redox reaction (see [12]). In the cases of PQ_9 and UQ_{10} this is seen in fig.1A only, our recording being to slow to measure corresponding half-times.

Reduction rates with ascorbate instead of dithionite as external reductant were slow in all cases, and not much of a difference between quinones of different chain length could be observed. This might reflect the need for a stronger reductant to form the intermediate semiquinone [15] than suggested from the redox

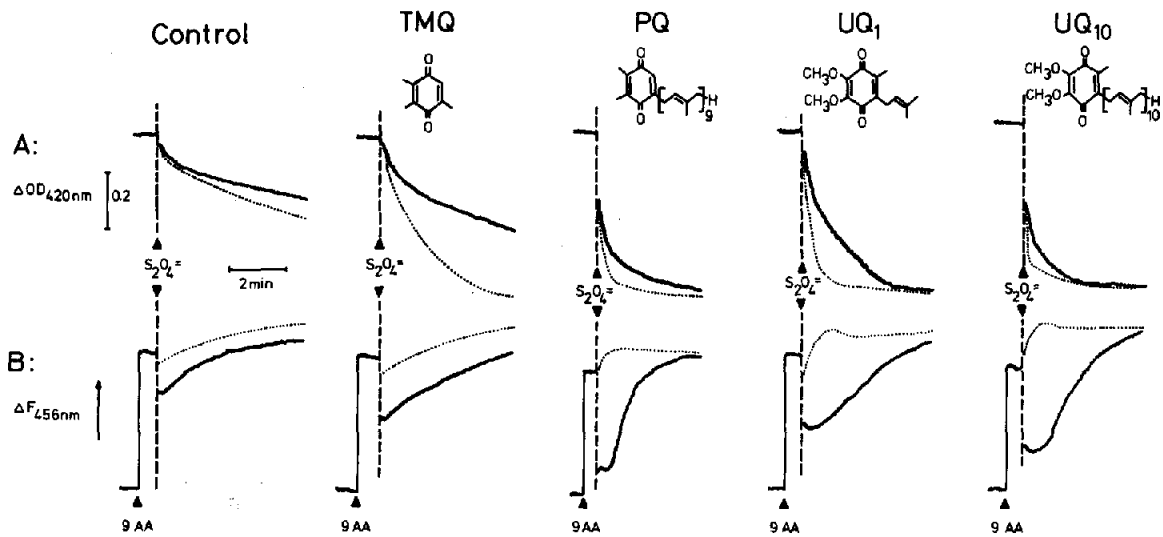

Fig.1. Ferricyanide reduction and fluorescence changes of 9-amino-acridine in quinone-containing liposomes. The conditions for the assay are described in table 1. The reaction was started by addition of dithionite as indicated by the arrows. In the case of fluorescence measurements 15 nmol 9-amino-acridine (9-AA) was added before dithionite. The dotted traces resemble the reactions in the presence of valinomycin and nigericin, 3 μg each. For abbreviations see table 1.

potentials of ascorbate and the substituted benzoquinones, which are close.

Figure 1B depicts the transient quenching of 9-amino-acridine following addition of dithionite, which is sensitive to valinomycin plus nigericin, and is a measure of internal acidification [13,16] during oxidation of the hydroquinone by ferricyanide. Also the control shows some quenching, suggesting that dithionite itself acts as a hydrogen carrier at a slow rate.

In conclusion the results presented here demonstrate that plastoquinone and ubiquinone per se are efficient translocating systems of electrons and protons through lipid membranes, the isoprenoid side chain against expectation even facilitating this action. The dynamics and organisation of the quinone molecules during this action remain to be elucidated, but it seems established that they do not just 'bob up and down' on one surface of the membrane [11] and there is no need to assume special proton carriers associated with the quinone pools in chloroplasts and mitochondria [6,7,11].

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