ORGANIC STRUCTURAL SPECIFICITY OF COENZYME Q FOR BIOLOGICAL ACTIVITY*

K. Folkers and H. W. Moore+

Stanford Research Institute, Menlo Park, California

and

G. Lenaz and L. Szarkowska

Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin

Received March 11, 1966

The biological activity of coenzyme Q has certain structural specificity in the succinoxidase and NADH-oxidase systems; the variations in structure which permit retention of activity are limited. Notably, vitamins E and K are inactive in these two systems. The biological activity of coenzyme Q is restricted to compounds having the oxygen and lipidic functionalities of the CoQ group, according to our studies, and the *in vitro* assays in the succinoxidase system have been consistent in terms of relative activity for upwards of seven years. Ozawa *et al.* (1965) have reported coenzymatic activity like that of coenzyme Q for compounds which are completely unrelated in structure and it appears that their system is not equivalent to ours.

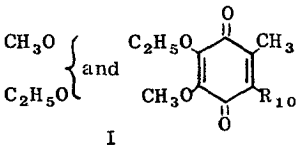
Green and Lester (1959) reported early tests which revealed a gross structural specificity and an absolute requirement for CoQ in the succinic-oxygen and the succinic-cytochrome *c* systems; others (Crane, 1960; Ambe and Crane, 1960; Crane *et al.*, 1959) also reported related tests. The low activity of the monoethoxy and diethoxy homologs of CoQ_{10}, I and II, respectively, and the inactivity of the 6-chromanol of hexahydrocoenzyme Q_4, III, and of coenzyme Q_{10}, IV, were reported (Hendlin and Cook, 1960; Shunk *et al.*, 1960; Hoffman *et al.*, 1960).

---

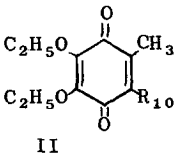
*Coenzyme Q. LXVII.

+Present address: Department of Chemistry, University of California, Irvine.

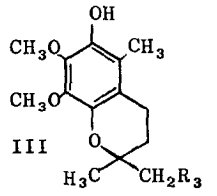
It has been recently discovered by Szarkowska (1966) that when mitochondria are lyophilized and extracted with pentane, the NADH-oxidase activity is not irreversibly lost as it is when mitochondrial preparations are extracted with acetone, and with the pentane-extracted system it was possible to show that the enzymatic activity could be restored by the addition of CoQ.



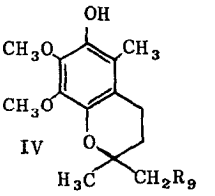
I



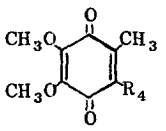
II



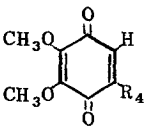
III



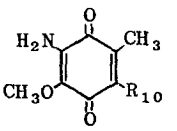
IV



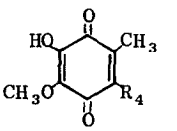
V



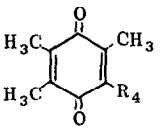
VI



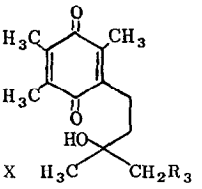
VII



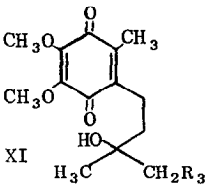
VIII



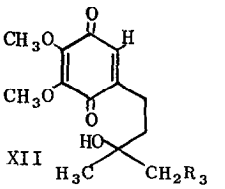
IX



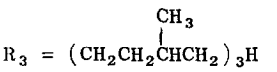
X

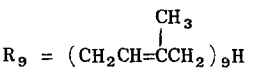


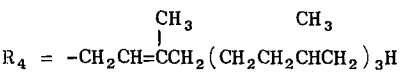
XI

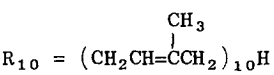


XII









The availability of the animal data on the biological response of the rabbit (Wagner et al., 1964; Smith et al., 1965) and the monkey (Fitch et al., 1965) to both CoQ and vitamin E made desirable a reexamination of the chemical specificity of CoQ for coenzymatic activity, particularly in respect to some structural features of the molecule which had not been previously examined for retention or loss of activity. New data on the restoration of succinoxidase and NADH-oxidase activities are summarized in Table I.

Although it had been known previously (Crane, 1959) that the length of the isoprenoid side chain of CoQ can be decreased with retention of coenzymatic

activity, it is now evident that (1) alteration of the trans geometry of the

Table I

RESTORATION OF SUCCINOXIDASE AND NADH-OXIDASE ACTIVITIES

Compound	Restoration of Succinoxidase Activity % of CoQ_{10} Activity	Restoration of NADH-Oxidase Activity % of CoQ_{10} Activity
Coenzyme Q_2	-	100
Coenzyme Q_{10}	100	100
Coenzyme Q_{10} (cis-trans side chain)[a]	100	-
Rhodoquinone (natural)	5-10	-
2-Methoxy-3-hydroxy-5-methyl-6-phytyl-1,4-benzoquinone	(inhibition)	0
(γ-Hydroxy-5-desmethyl-hexahydrocoenzyme Q_4)[b]	0	0
(γ-Hydroxyhexahydrocoenzyme Q_4)[b]	0	0
α-Tocopherylquinone	0	0
Vitamin E	-	0
2,3,5-Trimethyl-6-phytyl-1,4-benzoquinone	0	-
Hexahydrocoenzyme Q_4	100	-
2,3-Dimethoxy-5-phytyl-1,4-benzoquinone	100	-
Vitamin K_1	-	0
α-Carotene	-	0
β-Carotene	-	0

[a] Kindly prepared by Dr. Morton A. Golub of Stanford Research Institute by SO_2-catalyzed isomerization of the side chain of CoQ_{10}.
[b] "γ-Hydroxy" signifies that the isoprenoid side chain is 3'-hydroxy-3',7',11',15'-tetramethylhexadecyl.

10-unit isoprenoid side chain of CoQ_{10} retains activity; (2) decreasing the isoprenoid side chain to two units retains full activity for NADH-oxidase; and (3) hexahydrocoenzyme Q_4, V, with a side chain which is both shorter and more saturated than CoQ_{10} is fully as active as CoQ_{10} in the succinoxidase system. Because of its biological activity, chemical stability, and ease of adminis-

tration, hexahydrocoenzyme Q_4 has been extensively studied in rabbits and monkeys.

The 5-desmethyl analog of hexahydrocoenzyme Q_4, V (Moore et al., 1964), is comparable in activity to hexahydrocoenzyme Q_4. Rhodoquinone, newly proven to have structure VI (Moore and Folkers, 1966) and previously shown (Glover and Threlfall, 1962) to be produced by Rhodospirillum rubrum, apparently has a low order of activity, such as 5-10% that of CoQ_{10}; this amino analog of CoQ_{10} may have a unique coenzymatic function for the metabolism of this photosynthetic organism.

2-Methoxy-3-hydroxy-5-methyl-6-phytyl-1,4-benzoquinone (the phytyl derivative of fumigatin), VIII (Shunk et al., 1966), differs structurally from hexadrocoenzyme Q_4, V, in that V has a 3-methoxy group and VIII has a 3-hydroxy group. This fumigatin derivative appears to have some inhibitory activity since the rate of succinate oxidation was lower when it was added to the system than in the controls without supplementation; however, it appeared to be inert in the NADH-oxidase system.

Chemical or enzymic oxidation of a 6-chromanol (of CoQ, α-tocopherol) gives an oxidation product having a tertiary hydroxy group in the isoprenoid side chain. For interpreting the in vivo activity of the 6-chromanol of hexahydrocoenzyme Q_4 in rabbits and monkeys, it was necessary to know the coenzymatic activity of the tertiary alcohol, XI, and for other reasons that of the corresponding desmethyl analog, XII. Both of these compounds with the tertiary hydroxy group are inactive in the succinoxidase system, although the corresponding quinones without the tertiary hydroxy group (i.e., the dehydration products) are fully active. Consequently, the in vivo activity of the 6-chromanol of hexahydrocoenzyme Q_4 cannot be ascribed to its oxidation product, XI. Quinones with isoprenoid side chains having tertiary hydroxy groups are now well recognized to occur in nature, and α-tocopherylquinone, X, has been reported from animal tissue (Csallany et al., 1962).

Since the exchange of the two methoxy groups in hexahydrocoenzyme Q_4, V,

for two methyl groups as in 2,3,5-trimethyl-6-phytyl-1,4-benzoquinone, IX (the quinone corresponding to α-tocopherol), leads to complete loss of activity, it is to be expected that the hydration product (which is α-tocopherylquinone) would be inactive.

It is remarkable that the presence of a tertiary hydroxy group in the side chain completely inactivates the quinone for coenzymatic activity. It seems likely that the functionality of the quinone for electron transfer would be only slightly modified by the distant tertiary hydroxy group in the side chain. Perhaps the biological inactivation is due to a difference in the geometry and/or the lipidic characteristics of the substance when the tertiary hydroxy group is present so that the molecule can no longer occupy a juxtaposition with overlapping orbitals in the macromolecular structure for electron transfer.

Succinoxidase activity in mitochondria, extracted with 90% acetone, was measured as described (Lester and Fleischer, 1961) in standard manometric assays at 30° for 20-30 min, depending on the activity of the different enzyme preparations. Each flask contained a total volume of 3 ml: Tris-HCl, pH 7.5, 33 mM; sucrose 166 mM; cytochrome c 66 μg/ml; asolectin (soybean phospholipids) about 0.33 mg/ml, or mitochondrial phospholipids 0.25 mg/ml; acetone-extracted mitochondria 1 mg of protein per flask. The reaction was started by addition of 150 μmoles of succinate from the side arm. The basal rate without CoQ or addition of analogs was 0.05-0.10 μatoms O_2/min/mg of protein. The rate after addition of CoQ_{10} ranged between 0.3 and 0.8 μatoms/min/mg of protein, depending on the mitochondrial preparations which were used.

NADH-oxidase activity was measured (Lester and Fleischer, 1961) by standard manometry at 30°. The final concentrations of the reactants in the Warburg flask were: Tris-HCl, pH 7.5, 33 mM; sucrose 166 mM, EDTA 0.027 mM; lyophilized mitochondria extracted with pentane 0.25 mg of protein/ml; cytochrome c 33 μg/ml; mitochondrial phospholipids 342 μg/ml. The reaction was started by addition of 4 mM NADH from the side arm. The basal oxidation rate without CoQ or analogs was 0.02-0.05 μatoms O_2/min/mg of protein. The oxidation rate after

addition of CoQ_{10} ranged between 0.5 and 1.0 μatoms/min/mg of protein.

References

Ambe, K. S., and Crane, F. L., Biochim. Biophys. Acta 43, 30 (1960).

Crane, F. L., Arch. Biochem. Biophys. 87, 198 (1960).

Crane, F. L., Shunk, C. H., Robinson, F. M., and Folkers, K., Proc. Soc. Exptl. Biol. Med. 100, 597 (1959).

Csallany, A. S., Draper, H. H., and Shah, S. N., Arch. Biochim. Biophys. 98, 142 (1962).

Fitch, C. D., Dinning, J. S., Porter, F. S., Folkers, K., Moore, H. W., and Smith, J. L., Arch. Biochem. Biophys. 112, 488 (1965).

Glover, J., and Threlfall, D. R., Biochem. J. 85, 14P (1962).

Green, D. E., and Lester, R. L., Federation Proc. 18, 987 (1959).

Hendlin, D., and Cook, Th. M., J. Biol. Chem. 235, 1187 (1960).

Hoffman, C. H., Trenner, N. R., Wolf, D. E., and Folkers, K., J. Am. Chem. Soc. 82, 4744 (1960).

Lester, R. L., and Fleischer, S., Biochim. Biophys. Acta 47, 358 (1961).

Moore, H. W., Schwab, D. E., and Folkers, K., Biochemistry 3, 1586 (1964).

Moore, H. W., and Folkers, K., J. Am. Chem. Soc. 88, 567 (1966).

Ozawa, H., Natori, S., and Momose, K., Chem. Pharm. Bull. (Tokyo) 13, 1029 (1965)

Shunk, C. H., Trenner, N. R., Hoffman, C. H., Wolf, D. E., and Folkers, K., Biochem. Biophys. Res. Commun. 2, 427 (1960).

Shunk, C. H., McPherson, J. F., and Folkers, K., J. Org. Chem., in press (1966).

Smith, J. L., Moore, H. W., and Folkers, K., Proc. Soc. Exptl. Biol. Med. 118, 782 (1965).

Szarkowska, L. Arch. Biochem. Biophys., in press (1966).

Wagner, A. F. Stopkie, R. J., and Folkers, K., Arch. Biochem. Biophys. 107, 184 (1964).