Vol. 85, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1978 Pages 1047-1053

ON THE SIDEDNESS OF THE UBIQUINONE REDOX CYCLE. KINETIC STUDIES IN MITOCHONDRIAL MEMBRANES

G. Lenaz, L. Landi, L. Cabrini, P. Pasquali, A.M. Sechi and T. Ozawa*

Istituto di Biochimica, University of Ancona, Italy, and Istituto di Chimica Biologica, Faculty of Pharmacy, University of Bologna, Italy

Received September 28, 1978

Summary. The inhibition of NADH oxidation but not of succinate oxidation by the low ubiquinone homologs UQ-2 and UQ-3 is not due to a lower rate of reduction of ubiquinone by NADH dehydrogenase: experiments in submitochondrial particles and in pentane-extracted mitochondria show that UQ-3 is reduced at similar rates using either NADH or succinate as substrates. The fact that reduced UQ-3 cannot be reoxidized when reduced by NADH but can be reoxidized when reduced by succinate may be explained by a compartmentation of ubiquinone.

Using reduced ubiquinones as substrates of ubiquinol oxidase activity in intact mitochondria and in submitochondrial particles we found that ubiquinol-3 is oxidized at higher rates in submitochondrial particles than in mitochondria. The initial rates of ubiquinol oxidation increased with increasing lengths of isoprenoid side chains in mitochondria, but decreased in submitochondrial particles. These findings suggest that the site of oxidation of reduced ubiquinone is on the matrix side of the membrane; reduced ubiquinones may reach their oxidation site in mitochondria only crossing the lipid bilayer: the rate of diffusion of ubiquinol-3 is presumably lower than that of ubiquinol-7 due to the differences in hydrophobicity of the two quinones.

Introduction

We have previously observed that NADH oxidation in pentane-extracted mitochondria is only restored by the ubiquinone (UQ) homologs having relatively long isoprenoid side chains, while all homologs have considerable activity in restoring succinate

*Visiting professor from the Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Nagoya, Japan.

0006-291X/78/0853-1047$01.00/0

oxidation (1, 2); we have then observed that the low ubiquinone homologs act as competitive inhibitors of NADH oxidation but not of succinate oxidation in non-extracted mitochondrial membranes (3). The reduction of cytochrome b by NADH in submitochondrial particles is slower in presence of UQ-2 while the reduction of cytochrome b by succinate is stimulated (4). The inhibition of NADH oxidation observed in our systems might be due to either of the following effects.

a) The low homologs in the membrane are reduced by NADH dehydrogenase less efficiently than the high homologs because the sites of interaction for UQ have steric or polarity differences in NADH dehydrogenase and in succinate dehydrogenase.

b) The low homologs are reduced by NADH dehydrogenase but their reoxidation by the following redox acceptor in the chain is impaired. This possibility, involving the idea that UQ reoxidation is impaired only when UQ has been previously reduced by NADH but not by succinate, has been proven correct.

Materials and Methods

Bovine heart mitochondria (BHM) and submitochondrial particles (SMP) have been prepared as described previously (3). Ubiquinone-depleted mitochondria were prepared by pentane extraction after lyophilization according to Szarkowska (5). NADH-UQ reductase activity was assayed by following the decrease in absorbance of NADH at 340 nm; the activity was rotenone-sensitive in all systems tested.

Succinate-UQ reductase was assayed by following the decrease in absorbance at 275 nm due to ubiquinone reduction, using an extinction coefficient Δ_{ox-red} of 12.5×10^3 M^{-1} cm^{-1}.

All assays were accomplished at room temperature (22°C). The reaction was initiated by adding the ubiquinone in ethanolic solution. Ubiquinol oxidase activity was assayed by measuring the O_2 consumption polarographically with an oxygen electrode (YSI 5331 OXYGEN PROBE).

The medium contained in a total volume of 3 ml: 200 mM sucrose, 30 mM KCl, 8.3 mM Hepes, pH 6.8, 5 mM malonate and either SMP (0.65-0.70 mg of protein) or BHM (0.65-0.70 mg of protein); the low pH was chosen to prevent autoxidation. These assays were accomplished at different temperatures. The reaction was initiated by adding the particles after equilibration of the so-

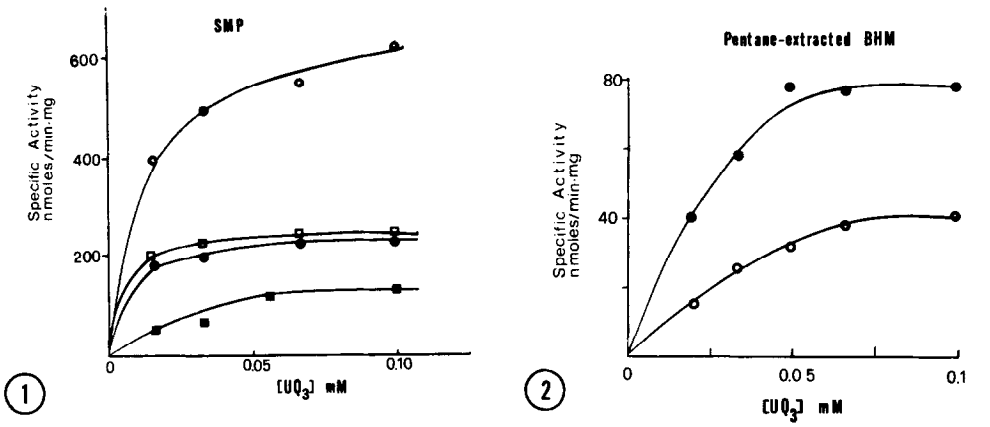

Fig. 1. NADH-UQ reductase and succinate-UQ reductase in SMP. o——o, NADH-UQ-1 reductase; ●——●, NADH-UQ-3 reductase; □——□, succinate-UQ-1 reductase; ■——■, succinate-UQ-3 reductase. The system contained in a total volume of 3 ml: 0.25 M Sucrose; 33 mM K-phosphate buffer, pH 6.8; 0.16 mM NADH, SMP (0.345 mg protein), Antimycin A 1 µg.

Fig. 2. NADH-UQ-3 reductase and succinate-UQ-3 reductase in pentane-extracted mitochondria. ●——●, NADH-UQ-3 reductase; o——o, succinate UQ-3 reductase. Experimental details are reported in the legend of Fig. 1.

lution with the ubiquinols previously added in ethanolic solution (20-40 µl per assay). The activity was Antimycin A and KCN sensitive.

Ubiquinols were prepared from the corresponding ubiquinones by the method of Rieske (6). Ubiquinones were kind gifts of Hoffman La Roche (Basel); Antimycin A was purchased from Sigma. Protein was determined with a Biuret method (7).

Results and Discussion

The rate of reduction of exogenous UQ-1 and UQ-3 in SMP is shown in Fig. 1. Using either NADH or succinate as substrates in incubation system, UQ-1 is a better acceptor than UQ-3; the latter however is reduced at comparable rates by NADH and succinate; the rates are actually higher with NADH.

Higher quinones could not be tested adequately as oxidants in this system, due to their low water solubility and subsequent

low incorporation into or interaction with the particles (cf. 8). Similar effects have been shown in pentane-extracted mitochondria, that are depleted of their endogenous ubiquinone (Fig. 2).

The rate of reduction of UQ-3 is higher using NADH than succinate as electron donor and the reduction is rotenone-sensitive.

It must therefore be concluded that UQ-3 is reduced by NADH through Complex I of the respiratory chain. The K_m for UQ-3 of NADH-UQ reductase in pentane-extracted mitochondria was 43 μM, in accordance with that in isolated Complex I, while the corresponding value for succinate-UQ reductase was 74 μM.

These experiments allow to conclude that the second hypothesis postulated in the introduction is the correct one.

The fact that UQ-3 reduced by NADH cannot be reoxidized by the cytochrome chain, whereas UQ-3 reduced by succinate is reoxidized is not of obvious explanation and may involve a sidedness of UQ reduction and oxidation in the membrane. To test this possibility we have studied electron transfer using exogenous reduced ubiquinones of different chain lengths as substrates of ubiquinol oxidase in both BHM and SMP which have opposite polarity: as is well known, SMP are inside-out and face the medium with the "matrix" side (M-side)(9). The results are shown in Table I. We can observe that ubiquinol-3 is a good substrate in SMP, but is oxidized at lower rates in BHM; on the other hand, ubiquinols-7 and -8, at similar concentrations, are oxidized at increasingly higher rates in BHM but not in SMP, where there is rather a decrease of oxidation rate with the longer quinols. Only at 15°C there was a lower activity for UQ-7 than for UQ-3 in BHM; a decreased solubility of the long chain quinol could be responsible for the observed effect. The table also shows that the oxidation rate relative to UQ-3 taken as 100%, increases with increasing chain length in BHM, but decreases in SMP.

The relatively low rate of oxidation of ubiquinol-7 and -8 in SMP might be due to the higher protein content of the inner face of the mitochondrial membrane (10) in comparison with the

Table I. Ubiquinol Oxidase in BHM and SMP

Assay temperature	Ubiquinol	BHM		SMP	
		Specific activity (μatoms/min.mg protein)	Relative efficiency (% of UQ-3)	Specific activity (μatoms/min.mg protein)	Relative efficiency (% of UQ-3)
I. 30°C	UQ-3	0.230	100	0.380	100
	UQ-4	0.260	113	0.127	33.4
	UQ-7	0.285	124	0.150	39.5
II. 25°C	UQ-3	0.104	100	0.202	100
	UQ-7	0.167	160	0.037	18.3
III. 15°C	UQ-3	0.151	100	0.110	100
	UQ-7	0.100	66.2	0.022	20

outer face so that the more hydrophobic quinols cannot interact efficiently with the membrane lipids.

In order to explain the difference among membranes of opposite sidedness we suggest that the side of oxidation of ubiquinol is near the M-side of the membrane, and therefore ubiquinol-7 and -8 added to mitochondria reach the oxidation site by crossing the membrane lipids, whereas ubiquinol-3 moves in the hydrophobic interior of the membrane at much lower rate.

We suggest therefore that UQ is reduced by NADH dehydrogenase at the cytoplasmic side and must cross the membrane to the M-side to be reoxidized by Complex III, whereas UQ is reduced by succinate on the M-side and reoxidized on the same side (11).

The rate of diffusion of reduced ubiquinones across lipid bilayers increases proportionally to the length of the isoprenoid chains (12), giving support to our view that reduced UQ-3 cannot be oxidized in mitochondria because of its incapability to cross the membrane.

We have confirmed this idea by spin label EPR experiments showing that oxidized UQ-3 (and both reduced and oxidized UQ-9) have a disordering effect on lipid bilayers, while reduced UQ-3 has a pronounced ordering effect, suggesting its insertion in the bilayer with the quinol group bound to the polar heads of the phospholipids (13); an ordering effect of reduced quinones has been found by Cain et al. (14) by X-ray diffraction and by Chance et al. (15) using the fluorescent probe 12 (9-antroyl) stearic acid.

Acknowledgements

This work was supported in part by a grant from CNR, Rome, Italy. The samples of the ubiquinones were kind gifts of Hoffman La Roche, Basel, Suisse.

References

1. Lenaz, G., Daves, D.G., and Folkers, K. (1968) Arch. Biochem. Biophys. 123, 539-550.
2. Castelli, A., Bertoli, E., Littarru, G.P., Lenaz, G., and Folkers, K. (1971) Biochem. Biophys. Res. Commun. 42, 806-812.
3. Lenaz, G., Pasquali, P., Bertoli, E., and Parenti-Castelli, G. (1975) Arch. Biochem. Biophys. 169, 217-226.
4. Lenaz, G., Pasquali, P., and Bertoli, E. (1975) In: Electron Transfer Chains and Oxidative Phosphorylation (E. Quagliariello, S. Papa, F. Palmieri, E.C. Slater and N. Siliprandi, eds), pp. 251-256, North-Holland, Amsterdam.
5. Szarkowska, L. (1966) Arch. Biochem. Biophys. 113, 519-525.
6. Rieske, J.S. (1967) Methods Enzymol. 10, 239-245.
7. Gornall, A.G., Bardawill, C.J., and David, M.M. (1949) J. Biol. Chem. 177, 751-766.
8. Hatefi, Y., and Stempel, K.E. (1969). J. Biol. Chem. 244, 2350-2357.
9. Lee, C.P., and Ernster, L. (1966) Symposium: Regulation of Metabolic Processes in Mitochondria, vol. 7, pp. 218-234, BBA Library.
10. Packer, L., Mehard, C.W., Meissner, G., Zahler, W.L., and Fleischer, S. (1974) Biochim. Biophys. Acta 363, 159-181.
11. Lenaz, G., Mascarello, S., Landi, L., Cabrini, L., Pasquali, P., Parenti-Castelli, G., Sechi, A.M., and Bertoli, E. (1977) In: Bioenergetics of Membranes (L. Packer et al., eds), pp. 189-198, Biomedical Press, Elsevier, North-Holland.
12. Hauska, G. (1977) FEBS Letters 79, 345-347.
13. Spisni, A., Masotti, L., Lenaz, G., Bertoli, E., Pedulli, G., and Zannoni, C. (1978) Arch. Biochem. Biophys., in press.
14. Cain, J., Santillan, G., and Blasie, J.K. (1972) In: Membrane Research (C.F. Fox, ed.), pp. 3-14, Academic Press, New York.
15. Chance, B., Erecinska, M., and Radda, G. (1975) Eur. J. Biochem. 54, 521-529.