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STUDIES ON THE ELECTRON TRANSPORT SYSTEM

XXVI. SPECIFICITY OF COENZYME Q AND COENZYME Q DERIVATIVES

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SUMMARY

In succinic dehydrogenase complex there are three alternative pathways by which quinones mediate the oxidation of succinate by cytochrome *c* or oxygen. The first, mediated by menadione, 2,3-dimethoxy, 5-methyl benzoquinone and the short chain coenzyme Q analogues terminates at external cytochrome *c* and is antimycin insensitive. The second, mediated only by lipophilic homologues of coenzyme Q (Q_2, Q_3, Q_6, Q_{10}) terminates at external cytochrome *c* and is antimycin sensitive. The third, mediated by coenzymes Q_2, Q_3, Q_6, Q_{10} and in part by Q_0, Q_1, plastoquinone and heptyl and heptadecyl coenzyme Q terminates at molecular oxygen, involves bound cytochrome *c* and is antimycin sensitive. There is a close correlation between the effects of Q analogues on the succinoxidase activity of acetone extracted SDC and the effects on the succinoxidase activity of acetone extracted electron transport particle ETP. This suggests that the reconstituted succinoxidase system of succinic dehydrogenase complex closely resembles that of electron transport particle from which it was derived. Succinic cytochrome *c* reductase activity of succinic dehydrogenase complex, in principle, is a measure of the segment of the electron transfer chain which has been detached from the cytochrome *a* terminus of the chain. This segment by virtue of changes induced by the cleavage process shows a new pattern of quinone specificity. Only derivatives of 2,3-dimethoxy, 5-methyl benzoquinone with two or more isoprenoid units in the side chain at carbon 6 are able to serve as mediators in the antimycin sensitive oxidation of succinate by cytochrome *c*. The antimycin insensitive pathway involving cytochrome *c* *via* other quinones involves transfer of electrons from a site in the electron transfer chain prior to the locus for antimycin action.

Abbreviations: ETP, electron transport particle; SDC, succinic dehydrogenase complex.

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INTRODUCTION

In a previous communication of this series[1] it has been shown that coenzyme Q can be extracted by isooctane from particles with electron transport activity and that the loss of coenzyme is related to the loss of succinoxidase activity. The present communication deals with the specificity of coenzyme Q and homologues of coenzyme Q in the restoration of the activity of such solvent-extracted particles.

Cytochrome *c* reductase activity is generally assumed to be a measure of a segment of the same electron transfer chain which is involved in the oxidation of substrate by oxygen. The studies of GREEN AND MACKLER[2] in this laboratory were the first to cast doubt on this assumption. They showed that particles capable of oxidizing DPNH by oxygen did not interact with external cytochrome *c* and that the bound form of cytochrome *c* was not identical with the classical, water soluble form of cytochrome *c*. Later it was demonstrated that the ETP could be prepared in a form which has succinoxidase activity but no cytochrome *c* reductase activity[3]. The recognition of lipide cytochrome *c*[4,5] as the bound form of cytochrome *c* in ETP defined more precisely the basis for this divergence. Studies reported below on the reactivation of succinic cytochrome *c* reductase and succinoxidase activity by coenzyme Q show: (a) That the requirements for restoration of these two activities are different; (b) that cytochrome *c* reduced external to the particle cannot penetrate or penetrates too slowly to the site in the particle where oxidation by molecular oxygen in the terminal segment of the chain takes place; (c) that antimycin sensitivity of electron transport is closely related to the lipophilic nature of the coenzyme quinone which precedes cytochrome *c* in the chain; and (d) only 2,3-dimethoxy-5-methyl benzoquinones with one or more isoprenoid units in the 6 position can serve as a coenzyme in this pathway.

EXPERIMENTAL PROCEDURE

Enzyme preparations

Three enzyme preparations from beef-heart mitochondria were employed to determine the location of coenzyme Q in the electron transport system. The ETP which catalyzes the overall oxidation of succinate and DPNH by molecular oxygen without addition of cytochrome *c* was prepared by alkaline extraction of mitochondria[3]. This particle contains lipide cytochrome *c*, but does not interact with aqueous cytochrome *c* as determined by the fact that it shows very little cytochrome *c* reductase or reduced cytochrome *c* oxidase activity unless it is treated with reagents which remove lipide cytochrome *c*. The residue or insoluble fraction which is removed prior to the sedimentation of ETP has been referred to as the ETP-residue. This particle also catalyzes the oxidation of succinate and DPNH by molecular oxygen, but is inactive without the addition of cytochrome *c*. The electron transport system in this particle has been described as an "open" system in that it is readily capable of interaction with external cytochrome *c* and without any pretreatment shows succinic and DPNH cytochrome *c* reductase activity as well as reduced cytochrome *c* oxidase activity. The ETP-residue obtained during separation of ETP from mitochondria by alkaline treatment was used in these experiments.

When ETP is treated with amyl alcohol or deoxycholate a red particle is formed which has succinic cytochrome *c* reductase activity. This type of particle is designated

as SDC[6,7]. The SDC particles prepared by either method are similar in their basic properties, but differ in some details. In the experiments reported here SDC was prepared by deoxycholate treatment of ETP. This preparation which has previously been referred to as the "red fraction"[6] contains a small amount of cytochrome *a* and when supplemented with cytochrome *c* it catalyzes the oxidation of succinate by oxygen. We have used this type of preparation to compare the effect of solvent extraction on succinic cytochrome *c* reductase and succinoxidase activity.

Extraction procedures

Two extraction procedures were used to remove coenzyme Q from the particles, and a number of polar and non-polar solvents were tried. The first procedure (wet extraction) involved extraction of the particles suspended in 0.25 *M* sucrose solution while the second involved extraction of dry lyophilized enzyme preparations with the solvent (dry extraction). Following extraction, as much solvent as possible must be removed from the particles to prevent solvent inhibition of electron transport. We find that exposure of the suspension to a vacuum for 0.5 h at room temperature, followed by centrifugation of the extracted particles diluted in sucrose solution, removes sufficient solvent to rule out solvent inhibition effects.

In the wet extraction, the sucrose suspension of the enzyme preparation (15–20 mg protein/ml) was shaken in a glass stoppered tube with four volumes of the solvent at room temperature (25°). Shaking was carried out on a reciprocal shaking machine at 300 strokes/min with stroke length of 30 cm. After the necessary time of shaking, the mixture was spun in a clinical centrifuge and separated into three phases. The particulate phase was then separated, exposed to vacuum for about 30 min and resuspended in fresh sucrose solution. The suspension was then homogenized and centrifuged at 40,000 RPM for 30 min. The sediment was taken up in sucrose solution and the homogenized suspension was then used directly.

The details of extraction of ETP with isooctane have been described previously[1,8]. We have followed essentially the same method except that Versene and acetate buffer were omitted during extraction of SDC since activity is irreversibly lost when these reagents are present.

In the dry extraction, 150 mg of enzyme powder were mixed with 15 ml solvent, and the suspension was shaken for 30 to 90 min on the reciprocal shaker as described above. The particulate material was then separated by centrifugation and the solvent decanted. Excess solvent was removed at room temperature under vacuum. The particles were washed in sucrose by high speed centrifugation and resuspended in sucrose for activity determination. The lyophilized powder of ETP or SDC was resuspended in a requisite amount of distilled water to achieve the sucrose concentration of the original suspension prior to lyophilization. These suspensions served as controls for the effect of solvent extractions.

Homologues and analogues of coenzyme Q

Coenzyme Q_{10} was isolated from heart muscle by the method of CRANE *et al.*[9] and coenzyme Q_6 by a modification* of the same procedure followed for coenzyme

* Coenzyme Q_6 was prepared by saponification of bakers yeast and isolation from the non-saponifiable fraction by the method used for coenzyme Q_{10} from beef heart. Preliminary crystallization was effected from ethanol–methanol (1:1, v/v) followed by crystallization from ethanol at —15°.

Q_{10}. CRANE[10] has described the method of isolation of plastoquinone (Q_{254}) from alfalfa meal. The following synthetic homologues and analogues of coenzyme Q were made available to us through the courtesy of Dr. K. FOLKERS of the Merck Sharp and Dohme Laboratories in Rahway, New Jersey: coenzymes Q_0* (aurantiogliocladin), Q_1, Q_2, Q_3; heptyl, phytyl and heptadecyl coenzyme Q; eicosahydrocoenzyme Q_{10}; coenzyme Q_{10} diacetate; 2,3-dimethoxy, 5-methylbenzoquinone, and various other substituted benzoquinones. The methods used for the synthesis of some of these compounds are described by WOLF *et al.*[11].

Chemicals

Cytochrome *c* was obtained from Sigma Chemical Laboratories; menadione, Vitamin K_2 from mycobacteria was a generous gift of Dr. H. NOLL, University of Pittsburgh. Synthetic vitamin K_2 derivatives containing side chains with varying number of isoprenoid units were obtained through the courtesy of Dr. A. BRODIE, Harvard University, and Prof. L. FIESER of Harvard University kindly provided several naphthoquinones. The acetone used for extraction purposes was an analytical grade supplied by Fischer Scientific Co.

Methods of assay

The assay of enzyme activity was carried out essentially by the methods which we have previously described[3].

Succinoxidase activity was determined manometrically in the Warburg apparatus. The reaction mixture contained 100 μmoles of succinate, 40 μmoles of potassium phosphate pH 7.4, 1.3 μmoles of sodium Versenate, and approx. 1 mg of enzyme in a volume of 1.3 ml; 0.2 ml of 6 *N* KOH was added to the center well. Quinones and other lipid supplements were added in 0.05 ml of ethanol and ethanol was added to the control flasks.

DPNH oxidase activity was determined spectrophotometrically. The reaction mixture contained 0.08 mg of DPNH and 80 μM of potassium phosphate pH 7.4 in a total volume of 1.0 ml, sufficient enzyme was added to give a change in absorbancy from 0.02 to 0.10/min.

Succinate cytochrome *c* reductase was determined spectrophotometrically by decrease in O.D. at 550 mμ. The reaction mixture contained 80 μM of potassium phosphate pH 7.4, 100 μM of potassium succinate pH 7.4, 0.4 μM of potassium cyanide and 0.2 mg of cytochrome *c* in a total volume of 1.0 ml. Sufficient enzyme was used to give a change in absorbancy of from 0.02 to 0.1/min.

RESULTS

Restoration of succinic-cytochrome c *reductase activity of SDC*

When the lyophilized powders of the various particles are extracted for 30 min or more with acetone, some 80% of the coenzyme Q content is removed and the addition of coenzyme Q alone suffices to restore cytochrome *c* reductase activity. Both coenzyme Q and cytochrome *c* are required for restoration of succinoxidase

* The subscript in each case indicates the number of monounsaturated isoprenoid units in the side chain at position 6. Other substitutions at position 6 are described by naming the side chain e.g. heptyl coenzyme Q with a heptyl side chain replacing the isoprenoid side chain.

activity. When the extraction period is extended beyond 30 min an additional lipide factor is required for succinoxidase activity. This factor has been mentioned previously[12] and will be discussed more extensively in a later communication. Suspensions prepared from lyophilized SDC which has been extracted with acetone for 90 min no longer catalyze the reduction of cytochrome *c* by succinate. This activity can be restored by homologues and analogues of coenzyme Q to the degree indicated in Table I. The positive results obtained with heptyl and phytyl coenzyme Q and eicosahydrocoenzyme Q_{10} suggest that the degree of unsaturation in the side chain is not critical for activity although it has a definite influence.

TABLE I

RESTORATION OF SUCCINIC CYTOCHROME *c* REDUCTASE AND SUCCINOXIDASE ACTIVITIES OF SDC* BY COENZYME Q AND RELATED QUINONES

*Compound added***	*Mg added*	*Succinic cytochrome c reductase***		*Succinoxidase*	
		No antimycin	*Antimycin§ added*	*No antimycin*	*Antimycin added*
None		0.00	0.00	0.16	0.00
Q_0	0.05	0.22	0.24	0.32	0.09
Q_1	0.05	0.31	0.25	0.62	0.05
Q_2	0.05	0.37	0.09	0.72	0.00
Q_3	0.05	0.42	0.00	0.78	0.00
Q_6	0.05	0.55	0.00	0.68	0.00
Q_{10}	0.10	0.61	0.00	0.50	0.00
Heptyl Q	0.05	0.31	0.31	0.29	0.00
Heptadecyl Q	0.05	0.09	0.00	0.37	0.00
Phytyl Q	0.05	0.35	0.05	0.44	0.00
2,3-dimethoxy, 5-methyl benzoquinone	0.05	0.15	0.18	0.00	0.00
Menadione	0.05	0.18	0.18	0.36	0.13
Vitamin K_1	0.05	0.00	0.00	0.00	0.00
2,3-dimethyl-1,4-naphthoquinone	0.05	0.00	0.00	0.00	0.00
Plastoquinone	0.10	0.00	0.00	0.24	0.00

* Lyophilized SDC (specific activity before extraction: cytochrome *c* reductase, 0.6; succinoxidase, 0.80) extracted with acetone for 90 min and processed as described.

** All activities in presence of added cytochrome *c*; concentration of supplement/mg protein: cytochrome *c*, 1.0 mg; Q_{10} and Q_{254}, 0.1 mg; other compounds 0.05 mg each.

*** All compounds reported at levels which gave optimum activity; range of concentration tested 10–50 μg; the inactive compounds were tested in the range 0.01–0.10 mg, numericals suffixed to Q denote the number of isoprenoid units in the side chain at 6 position as homologue of coenzyme Q. Plastoquinone (Q_{254}) is a quinone common to green plants and was prepared from alfalfa.

§ Antimycin, wherever inhibitory, was active even at 1 μg/100 μg enzyme protein, range tested 1–10 μg/100 μg protein.

The activity induced by the higher coenzyme Q homologues (Q_3–Q_{10}) and by heptadecyl coenzyme Q is completely antimycin sensitive whereas that of the following homologues and analogues is sensitive only in part: Coenzyme Q_1 (20% sensitive); coenzyme Q_2 (75% sensitive); and phytyl coenzyme Q (85% sensitive). The activity induced by coenzyme Q_0, menadione and 2,3-dimethoxy, 5-methyl-benzoquinone is completely insensitive to antimycin.

Table I also shows that coenzyme Q cannot be replaced by plastoquinone (Q_{254}) and vitamin K_1; α-tocopherol, beef serum albumin, mitochondrial carotene, phospho-

lipides and cholesterol do not restore activity. The diacetate of reduced coenzyme Q_{10} is also inactive whereas the reduced form of coenzyme Q_{10} has the same activity as the oxidized form. The Q lipoprotein[13] which contains coenzyme Q in an available form is fully as active as free coenzyme Q_{10}. The amount of Q required for maximal restoration is, however, less when the coenzyme is added in the form of the lipoprotein. The acetone extract of lyophilized SDC after removal of material insoluble in cold alcohol has comparable activity to that of coenzyme Q_{10} when compared at the same concentration level of coenzyme Q_{10}. The crude acetone extract contains inhibitory substances which obscure the restoration effect.

The increase in cytochrome *c* reductase activity is a function of coenzyme Q_{10} concentration. When the ratio of SDC to coenzyme Q_{10} (mg protein/μg of coenzyme) is 1.7, the system is half saturated. At saturation the ratio is 0.8.

The residue fraction which is a byproduct in the isolation of ETP[3] (referred to as ETP residue) has considerable succinic-cytochrome *c* reductase activity. Since this particle has an open electron transfer chain compared to the closed chain of ETP[2,3] it was of interest to determine the requirements for the cytochrome *c* reductase activity of the ETP residue. The results in Table II show that the activity is largely lost following extraction of the lyophilized particles with acetone, and that coenzyme Q can restore the lost activity, whereas α-tocopherol, vitamin K_1 or albumin are inactive in this respect. The pattern for the ETP residue closely resembles that for SDC.

TABLE II

RESTORATION OF CYTOCHROME *c* REDUCTASE ACTIVITY OF ETP-RESIDUE

State of particle	*Additions*	*Succinic cytochrome c reductase activity μM succinate/min/mg protein*
Original (fresh)	None	0.21
Lyophilized	None	0.26
Lyophilized	CoQ	0.25
Extracted*	None	0.06
Extracted	CoQ, 12 μg	0.16
Extracted	CoQ, 25 μg	0.29
Extracted	Acetone extract (20 μg CoQ)	0.28
Extracted	*d*-α-tocopherol, 25 μg	0.06
Extracted	Vitamin K_1, 25 μg	0.05
Extracted	Beef serum albumin, 0.1–1.0 mg	0.08

* The ETP-residue was extracted for 60 min with acetone and processed as described in the text.

Restoration of succinoxidase activity of SDC

During acetone extraction of SDC, succinic-cytochrome *c* reductase activity is the first to disappear and then later succinoxidase activity. Providing the period of extraction is not excessive only two components are required for restoration of succinoxidase activity viz. coenzyme Q and cytochrome *c*. The various homologues and analogues of coenzyme Q which can restore succinoxidase activity are listed in Table I. These include some homologues of coenzyme Q from Q_0 to Q_{10}, heptyl, heptadecyl and phytyl coenzyme Q. Coenzyme Q_3 is the most active of the compounds tested. The activity induced by all but two of the compounds mentioned is antimycin

sensitive. The activity in presence of aurantiogliocladin and coenzyme Q_1 is respectively 72 % and 92 % inhibited by antimycin.

Heptyl coenzyme Q which induces an antimycin insensitive cytochrome *c* reductase activity, by contrast gives rise to succinoxidase activity which is completely antimycin sensitive. The menadione-induced oxidase activity is atypical in two respects: (a) It is insensitive to cyanide; and (b) does not require the mediation of cytochrome *c* (F. L. CRANE AND K. S. AMBE, unpublished data). Activity induced by coenzyme Q derivatives is both cyanide-sensitive and dependent upon the presence of externally added cytochrome *c*. Plastoquinone is inactive in the reductase system but shows appreciable activity in the oxidase system when cytochrome *c* is present. 2,3-dimethoxy, 5-methyl benzoquinone not only is inactive in the oxidase assay but it also inhibits the catalytic effect of coenzyme Q_{10}.

Restoration of succinoxidase activity of ETP

The degree of specificity of coenzyme Q compounds in restoring the succinoxidase activity of acetone extracted ETP is higher than in the restoration of the activity of acetone extracted SDC. Both aurantiogliocladin (Q_0) and menadione induce succinoxidase activity in the extracted SDC which to a limited extent is antimycin insensitive (*cf.* Table I and III). When added to extracted ETP, however, these compounds restore little activity and whatever activity reappears is entirely antimycin sensitive. In other respects the specificity of coenzyme Q compounds for restoration of succinoxidase activity in acetone extracted ETP is the same as observed in SDC,

TABLE III

RESTORATION OF SUCCINOXIDASE ACTIVITY OF ETP BY HOMOLOGUES AND ANALOGUES OF COENZYME Q

State of particle	*Coenzyme added*	*Succinoxidase activity* μM succinate/ min/mg protein*
Lyophilized	None	0.53
Extracted**	None	0.00
Extracted	Q_0	0.11
Extracted	Q_1	0.52
Extracted	Q_2	0.52
Extracted	Q_3	0.50
Extracted	Q_{10}	0.49
Extracted	Phytyl Q	0.40
Extracted	Heptyl Q	0.40
Extracted	Heptadecyl Q	0.22
Extracted	Menadione	0.03

* All activities were measured in presence of 1.0 mg cytochrome *c*. Concentration of supplements giving optimum activity added per assay per mg protein: Coenzyme Q_{10} and heptadecyl Q, 0.1 mg, all other forms of coenzyme Q and menadione 0.05 mg. There was no activity in the presence of antimycin.

** Lyophilized ETP was extracted with acetone for 30 min.

α-tocopherol, tetra-methoxy coenzyme Q_{10}, diacetyl dihydro coenzyme Q_{10}, vitamin K_1, vitamin K_2 from mycobacteria and synthetic forms of vitamin K_2 with isoprenoid side chains containing two to four isoprene residues respectively, as well as beef serum

albumin are inactive*. Only derivatives of 2,3-dimethoxy, 5-methyl benzoquinone with a fatty side chain in the 6 position are capable of restoring succinoxidase activity, and this restored activity is antimycin sensitive.

Succinoxidase versus *DPNH oxidase activity of ETP*

DPNH oxidase activity is destroyed by extraction of particles with acetone and cannot be restored by addition of cytochrome *c* or coenzyme Q or both (*cf.* Table IV). However, addition of the acetone extract back to the particle along with cytochrome *c*, results in a slight restoration of DPNH-oxidase and a very marked restoration of succinoxidase activity. In the case of ETP extracted with isooctane, cytochrome *c* alone could replace the requirements of coenzyme Q and phospholipid in reactivating succinoxidase[1]. Only under very drastic conditions of extraction, are all three components necessary for restoration. These phenomena could be explained in terms of a bypass of electrons from the succinic to the DPNH chain and vice versa as discussed previously[1].

TABLE IV

SUCCINOXIDASE VS DPNH-OXIDASE ACTIVITY OF ETP

State of particle	*Additions*	*Succinoxidase activity*	*DPNH-oxidase activity*
		μM substrate oxidized/min × mg protein	
Lyophilized	None	0.51	0.68
Lyophilized	Cytochrome *c* + CoQ + acetone extract	0.48	0.52
Extracted*	None	0.00	0.00
Extracted	Cytochrome *c*	0.00	0.00
Extracted	Cytochrome *c* + CoQ	0.18	0.00
Extracted	Cytochrome *c* + CoQ + acetone extract	0.42	0.04
Extracted	Cytochrome *c* + acetone extract	0.40	0.05
Extracted	Acetone extract	0.15	0.00

* Lyophilized powder of ETP extracted for 30 min with acetone. Concentrations of supplements in both assays per mg enzyme protein: Cytochrome *c*, 0.66 mg; coenzyme Q (CoQ), 0.066 mg; acetone extract, equivalent of the original enzyme.

DISCUSSION

There have been several recent reports on the restoration of the cytochrome *c* reductase activity of isooctane-extracted particles by various lipide materials[14–17]. Apart from the fact, that these effects were not specific there was in none of these instances any evidence that the loss in activity of the particles sustained by the solvent extraction, could be correlated with the extraction of the component from the particle. In the present studies[1] the loss of enzymic activity could be correlated with extraction

* In alternate experiments with isooctane extracted ETP, essentially the same specificity was observed in the restoration of succinoxidase. Various benzoquinone derivatives with the following substituents were tested and found inactive in these experiments: 2,3-dimethoxy; 2,5-dimethoxy, 3-pentadecyl; 2,5-dimethoxy, 3,6-dipentadecyl; 2,5-dimethoxy, 3,6-diphytyl; 2,5-dihydroxy, 3-pentadecyl; 2,5-dihydroxy, 3,6-dipentadecyl; 2,6-dimethoxy. 2,5-dimethoxy, phytyl benzoquinone, however, showed some trace activity.

of coenzyme Q and the concentration of the coenzyme required for reactivation of the particle was of the same catalytic order of magnitude as the concentration of coenzyme in the extract.

NASON *et al.*[17] have presented evidence that α-tocopherol is required for restoration of the succinic-cytochrome *c* reductase activity of isooctane extracted particles. These authors were in fact the originators of the immensely useful technique of wet solvent extraction of enzyme solutions and particle suspensions. POLLARD AND BIERI[16] interpret the effect of α-tocopherol in terms of the unspecific reversal of the inhibitory effects referable to residual traces of solvent in the particle suspension. DONALDSON *et al.*[17] were unable to correlate loss of activity with extraction of α-tocopherol.

WEBER, GLOOR AND WISS[14] and REDFEARN AND PUMPHREY[15] found that coenzyme Q_{10} was only one of a group of several isoprenoid compounds which could restore the succinic-cytochrome *c* reductase activity of isooctane-extracted particles. Under the extraction conditions used by these authors (extraction in the cold) there is minimal extraction of coenzyme Q_{10}. The action of coenzyme Q and the other isoprenoid compounds in such a system is often a reversal of solvent inhibition[15] and is not specific. Obviously, coenzyme effects are demonstrable only with particles from which the bulk of coenzyme Q has been extracted.

Under the conditions described in these studies, the specificity of coenzyme Q is very pronounced, especially in the succinoxidase system. α-Tocopherol, vitamin K_1, vitamin K_2 from mycobacteria and synthetic forms of vitamin K_2 with isoprenoid side chains containing 2–6 isoprene residues were all found to be inactive. Only derivatives of 2,3-dimethoxy, 5-methyl benzoquinone with a fatty side chain in the 6 position are capable of restoring antimycin sensitive succinoxidase activity which is mediated by cytochrome *c*. Aurantiogliocladin (coenzyme Q_0) and menadione show some activity, a minor fraction of which is antimycin insensitive only in the case of SDC.

A study of the restoration of succinic cytochrome *c* reductase and succinoxidase activity by derivatives of coenzyme Q reveals that activity in the two systems is not necessarily restored to the same extent by a given quinone. Unless cytochrome *c* is reduced at the antimycin sensitive site it does not contribute to succinoxidase activity. Thus, menadione and aurantiogliocladin restore in part the succinic cytochrome *c* reductase activity of acetone extracted SDC. But this activity unlike that induced by lipophilic forms of coenzyme Q is antimycin insensitive. It would appear that the pathway between succinate and cytochrome *c*, mediated by menadione and aurantiogliocladin, skips the antimycin-sensitive site. Probably cytochrome *c* reacts directly with the reduced forms of these two quinones. There is an analogous direct reaction between reduced coenzyme Q and cytochrome *c* which according to HATEFI[18] takes place in the lipoprotein in which coenzyme Q is localized. This reaction is also antimycin insensitive. However, this artificial interaction does not go on in a particle to which both coenzyme Q and cytochrome *c* have been added as shown by the complete antimycin sensitivity of the process. This result suggests that coenzyme Q is anchored to sites or components in the lipoprotein which either do not react with other quinones or which are inaccessible to them. It also appears that the portion of the externally added cytochrome *c* which functions in oxidase activity is restricted in its operation as if it were reincorporated within a "closed" system.

SDC can also catalyze an antimycin insensitive reduction of cytochrome *c* by coenzyme Q derivatives with short isoprenoid or alkane substituents in the 6 position of the benzenoid nucleus as well as by other quinones such as menadione and 2,3-dimethoxy, 5-methyl benzoquinone. RAMASARMA AND LESTER[19] have previously observed that many quinones of diverse structure are reduced by succinate in the presence of mitochondria. It has been shown by MAHLER *et al.*[20] that many reduced quinones are able to interact non-enzymically with ferri cytochrome *c*. Although the antimycin insensitive pathway is obviously artifactual, it is not without interest in view of the specificity of the particle with respect to the nature of the quinone which can participate in the interaction with cytochrome *c*. Benzoquinone, paraxylobenzoquinone and the fully substituted naphthoquinones are ineffective as catalysts for cytochrome *c* reductase.

The possibility of restoring antimycin sensitive cytochrome *c* reductase and oxidase activity with succinate as substrate is restricted specifically to forms of coenzyme Q with long side chains and is accomplished most efficiently by compounds which possess an unsaturated isoprenoid side chain containing more than two isoprene residues.

The low activity of coenzyme Q_{10} in the assay for succinoxidase activity may be surprising. However, as discussed previously[12] acetone-extracted particles show some requirement for a lipid component NL II, and this compound is essential for the activity only of coenzyme Q analogues which possess a side chain of more than two isoprenoid units. In presence of NL II, coenzyme Q_{10} is as active as coenzymes Q_2 or Q_3. By contrast coenzyme Q_{10} is maximally active without added NL II in the restoration of the cytochrome *c* reductase activity of the same particle. The heptadecyl derivative of coenzyme Q as well as plastoquinone are atypical in that the restoration of cytochrome *c* reductase activity which they induce is negligible compared to the restoration of oxidase activity.

In the case of SDC, the particle which was most extensively studied, the following pattern has emerged from the present work: SDC has three pathways involving cytochrome *c* (or oxygen) which respond to the addition of quinones. The first is the antimycin insensitive pathway to external cytochrome *c* induced by addition of menadione, 2,3-dimethoxy, 5-methyl benzoquinone and short chain coenzyme Q analogues. The second is the antimycin sensitive pathway that can be induced only by homologues of coenzyme Q_{10} (ranging from Q_{10} to Q_2). The third is the antimycin sensitive pathway to molecular oxygen which involves bound cytochrome *c* and which can operate only in the presence of members of the coenzyme Q family of compounds of analogues of coenzyme Q (heptyl and heptadecyl coenzyme Q), and plastoquinone.

The possibility has to be entertained that particles like SDC which are derived by deoxycholate fragmentation of ETP may have artifactual activities and that the requirement of any particular activity like cytochrome *c* reductase activity for coenzyme Q, may not necessarily reflect a coenzymic role for the quinone in overall electron transport. But the fact that particles like the ETP residue which are formed from mitochondria by a relatively mild procedure also show exactly the same requirement for reductase activity, lends support to the hypothesis of a true coenzymic role for the mitochondrial quinone.

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