EFFECT OF HYDROXY ANALOGS OF COENZYME Q ON DPNH- AND SUCCIN-OXIDASE ACTIVITIES OF YEAST MITOCHONDRIA

by

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SUMMARY

2,3-Dimethoxy-5-hydroxy-6-phytyl-and-6-farnesyl-1,4-benzoquinones (HPB and HFB) inhibit DPNH- and succin-oxidases of intact mitochondria from yeast. CoQ_2 reversed the inhibition in DPNH-oxidase, but there was little or no reversal in succin-oxidase. CoQ_6, the dominant CoQ of yeast, showed the same relative reversals as CoQ_2. For CoQ_6-deficient DPNH-oxidase, supplementation with: (a) HPB; (b) HFB; (c) HPB and CoQ_6; (d) HPB and CoQ_{10} showed unexpected increases in activity indicating coenzymatic activity for HPB and HFB; the inhibitory effects of HPB and HFB were apparent with supplementation with CoQ_2. HPB inhibited the CoQ_6-deficient succin-oxidase, and also in the presence of CoQ_2, CoQ_6 or CoQ_{10}.

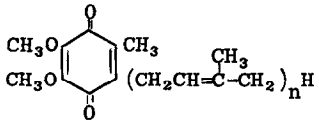
Our previous studies on the role of coenzyme Q in the electron transfer chain have revealed the existence of two sites for coenzyme Q. One is in the DPNH-oxidase system and the other is in the succin-oxidase system. The presence of the two sites was demonstrated both for beef heart mitochondria (1) and yeast mitochondria (2,3) which have two different homologs of coenzyme Q, CoQ_{10}(I) and CoQ_6 (II) respectively, in their respiratory chains.

Further evidence on the existence of the two different sites may be found by the use of inhibitors which act at the site or near the site of CoQ in the respiratory chain. Some examples of such evidence based on inhibitors have been described (4,5,6,7). Interesting inhibitors which were synthesized by Catlin et al.(5) are the hydroxy analogs of CoQ, 2,3-dimethoxy-5-hydroxy-6-phytyl-1,4-benzoquinone (HPB) (III) and 2,3-dimethyl-5-hydroxy-6-farnesyl-1,4-benzoquinone (HFB) (IV). These analogs were found to inhibit mitochondrial coQ-enzyme systems from beef heart (5,7). The significant structural difference between these analogs and CoQ is the replacement of the methyl group at position 5 of CoQ by the hydroxyl group as in the inhibitors. Presumably, the differences in the isoprenoid side chain between I-IV are considerably less significant than the difference between the methyl- and hydroxyl- groups. Changes in the isoprenoid

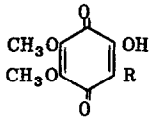
*Coenzyme Q. CXXXIII.

side chain do alter the degree of coenzymatic activity, but are less likely to change a coenzymatically active compound to an inhibitor. The replacement of the methyl- group with the hydroxyl- group can be expected to create an inhibitor at least in certain systems.

The important differences which have been observed between the respiratory chains of yeast and mammalian mitochondria (8) have prompted us to extend the study of the effect of HPB and HFB on the respiration of the mitochondria of Saccharomyces cerevisiae.



n = 10, I
n = 6, II



III, R = $CH_2CH{=}\overset{CH_3}{\underset{|}{C}}{-}CH_2(CH_2CH_2\overset{CH_3}{\underset{|}{C}}HCH_2)_3H$

IV, R = $(CH_2CH{=}\overset{CH_3}{\underset{|}{C}}{-}CH_2)_3H$

METHODS

The mitochondria were prepared by the method of Schatz (9) from S.cerevisiae, and were then lyophilized in a solution of potassium chloride (10). Aliquots of the mitochondrial preparations were extracted with pentane and the respiratory activities were assayed with the DPNH-oxidase and succin-oxidase enzyme systems (10). The homologs of coenzyme Q, HPB, and HFB were added as alcoholic solutions in the presence of the phospholipid micelles (Asolectin) as carriers for the quinones (3). The amount of mitochondrial protein which was used was generally 0.6 mg per flask.

RESULTS

Lyophilized Mitochondria. - The data in Table I show the effect of HPB and HFB on the DPNH- and succin-oxidase activities of lyophilized mitochondria of yeast which had not been extracted with a solvent. The activities of both CoQ-enzyme systems were progressively depressed by increasing levels of HPB and HFB.

CoQ_2 is a very effective homolog in yeast mitochondria (2,3). Increasing levels of CoQ_2 clearly effected a progressive restoration of the activity of DPNH-oxidase in the presence of HPB or HFB as shown in Table II. However, there was little or no restoration by CoQ_2 of the activity of succin-oxidase in the presence of HPB or HFB. Similar results were observed when CoQ_6 instead of CoQ_2 was used as shown in Table III.

CoQ_6-Deficient Mitochondria. - The extraction with pentane removes most of the endogenous CoQ_6 from the mitochondria. The inhibition by HPB and reversal by homologs of CoQ on such CoQ_6-deficient mitochondria were observed. In contrast HFB alone stimulated activity.

The data in Table IV show the effect of HPB and HFB on the DPNH-oxidase

TABLE I. Effects of HPB and HFB on DPNH- and Succin-Oxidase Activities of Non-Extracted Yeast Mitochondria

H P B (nmoles)	DPNH-Oxidase Specific Activity (a) (b)	%	(c)	%	Succin-Oxidase Specific Activity (a) (b)	%	(c)	%
-	1.031	100	0.712	100	0.402	100	0.363	100
50	0.759	73	0.421	59	0.277	69	0.124	34
100	0.524	50	0.382	53	0.199	50	0.074	20
200	0.480	46	0.267	37	0.098	25	0.074	20
500	0.232	22	0.204	28	0.012	3	0	0
H F B (nmoles)								
-	1.48	100			0.362	100		
100	1.07	72			0.163	45		
500	0.80	54			0.045	12		

(a) μatoms of oxygen consumed/min/mg mitochondrial protein.
(b) and (c) refer to two different mitochondrial preparations.

TABLE II. Effect of CoQ_2 on HPB and HFB Inhibition of DPNH- and Succin-Oxidase of Non-Extracted Yeast Mitochondria (Three Experiments)

H P B (nmoles) or H F B (nmoles)	CoQ_2 (nmoles)	DPNH-Oxidase (b) Specific Activity HPB (a)	%	HFB (a)	%	Succin-Oxidase Specific Activity HPB (a)	%	HFB (a)	%
-	-	1.555	100	1.79;1.57	100	0.363	100	0.338	100
-	100	2.59	-	2.47;2.81	-	0.444	-	0.478	-
-	500	3.00	-	3.38;3.26	-	0.464	-	0.464	-
100	-	0.535	35	1.14;1.13	70	0.190	50	0.184	55
100	100	0.750	50	1.93;1.55	100	0.217	60	0.221	65
100	200	0.920	60	2.32;1.90	125	0.217	60	0.227	65
100	500	1.124	70	2.30;1.80	120	0.207	60	0.203	60
-	-	1.085	100			0.181	100		
-	100	1.028	-			0.264	-		
-	500	1.167	-			0.272	-		
200	-	0.334	30			0.105	60		
200	100	0.494	45			0.141	80		
200	200	0.592	55			0.137	75		
200	500	0.787	70			0.137	75		
-	-	1.260	100			0.364	100		
-	100	2.48	-			0.486	-		
-	500	-	-			0.493	-		
200	-	0.545	45			0.181	50		
200	100	0.699	55			0.195	55		
200	200	0.795	65			0.206	55		
200	500	0.889	70			0.197	55		

(a) μatoms O_2/min/mg protein.
(b) The two columns under DPNH-oxidase refer to two different mitochondrial preparations.

TABLE III. Effect of CoQ_6 on HPB Inhibition of DPNH- and Succin-Oxidase of Non-Extracted Yeast Mitochondria

HPB (nmoles)	CoQ_6 (nmoles)	DPNH-Oxidase (Specific Activity) (a)	%	(a)	%	Succin-Oxidase (Specific Activity) (a)	%
-	-	1.61	100	1.59	100	0.218	100
-	100	1.61	-	-	-	0.180	-
-	500	1.85	-	-	-	0.170	-
100	-	0.72	45	1.08	70	0.122	55
100	100	0.871	55	1.21	75	0.091	40
100	200	0.965	60	1.22	75	0.087	40
100	500	1.04	65	1.37	85	0.089	40

(a) µatoms O_2/min/mg protein.

TABLE IV. Effect of CoQ_2 on HPB and on HFB Inhibition of DPNH- and Succin-Oxidase of Pentane-Extracted Yeast Mitochondria (Two Experiments)

Inhibitor nmoles	CoQ_2 nmoles	DPNH-Oxidase Specific Activity HPB (a)	%	HFB (a)	%	Succin-Oxidase Specific Activity HPB (a)	%	HFB (a)	%
-	-	0.233	100	0.300	100	0.087	100	0.078	100
-	100	1.96	840	2.12	700	0.191	220	0.138	175
-	500	2.43	1040	2.64	880	0.195	225	0.167	215
100	-	0.177	75	0.417	140	0.045	50	0.045	55
100	100	0.320	140	1.302	435	0.076	90	0.083	105
100	200	0.418	180	1.532	510	0.076	90	0.096	125
100	500	0.621	270	1.935	645	0.086	100	0.107	135
-	-	0.343	100	0.317	100	0.107	100	0.101	100
-	100	2.18	635	-	-	0.162	150	-	-
-	500	2.47	720	-	-	0.195	180	-	-
100	-	0.321	95	0.449	140	0.063	60	0.046	45
100	100	0.573	170	1.429	450	0.112	105	0.097	100
100	200	0.738	215	1.566	495	0.120	110	0.107	105
100	500	1.15	335	1.990	625	0.133	125	0.118	115
-	-							0.093	100
100	-							0.057	61
100	100							0.078	85
100	200							0.081	85
100	500							0.106	115

(a) µatoms O_2/min/mg protein.

system of pentane-extracted mitochondria which were deficient in CoQ_6 or supplemented with exogenous homologs of CoQ. The inhibitory effect of HPB and HFB is apparent in the CoQ_2-supplemented mitochondria. However, using HPB alone and using HPB with a supplement of CoQ_6 or using HPB and CoQ_{10}, there were increases of activity which were in proportion to the level of HPB, as shown in Table V.

It appears that HPB shows coenzymatic activity like that of CoQ_6 in the CoQ_6-deficient DPNH-oxidase system of yeast.

TABLE V. Effect of HPB on DPNH-Oxidase Activity of Pentane-Extracted Yeast Mitochondria

H P B (nmoles)	No CoQ Added	%	Specific Activity CoQ_2(b) (a)	%	CoQ_6(b) (a)	%	CoQ_{10}(b) (a)	%
-	0.078 0.075	100	1.330	100	0.095	100	0.105 0.117	100
50	-		1.074	80	0.120	125	0.125 0.133	115
100	0.080	100	0.756	55	0.145	150	0.151 0.171	145
200	0.169	220	0.494	35	0.195	205	0.202 0.236	200

(a) μatoms O_2/min/mg protein.
(b) 100 nmoles per assay.

TABLE VI. Effect of HPB on Succin-Oxidase Activity of Pentane-Extracted Yeast Mitochondria

H P B (nmoles)	-	Specific Activity CoQ_2(b) (a)	%	CoQ_6(b) (a)	%	CoQ_{10}(b) (a)	%
-	0.064 0.033	0.188 0.153	100	0.138	100	0.088	100
50	-	0.098 0.085	55	0.093 0.068	60	0.079	90
100	0.046	0.069 0.058	35	0.075 0.058	50	0.067	75
200	-	0.079 0.063	40	0.051 0.033	30	0.067	75

(a) μatoms O_2/min/mg protein.
(b) 100 nmoles per assay.

It is interesting that HPB shows inhibition in the presence of CoQ_2 and yet apparent coenzymatic activity alone or in the presence of CoQ_6 or CoQ_{10}. It is known (3) that the higher CoQ homologs have low activity in the DPNH-oxidase system of yeast mitochondria which is apparently due to difficulties of transport to the enzymic site even in the presence of micelles of phospholipid as carriers. Since HPB has a side-chain with only four isoprenoid units, it might more effectively reach the enzymic site.

For the succin-oxidase system, HPB acts as an inhibitor in all systems as shown in Table VI.

DISCUSSION

2,3-Dimethoxy-5-hydroxy-6-phytyl-and-6-farnesyl-1,4-benzoquinones (HPB and HFB) are analogs of coenzyme Q in which the 5-methyl- group of coenzyme Q is replaced by a 5-hydroxyl- group as the apparent significant structural change. The change in the isoprenoid side chain is considered of lesser significance.

It has been found that HPB and HFB are inhibitors of both DPNH- and succin-oxidase systems of yeast mitochondrial preparations, and they appear to compete with the endogenous CoQ_6 for the same enzymic sites. However, there is a difference of action in the two enzyme systems as measured by the percent change. Succin-oxidase appears to be slightly more sensitive to the inhibition, and the inhibition appears to be irreversible in succin-oxidase. In contrast, exogenous CoQ homologs can reverse the inhibition of HPB and HFB in DPNH-oxidase. It has been reported (1,11) that the endogenous coenzyme Q is located in two different molecular environments and that two different pools are associated with Complex I and Complex II and which react at two different sites. A consequence of these two molecular compartments for endogenous coenzyme Q may be the higher sensitivity to HPB and HFB and the stronger affinity for HPB and HFB of the site which reacts with the pool of CoQ associated with the succin-oxidase system. Similar uncertainties were reported by Jeng et al.(4) for the reversal of inhibition by piericidin A of succinate oxidation. In any case, these results confirm again and extend the assignment of two different enzymic sites for coenzyme Q and the dual compartments for CoQ in the respiratory chain of yeast mitochondria as in mammalian mitochondria.

Additional evidence for the existence of two enzymic sites for CoQ in yeast mitochondria is based on the studies of the effect of HPB and HFB in pentane-extracted particles. HPB appears to have actual CoQ-activity in this DPNH-system but no activity in the succinate-system. The apparent coenzymatic activity of HPB leads to enhancement of activity according to the results obtained from its addition with CoQ_6 or CoQ_{10} to the DPNH-oxidase system.

HPB and HFB appear to be inhibitors of the respiratory chain by competitively displacing the endogenous coenzyme Q_6 from its sites of action in Complex I and II.

Piericidin A (4,12) which has some structural resemblance to the coenzyme Q, acts like CoQ in the site of succinate dehydrogenase, but it is a specific high-affinity inhibitor of the DPNH-dehydrogenase site. HPB does not have such affinity either in yeast or in mammalian mitochondria.

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