RHODOQUINONE SPECIFICITY IN THE REACTIVATION OF SUCCINOXIDASE ACTIVITY OF ACETONE-EXTRACTED ASCARIS MITOCHONDRIA

Masashi Sato*, Koji Yamada and Hikaru Ozawa

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan.

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SUMMARY

Ubiquinone and rhodoquinone were assayed on the reactivation of succinoxidase activity of acetone-extracted rat liver mitochondria and of Ascaris muscle mitochondria. In the rat liver preparation ubiquinone restored the lost succinoxidase but rhodoquinone did not. On the other hand, in Ascaris preparation rhodoquinone alone restored the lost succinoxidase. From specific reactivation activity of two types of bioquinone it was suggested that rhodoquinone participates in Ascaris succinoxidase system just as ubiquinone does in the mammalian system.

INTRODUCTION

Ubiquinone is now recognized as an essential component in the electron transport chain of mitochondria for many forms of life (1). In contrast to ubiquinone, natural occurrence of rhodoquinone, a benzoquinone in which a methoxy group of ubiquinone is substituted with an amino group, has hitherto been restricted to few organisms and its biological activity has not been demonstrated (2). Until recently this family of compounds has been reported only in Rhodospirillum rubrum (3,4), other members of the Athiorhodaceae (5) and Euglena gracillis (6).

Our finding(7) that swine roundworm, Ascaris lumbricoides var. suis, contains rhodoquinone in the place of ubiquinone extended the distribution of rhodoquinone in nature from microorganisms to metazoa, which was followed by the finding of rhodoquinone in the same family of parasitic nematode, Stephanurus dentatus(8).

*Present adress: Department of Biology, Miyagi College of Education, Aobayama, Sendai, Japan.

Compared with the above mentioned microorganisms, which are known to have both ubiquinone and rhodoquinone, Ascaris worm has rhodoquinone only. Therefore Ascaris worm has some experimental advantages for elucidating biological activity of rhodoquinone.

In the present experiment biological activity of rhodoquinone and ubiquinone were examined on the reactivation of succinoxidase of acetone-extracted Ascaris muscle mitochondria as well as of rat liver mitochondria.

MATERIALS AND METHODS

Rat liver mitochondria and Ascaris muscle mitochondria were obtained by the differential centrifugation by the procedure as described by Schneider and Hogeboom (9). Final concentrations of rat liver mitochondria and Ascaris muscle mitochondria were adjusted to 50 mg protein per ml of isolation medium (0.25 M sucrose, 0,1 mM EDTA and 10 mM Tris-HCl, pH 7.4) and to 30 mg protein per ml of isolation medium (0.3 M sucrose, 1mM EDTA and 10 mM Tris-HCl, pH 7.4) respectively. Mitochondrial preparations thus obtained were extracted with 96% cold acetone (10), which resulted in the considerable loss of succinoxidase activity as well as in almost complete depletion of ubiquinone or rhodoquinone in them.

Succinoxidase activity in mitochondria, extracted with 96% acetone, was measured in standard manometric assays at 37° for 30 min.. Reaction systems adopted were as described in tables. As a lipid factor sonicated lecithin (soybean phospholipids) was used. Quinones were added in ethanol solution, and ethanol concentration was kept constant in a total volume of 2 ml (0.05 ml of ethanol in 2 ml of reaction mixture).

The reaction was started by addition of succinate from side arm after 10 minute balance.

Protein was determined by the biuret method (11), and on the turbid Ascaris mitochondrial preparation some modification of the method (12) was needed.

RESULTS AND DISCUSSION

Biological activity of ubiquinone and rhodoquinone examined on the reactivation of acetone-extracted rat liver mitochondria

were shown in table 1. Ubiquinone-9 and phytylubiquinone restored the lost activity, but ubiquinone-0, rhodoquinone-9, phytylrhodoquinone as well as rhodoquinone-0 did not. Some of them rather inhibited the residual low succinoxidase activity of the preparation. The results obtained with rat liver preparation are in accord with those reported by Lenaz et al. (13) on ubiquinone homologs and rhodoquinone-10.

Table 1

UBIQUINONE SPECIFICITY IN THE REACTIVATION OF SUCCINOXIDASE OF ACETONE-EXTRACTED RAT LIVER MITOCHONDRIA

Addition	Succinoxidase $\mu l\ O_2$/mg protein/hr.			
None	4.46	7.30	5.87	5.19
Ubiquinone-9	45.09	43.58	40.06	39.87
Phytylubiquinone	67.57			
Ubiquinone-0			2.21	
Rhodoquinone-9		6.42		4.62
Phytylrhodoquinone			3.61	
Rhodoquinone-0				2.41

The reaction system contained 500 μmoles sucrose; 40 μmoles Tris-HCl, pH 7.4; 2 μmoles $MgCl_2$; 100 μmoles sodium siccinate; 1 mg cytochrome c; about 1 mg sonicated lecithin; 250 μg quinone as shown in the column of addition and about 5 mg protein of acetone-extracted mitochondria in a total volume of 2 ml.

As for Ascaris preparation, the situation was the reverse. In this case (table 2), rhodoquinone-9 and phytylrhodoquinone restored the lost activity, but ubiquinone-9 and rhodoquinone-0 did not. Furthermore, succinoxidase activity restored by addition of rhodoquinone-9 is almost equal to that of untreated normal Ascaris mitochondria.

The present results, together with the previous data that in its subcellular distribution study rhodoquinone in mitochondrial fraction accounted for more than 50% of the total rhodoquinone

in Ascaris muscle (details are to be published elswhere), strongly support the idea that rhodoquinone participates in Ascaris succinoxidase system just as ubiquinone does in the mammalian system.

As seen from the effect of ubiquinone-0 and rhodoquinone-0, existence of isoprenoid side chain in the structure is likely to be essential for biological activity of rhodoquinone also.

Based upon the organic structural specificity of ubiquinone for the activity in the succinoxidase and NADH-oxidase systems

Table 2

RHODOQUINONE SPECIFICITY IN THE REACTIVATION OF SUCCINOXIDASE OF ACETONE-EXTRACTED ASCARIS MUSCLE MITOCHONDRIA

Addition	Succinoxidase $\mu l\ O_2$/mg protein/hr.	
None	1.5	1.2
Ubiquinone-9	2.5	2.4
Rhodoquinone-9	20.6	14.8
Phytylrhodoquinone	20.6	17.5
Rhodoquinone-0	2.5	4.1

The reaction system contained 140 μmoles potassium phosphate buffer, pH 7.4; 5 μmoles $MnCl_2$; 100 μmoles sodium succinate; 1 mg cytochrome c; about 1mg sonicated lecithin; 250 μg quinone as shown in the column of addition and 3 mg protein of acetone-extracted mitochondria in a total volume of 2 ml.

of beef heart Lenaz et al. (13) have proposed two sites for the function of ubiquinone, in which the site for ubiquinone in mammalian succinoxidase system is characterized by an electron functionality that is met by ubiquinone but not at all by rhodoquinone. In Ascaris succinoxidase system, the possible site of interaction of rhodoquinone may be characterized by an electron functionality that is met by rhodoquinone but not at all by ubiquinone.

Further studies on the effect of rhodoquinone analogs on Ascaris systems may advance the understanding not only of the

role of rhodoquinone in parasitic Ascaris worms but also of the functional mechanism of ubiquinone in the mammalian systems.

This knowledge in turn may facilitate the design of new types of anthelmintics.

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