LOW MOLECULAR WEIGHT ANALOGS OF COENZYME Q AS HYDROGEN ACCEPTORS AND DONORS IN SYSTEMS OF THE RESPIRATORY CHAIN

Yieh-Ping Wan, Robert H. Williams, and Karl Folkers*

Institute for Biomedical Research
The University of Texas at Austin
Austin, Texas 78712

Kam Hung Leung and Efraim Racker

Section of Biochemistry, Molecular and Cell Biology
Cornell University
Ithaca, New York 14850

Received December 17, 1974

SUMMARY

2,3-Dimethoxy-5-methyl-6-n-pentyl-, -6-n-decyl-, and -6-n-pentadecyl-1,4-benzoquinones were synthesized as analogs of coenzyme Q_1, Q_2, and Q_3, respectively. The 6-substituents of the three pairs of quinones have 5, 10, and 15 carbon atoms. These analogs are more stable than the isoprenoid Q's, and have advantages in biochemical experiments. These low molecular weight analogs have inadequate lipoidal characteristics to simulate the higher molecular weight CoQ's including CoQ_{10}. However, if lipoidal characteristics are not essential, the pentyl and decyl analogs are highly effective as evidenced by their functioning like CoQ_1 and Q_2 as hydrogen acceptors with ETPH particles for oxidative phosphorylation at site I, and after reduction as hydrogen donors for complex III.

INTRODUCTION

In nature, coenzyme Q_1 through Q_{10} exist. CoQ_1, Q_2, Q_3, Q_4 were identified by high-sensitivity mass spectrometry by Daves, et al.(1) in paper chromatographically pure samples of the dominant coenzyme Q of diverse organisms. In other words, these four CoQ's exist in "micro-trace" concentrations. Apparently, CoQ_5 exists in nature in somewhat higher concentration, because it could be isolated in pure form; Friis et al.(2). Coenzyme Q_6 through Q_{10} have all been isolated from microorganisms and occur in far higher concentrations than the low molecular weight CoQ's. Coenzymes Q_9 and Q_{10} exist in certain rodent tissues. Coenzyme Q_{10} is the "human CoQ". It appears likely that CoQ_1 through Q_5 have no intrinsic function in life processes, but merely result from the absence of absolute specificity of biosynthesis.

CoQ_1 and CoQ_{10} have molecular weights of 250 and 862, respectively. For over a decade, biochemists have frequently preferred to conduct studies with CoQ_1, Q_2 and Q_3 because their lower molecular weights (i.e., minimal lipoidal nature) facilitate their functioning in diverse systems of in vitro nature. The relative chemical instability of low molecular weight isoprenoid quinones

*Coenzyme Q 187.

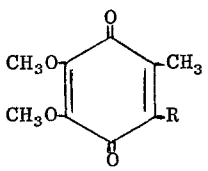
including CoQ_1, Q_2 and Q_3 can be a difficulty, and partially decomposed samples can be inadvertently used and presumed to be satisfactory. An extreme example is plastoquinone-3 which was surprisingly and extremely unstable; Misiti et al. (3).

To circumvent the potential and frequently overlooked aspect of instabilities of CoQ_1, I; CoQ_2, II; CoQ_3, III; three analogs have been synthesized where the three isoprenoid groups have been replaced with saturated straight-chain alkyl groups. In other words, the 6-pentyl derivative, IV, "simulates" CoQ_1, and the 6-decyl derivative V, and the 6-pentadecyl derivative, VI, simulate CoQ_2 and CoQ_3, respectively. Studies of the biological activity of the pentadecyl-derivative have been reported (4).

CoQ_1, I, R = $-CH_2CH=\overset{CH_3}{\overset{|}{C}}-CH_3$

CoQ_2, II, R = $-(CH_2CH=\overset{CH_3}{\overset{|}{C}}-CH_2)_2H$

CoQ_3, III, R = $-(CH_2CH=\overset{CH_3}{\overset{|}{C}}-CH_2)_3H$



IV. R = $-(CH_2)_4CH_3$; PB

V. R = $-(CH_2)_9CH_3$; DB

VI. R = $-(CH_2)_{14}CH_3$; PDB

The synthesis and chemical characterization of these three 6-alkyl analogs are described herein, and they have been appropriately compared with CoQ_1 and Q_2 in the assay for oxidative phosphorylation at site I.

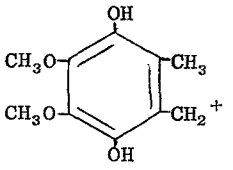
MATERIALS AND METHODS

The general method for synthesis of these three 2,3-dimethoxy-5-methyl-6-alkyl-1,4-benzoquinones was as follows, and the method is better than that of previous procedures (5). To a solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone in acetic acid was added, in portions, the appropriate diacyl peroxide with stirring at 90-95° C for 1-2 hours under nitrogen. The reaction mixture was heated for another 10-20 hours. The solution was concentrated, in vacuo, to dryness, and residue was subjected to silica gel column chromatography. The collected orange fraction was further purified on preparative thin layer plates to give a pure product. These 2,3-dimethoxy-5-methyl-6-alkyl-1,4-benzoquinones were dark orange oils, and the yields were 40-50%. The elemental analyses were carried out by Chemalytics, Inc., Tempe, Arizona, and all values were within 0.4% of the calculated values. The composition of the 6-pentadecyl derivative agreed with 0.04% of the theoretical values. The synthesis of 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (DB) is described in detail.

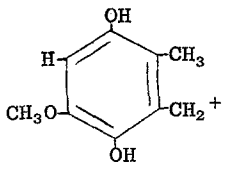
2,3-Dimethoxy-5-methyl-6-decyl-1,4-benzoquinone. - 2,3-Dimethoxy-5-methyl-1,4-benzoquinone (1.82 g., 10 mmoles) was dissolved in 50 ml glacial acetic acid, and the mixture was heated to 90-95° C under nitrogen. Then, a mixture of diundecanoyl peroxide (7.4 g., ~ 20 moles) in 5 ml of acetic acid was added dropwise over a 2-hour period. The reaction mixture was allowed to stir for another 20

hours at 90-95° C, and then evaporated to dryness in vacuo. The residue was subjected to a silica gel column and eluted with a mixture of hexane and chloroform. The collected orange fraction was concentrated and further purified on preparative thin layer plates developed with a mixture of chloroform: hexane: ether (10:10:1) to give a total of 1.4 g of pure product; yield, 43%.

Identity by Mass Spectometry and NMR Spectra. - The mass spectra of the analogs were obtained at 70 eV using a CEC-21-491 mass spectrometer. Molecular ions at m/e 252, 322, and 392 for the three analogs, were the largest peaks in the high-mass portion of the spectra. The next highest-mass peak of prominence in each spectrum appeared at m/e 197, corresponding to tropylium ion VII, which is a characteristic fragment in the mass spectra of coenzyme Q and analogs (6). A second characteristic peak which appears in the mass spectra at m/e 167 corresponds to the monomethoxyl tropylium ion VIII, which is produced by demethoxylation of analogs of coenzyme Q under the conditions required to obtain mass spectra (7).



VII



VIII

Nmr spectra (A-60) of the analogs were entirely consistent with the structural assignments. The solvent and internal standard were $CDCl_3$ and TMS, respectively. Resonance of the methoxyl hydrogens gives rise to a singlet at δ 4.0 ppm, and the methyl group which is bonded to the ring carbon atom gives rise to a resonance at δ 2.0. The alkyl group resonance signals appear at δ 2.5 (ring-bound CH_2), ca. 1.3 ($(CH_2)n$), and 0.9 (CH_3).

Purity by TLC. - Purity of the analogs was established by chromatographic homogeneity; the Rf's which are observed for tlc on silica gel plates (using 10:10:1 chloroform: hexane: ether for development) are 0.44, 0.46, and 0.50 for the pentyl-, decyl-, and pentadecyl-compounds, respectively.

RESULTS AND DISCUSSION

Organic Synthesis. - The syntheses of the 2,3-dimethoxy-5-methyl-6-pentyl-, -6-decyl- and -6-pentadecyl-1,4-benzoquinones were accomplished by treating the appropriate diacyl peroxide with 2,3-dimethoxy-5-methyl-1,4-benzoquinone in acetic acid. The di-n-hexanoyl, di-n-undecanoyl, and di-n-hexadecanoyl peroxides were prepared from the corresponding acids. The appropriate acids were converted to acid chlorides by reacting with thionyl chloride, and the diacyl peroxides were obtained by treating the acid chlorides with hydrogen peroxide

TABLE I ASSAY OF CO Q AND ANALOGS FOR OXIDATIVE PHOSPHORYLATION AT SITE I

CoQ or Analogs	NADH Reduction Rate ----	with Rotenone	Inhibition	Δ NADH	G6P	P/NADH
	μmoles/min/mg	μmoles/min/mg	%			
Q_1	0.45	0.03	93	0.088	0.046	0.52
Q_2	0.25	0.02	92	0.079	0.045	0.57
PB*	0.47	0.02	95	0.082	0.036	0.44
DB**	0.52	0	100	0.085	0.039	0.46

Assays were performed with 200 μg of ETPH particles as described by Schatz and Racker (10). The concentration of the CoQ compounds was 0.12 mM. Rotenone was added to provide a final concentration of 2 μM.

*2,3-dimethoxy-5-methyl-6-pentyl-1,4-benzoquinone.

**2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone.

TABLE II REDUCTION OF CYTOCHROME c

BY REDUCED CO Q AND ANALOGS CATALYZED BY COMPLEX III

Reduced CoQ or Analog	Namomoles Cytochrome c Reduced per Minute Without Complex III	With Complex III	With Complex III and Antimycin A
Q_1	0.6	13.0	0.6 (100)
Q_2	0.2	27.6	0.7 (98)
PB	0.2	14.8	0.2 (100)
DB	0.2	29.2	0.8 (98)

Reduction of cytochrome c was measured at 550-540 nm using a reduced minus oxidized extinction coefficient of 19 mM^{-1}. The reaction mixture contained in 1.0 ml: 25 mM KP_i, pH 7.5; 0.05 mM EDTA; 8 μM cytochrome c; 10 μM reduced CoQ or analog and 2 μg complex III (11). When indicated, antimycin A was added to the assay medium at 1 μg/ml. The percent inhibition by antimycin is shown in parenthesis.

and pyridine (8,9). The three analogs were characterized in terms of identity and purity by tlc, microanalytical data, and nmr and mass spectral data.

Biochemical Assay. - 2,3-Dimethoxy-5-methyl-6-n-pentyl-1,4-benzoquinone (PB) and the corresponding 6-n-decyl-derivative (DB) were as biochemically effective as CoQ_1 in the systems used, as indicated in Table I. The corresponding 6-n-pentadecyl-derivative (PDB) was not suitable or as effective, because of its lower solubility in water. In this assay system, CoQ_{10} is also too insoluble and even the somewhat more soluble CoQ_6 is not adequate.

One may observe that any biochemical assay system, in vitro, for research

on respiration, in which the intrinsic role of coenzyme Q is important, must necessarily be responsive to forms and analogs of coenzyme Q which are sufficiently lipoidal in nature. The lipoidal nature of the analog permits simulation of the intrinsic coenzyme Q for both coenzymatic activity and for inhibition. However, a form or analog of coenzyme Q with negligible or minimal lipoidal character, and an assay system which is not responsive to the lipoidal analogs and forms of CoQ, can give very useful biochemical information. Such information may or may not bear directly on the intrinsic role of the dominant form of coenzyme Q in the given species, i.e., CoQ_6 or Q_7 in yeast, CoQ_8 in E. coli., CoQ_{10} in mammalian mitochondria, etc.

The apparent greater stability of PB and DB and the observation that they serve as well as CoQ_1 in this assay for oxidative phosphorylation at site I, document their utility. Also, after reduction and extraction, both PB and DB were excellent substrates in the assay with complex III. It can be seen from Table II that both gave very low blank reduction of cytochrome c and reacted as well as CoQ_1 and CoQ_2 with complex III. Again, this system of complex III was virtually unresponsive to the 6-n-pentadecyl derivative, and there was no inhibition, and doubtless because of the increased lipoidal nature of this 15-carbon (side chain) analog in comparison with that of the 5- and 10- carbon analogs, PB and DB, respectively. It could be misleading to consider the pentadecyl-derivative "inactive" in this system of complex III, because the analog is as potentially functional in redox reactions as are the pentyl- and decyl- derivatives. Such "inactivity" is really due to the incompatibility of the assay system with the more lipoidal analog.

A grant from the Robert A. Welch Foundation provided equipment used in this research, and a United States Public Health Service Grant CA-08964 (E.R.) from the National Cancer Institute are gratefully acknowledged.

1. Daves, Jr., G.D., Muraca, R.F., Whittick, J.S., Friis, P., and Folkers, K., Biochemistry, 6, 2861 (1967).
2. Friis, P., Daves, Jr., G.D., and Folkers, K., Biochem.Biophys. Res. Commun., 24, 252 (1966).
3. Misiti, D., Moore, H.W., and Folkers, K., J. Amer. Chem. Soc., 87, 1402 (1965).
4. Ramasarma, T. and Lester, R.L., J. Biol. Chem., 235, 3309 (1960); Hendlin, D. and Cook, T.M., ibid., 235, 1187 (1960); Crane, F.L. and Ehrlich, B., Arch. Biochem. Biophys., 89, 134 (1960).
5. Caltin, J.C., Daves, Jr., G.D., and Folkers, K., J. Med. Chem., 14, 45 (1971).
6. Muraca, R.F., Whittick, J.S., Daves, Jr., G.D., Friis, P., and Folkers, K., J. Amer. Chem. Soc., 89, 1505 (1967).
7. Farley, T.M., Daves, Jr., G.D., and Folkers, K., J. Org. Chem., 33, 905 (1968).
8. Cason, J., Org. Syn., Coll. Vol., 3, 169 (1955).
9. Silbert, L.S., and Swern, D., J. Amer. Chem. Soc., 81, 2364 (1959).
10. Schatz, G., and Racker, E., J. Biol. Chem., 241, 1429 (1966).
11. Rieske, J.S., Methods in Enzymology, 10, 239 (1967).