Biochemical Pharmacology, 1961, Vol. 5, pp. 381–388. Pergamon Press Ltd., Printed in Great Britain.

LIPOPROTEINS AND ELECTRON TRANSPORT

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Abstract—Mitochondrial enzymes are arranged according to a specific pattern. Lipids have been implicated as important components in mitochondrial organization. Some properties of three isolated mitochondrial lipoproteins, the coenzyme Q-lipoprotein, the cytochrome c_1-lipoprotein and the lipoflavoprotein are reviewed, and their role in the function of the electron transport system is discussed.

OUR current concepts of mitochondrial structure are twofold: the one is based on evidence obtained with the electron microscope, the other on biochemical evidence. It is now very firmly established that mitochondria carry out a great number of fundamental biochemical reactions, the proper functioning of which is essential to all life. Thus a great number of reactions take place in mitochondria, and the appropriate enzymes catalysing these reactions are located within them. The question arises whether these enzymes are randomly distributed in the mitochondrion, or whether they are arranged according to some pattern. The available evidence strongly favours the latter view of a rigid and meaningful structural organization of the mitochondrial enzymes.

Let us not consider this evidence in detail, since it has been discussed elsewhere[1] and earlier at this Symposium[2], but rather move on to some particular aspects of mitochondrial organization, which have arisen in connexion with studies on mitochondrial lipids. It is well known that in addition to a number of redox components, the flavoproteins and the cytochromes, the mitochondria and particles derived from them contain high proportions of lipid material. The electron transport system, when isolated in the form of the ETP_H-particle,[3] which oxidizes DPNH* and succinate by molecular oxygen and concomitantly esterifies phosphate to form ATP, contains about 30 per cent of lipid.

Coenzyme Q

Among the mitochondrial lipids there are specific components that may take part in oxidation–reduction reactions. One such, namely coenzyme Q[4–6] has recently been characterized and studied in detail, and found to be an essential component of the system bringing about oxidative phosphorylation. Coenzyme Q is a tetrasubstituted benzoquinone with two methoxy groups, one methyl group and a long chain consisting of isoprene units as the substituents. The number of isoprene units in the long chain varies from one species to another: mammalian coenzyme Q contains ten isoprene units. The absolute requirement for coenzyme Q in the succinoxidase system and its

* The following abbreviations are used: ATP, adenosine triphosphate; CoQ, coenzyme Q; $CoQH_2$, dihydrocoenzyme Q; cyt. *c*, cytochrome *c*; cyt. c_1, cytochrome c_1; DPNH, reduced diphosphopyridine nucleotide; ETP and ETP_H, the electron transport particle; FAD, flavin adenine dinucleotide; SDC, the succinic dehydrogenase complex.

specificity has been beautifully demonstrated by Lester and Fleischer[7]. Extraction of ETP-particles with organic solvents, such as *iso*octane or acetone abolishes their succinoxidase activity. Coenzyme Q fully restores this activity. That the restored activity closely resembles the activity originally present is shown by the fact that it is completely inhibited by antimycin A. The specificity for coenzyme Q in this system is high; a number of other compounds reported to restore the activity of solvent extracted succinoxidase particles, including some simple derivatives of coenzyme Q do not restore any activity at all.[7]

That coenzyme Q is an equally important link in the electron transport chain, such as the flavins and cytochromes, has been conclusively shown by Hatefi[8]. Thus mitochondrion-bound coenzyme Q shows changes corresponding in its oxidation–reduction levels to those of these other components, and it conforms very nicely with the different steady states given by Chance and Williams[9]. Its involvement in electron transport and oxidative phosphorylation in intact mitochondria seems well established.

Lipoproteins

Recently a number of lipoproteins have been isolated from mitochondria. This advance has thrown much light on the structure and function of the electron transport system. Each of these lipoproteins contains one of the principal components of the electron transport system and this fact, of course, makes them especially interesting from the standpoint both of the structure of the system and of its function.

The isolation of these lipoproteins from mitochondrial suspensions has been made possible by the use of a number of reagents, which readily interact with lipid materials. The successes obtained in the fragmentation of mitochondria by the use of *iso*butyl and amyl alcohols[10] and the salts of bile acids,[11] encouraged investigations on their further effect on submitochondrial fragments. The fraction most thoroughly studied in this respect is the succinic dehydrogenase complex (SDC).[10] Upon treatment with high concentrations of cholate and nearly saturating KCl, the SDC was fragmented into a number of discrete units, with different enzymatic and chemical characteristics.[12] During this fragmentation a lipoprotein was liberated, and it could be separated from the enzymatically active fragments by dialysis and ammonium sulphate fractionation. The combined use of alcohols, bile acids and high salt concentrations has proved to be effective in splitting submitochondrial particles with liberation of two different lipoproteins from them.[13] Furthermore, particles obtained from SDC through the use of cholate and salt can be extracted with solvents such as *iso*octane. Upon lyophilization the *iso*octane extract yields a water-soluble lipoprotein with a strong diaphorase activity.[14] Thus the use of lipid solvents and solubilizing agents has led to the isolation of three different lipoproteins from mitochondria. It is quite conceivable that the use of similar methods may in the future permit the isolation of other lipoproteins from various tissues.

Coenzyme Q–lipoprotein

The first mitochondrial lipoprotein to be isolated was the coenzyme Q–lipoprotein. It can be prepared from the succinic dehydrogenase complex (SDC) by either of two different procedures[15] and is stable upon storage at 4 °C but cannot be frozen. The coenzyme Q–lipoprotein contains large amounts of coenzyme Q; in fact a very large

proportion of the total coenzyme Q in the original succinic dehydrogenase complex (SDC) is recovered in the lipoprotein.

The coenzyme Q–lipoprotein has been studied in considerable detail, and it exhibits many interesting properties, which give it a key position in the electron transport mechanism and possibly also in oxidative phosphorylation. The first role assigned to the lipoprotein was that of a link between the succinic and DPNH dehydrogenase systems in SDC.[12] This interpretation was based on the observation that these two dehydrogenases were separated from each other during the isolation of the lipoprotein. It is now quite clear, however, that this cannot be the only, or even the main, function of the lipoprotein.

A very interesting property of the coenzyme Q–lipoprotein is its ability to take up and bind substances from the surrounding medium. In this ability the lipoprotein shows considerable specificity, as is shown in Table 1.[15] Coenzyme Q is taken up in

TABLE 1. UPTAKE OF SOME COMPOUNDS BY THE COENZYME Q LIPOPROTEIN

Compound	Uptake (μmoles/mg protein)
Coenzyme Q	0·50*
α-tocopherol	0·10
Vitamin A	0·04
Vitamin K_1	0·012
Cyt. *c* (mammalian)	0·0173
Cyt. *c* (*Azotobacter*)	0

* The lipoprotein, as isolated contains 0·15 μmoles coenzyme Q per mg protein.[15]

large amounts, whereas the three other lipid soluble compounds, α-tocopherol, vitamin A and vitamin K, are bound to a much smaller extent. The affinity for CoQ is thus much higher and gives further support to the concept that CoQ is the natural redox component of the lipoprotein.

Cytochrome *c* is also bound to a considerable extent. Here a very remarkable specificity for mammalian cyt. *c* is evident, as cytochrome *c* from *Azotobacter* is not bound. When cyt. *c* is bound by the lipoprotein it exhibits changes in properties, and can now be re-extracted from the lipoprotein with heptane. A similar lipid–cytochrome *c* can also be prepared by the use of particles such as ETP, as Widmer and Crane[16] have shown, since these particles contain the lipoprotein. The ability of the lipoprotein to bind CoQ and cyt. *c* will be seen to be of importance later on, when the functional role of the CoQ lipoprotein is evaluated.

Enzyme systems requiring the coenzyme Q–lipoprotein

A number of enzyme systems requiring the presence of the CoQ–lipoprotein for their proper function have been prepared. As a matter of fact, the *iso*octane extracted ETP is one such system.[4] Its succinoxidase activity is restored by the addition of the CoQ–lipoprotein and the amount of CoQ needed in the form of the lipoprotein is indeed much smaller than when free CoQ is added.

Coenzyme Q oxidase

An enzyme isolated from mitochondria, called CoQ oxidase,[17] also shows a requirement for the lipoprotein (Table 2). This enzyme, which contains only cytochrome *a*

of the cytochromes, catalyses the oxidation of dihydrocoenzyme Q by molecular oxygen. There is an absolute requirement for added cyt. *c*, and a very strong requirement for the lipoprotein. These two components can in part be replaced by the earlier mentioned lipid cytochrome *c*. Dihydrocoenzyme Q is thus oxidized only in presence of cyt. *c*, and especially its lipid soluble form or lipoprotein bound cyt. *c*. Therefore we must assume that cyt. *c* lies between coenzyme Q and cytochrome *a* in the oxidation chain.

Cytochrome c *oxidase*

Another very similar enzyme, cytochrome *c* oxidase, has also been isolated from mitochondria.[18] For maximum activity this enzyme shows a similar requirement

TABLE 2. THE REQUIREMENT FOR COENZYME Q LIPOPROTEIN AND CYTOCHROME *c* IN THE COENZYME Q OXIDASE AND CYTOCHROME *c* OXIDASE SYSTEMS

Conditions	CoQ oxidase	Cyt. *c* oxidase
(1) Enzyme alone	0*	7·12*
+ lipoprotein	0	21·36
+ cyt. *c*	5·7	—
+ cyt. *c* + lipoprotein	44·7	—
(2) Enzyme alone	0	10·68
+ lipoprotein	—	35
+ lipoprotein + cyt. *c*	67·5	—
+ lipid cyt. *c*	31·0	15·7

* The figures giving the rates of oxidation in the two systems should not be compared with each other, since the methods used for assay of the different enzymatic activities do not give comparable results. For details, see the original papers by Hatefi[17, 18].

TABLE. 3. COMPARISON OF CoQ OXIDASE AND CYT. *c* OXIDASE

Substrate	Rate of oxidation by	
	CoQ oxidase	Cyt. *c* oxidase
$CoQH_2$	rapid	slow
Ferrocyt. *c*	rapid	rapid

for the lipoprotein (see Table 2). Here again, lipid cytochrome *c* has an effect, but much smaller than in the previous case.

These two enzymes, CoQ oxidase and cyt. *c* oxidase, which are both isolated from beef heart mitochondria, thus show rather interesting properties with respect to the lipoprotein. Neither enzyme is capable of oxidizing its substrate maximally without the lipoprotein and both contain only cytochrome *a* of the haems. In Table 3, the two enzymes are compared in respect of their substrate specificity. Coenzyme Q oxidase rapidly oxidizes both dihydro-CoQ and ferrocytochrome *c*, whereas cyt. *c* oxidase is unable to oxidise dihydro-CoQ at a speed comparable to the rate of ferrocytochrome *c* oxidation. It therefore seems possible that cytochrome oxidase is a part of the CoQ oxidase. The latter can be stripped of an enzyme necessary for transferring electrons from dihydro-CoQ to cytochrome *c*. From all the data on the two

oxidases and their requirements for maximal activity, it is possible to place cyt. *c* with considerable confidence between coenzyme Q and cytochrome oxidase in the oxidation sequence.

So far it has not been possible to decide with any certainty which is the member of the electron transport chain from which CoQ receives electrons. An experiment by Hatefi[17] seems to rule out cytochrome c_1. Cytochrome oxidase supplemented with catalytic amounts of cyt. *c*, rapidly oxidises purified ferrocytochrome c_1. The rate of oxidation is almost the same as that obtained for ferrocytochrome *c* under the same conditions. Coenzyme Q does not seem to be implicated in this oxidation of ferrocytochrome c_1, and one may therefore conclude that cyt. c_1 can be oxidized through the mediation of cyt. *c* only.

Although much misunderstanding may arise from the presentation of pictures describing electron transport pathways, the data discussed above have been summarized in Fig. 1, which is based mainly on a discussion by Hatefi[17]. The encircled

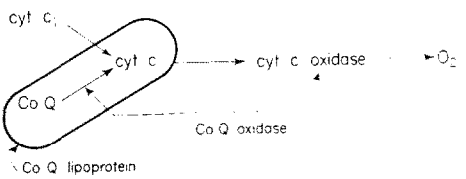

FIG. 1. The flow of electrons from cytochrome c_1 and coenzyme Q to oxygen.

area represents the coenzyme Q lipoprotein, which is shown to contain both CoQ and cyt. *c*. CoQ is there in the first place, when the lipoprotein is isolated and, as was shown, cyt. *c* is readily bound by the lipoprotein. The oxidation of added external $CoQH_2$ starts by equilibrium with the CoQ in the lipoprotein. Electrons are then transferred to cyt. *c* by an enzyme present in the CoQ oxidase , as indicated. Cyt. *c* is then oxidized by the cytochrome oxidase, which transfers the electrons to molecular oxygen. The oxidation pathway of cyt. c_1 is also shown, and passes again through cyt. *c*. Cyt. *c* thus seems to be at the cross-roads, collecting electrons from at least two directions and being oxidized by a single oxidase.

What there should be on the left side of this diagram is not fully known. So far it seems that there is insufficient evidence as to which components of the electron transport system directly precede CoQ and cyt. c_1. There are, of course, not many components left, but their arrangement still presents a puzzle.

Cytochrome c_1 *lipoprotein*

In addition to the CoQ lipoprotein, a lipoprotein containing or associated with cytochrome c_1 has been isolated.[13] In the first phase of the isolation of cyt. c_1 from SDC, this cytochrome is obtained in a form containing about 50 per cent lipid. Table 4 shows some data on this complex and the purified cyt. c_1. The fact that the lipoprotein–cyt. c_1 does not contain any CoQ and has a much lower phospholipid content than the CoQ–lipoprotein, makes it probable that the lipoprotein associated with cyt. c_1 is not identical with the CoQ–lipoprotein. Furthermore, the latter could be isolated in good yield by the same procedure as the cyt. c_1 lipoprotein but appears

during the isolation procedure in an entirely different fraction. The formidable difficulties of obtaining any sizeable amounts of the cyt. c_1–lipoprotein have so far made a more detailed study of its properties impossible.

Flavolipoprotein

The work on the isolation and characterization of mitochondrial components in their natural form has met with extremely interesting success in the isolation by

TABLE 4. THE TWO FORMS OF CYTOCHROME c_1 ISOLATED[13]

	Cytochrome c_1 lipoprotein complex	Free cytochrome c_1
Haem (mμmoles/mg protein)	6·05	14·7
Lipid (% of dry wt.)	52	0
Coenzyme Q	none	—
Phospholipid (% of total)	80	—

Ziegler *et al.*[14] of a flavolipoprotein showing a very high diaphorase activity.

One of the observations that led to this discovery was that the activity of ETP, when expressed as DPNH oxidized per unit of flavin, was very much higher than in the purified DPNH–cyt. *c* reductase preparations described by Mahler *et al.*[19] and by de Bernard[20]. This fact is shown in Table 5. The recently isolated lipoflavoprotein

TABLE 5. DIAPHORASE ACTIVITY PER UNIT OF ACID EXTRACTABLE FLAVIN

ETP	25–35 μmoles DPNH/mμmole flavin
Lipoflavoprotein	32 μmoles DPNH/mμmole flavin
Cyt. *c* reductase	2·2 μmoles DPNH/mμmole flavin

Data from Ziegler *et al.*[14]

shows an activity similar to that of ETP. All these activities were measured with ferricyanide as electron acceptor.

A number of different flavoproteins with diaphorase activity have been isolated from mitochondria.[16] With respect to flavin content those mentioned above, and the Straub[21] diaphorase are about equally purified. In the cytochrome reductase, none of the flavin, which is easily and quantitatively extracted by acid, can be assayed as FAD in the D-amino acid oxidase system.[19, 20] In the others, the Straub[21] diaphorase and the lipoflavoprotein,[14] all of the flavin is accounted for by FAD. There are also considerable differences in the activities of the different flavoproteins when different electron acceptors are used. The lipoflavoprotein shows the highest activity with ferricyanide, whereas the lipid-free flavoproteins are practically inactive with ferricyanide but show a very high activity with methylene blue as electron acceptor. The similarity in properties between the classical Straub diaphorase and a flavoprotein obtained from the lipoflavoprotein by complete removal of the lipid is most striking, as is the change in properties when this conversion takes place.

The question about the relation between the Straub[21] diaphorase and the cyt. *c* reductase[19, 20] now seems to be settled in the light of the evidence provided by the extraction of the lipoflavoprotein. Table 6 shows that the extraction method used for the liberation of the cyt. *c* reductase is capable of converting its flavin into a form which does not function as FAD in the D-amino acid oxidase assay, while the other extraction method does not lead to such a conversion. The form of flavin encountered in the cyt. *c* reductase is thus an artifact of preparation, and one may therefore ask

TABLE 6. RECOVERY OF FLAVIN IN THE PREPARATION OF DIAPHORASES FROM ETP_H BY TWO DIFFERENT EXTRACTION METHODS

Enzyme	Total acid extractable flavin (mμmoles/mg)	(% recovery)	FAD %
ETP_H	0·2	—	100
Lipoflavoprotein (first extract)	0·9	80	98
Cyt. *c* reductase (first extract)	9·0	80	2–5

From Ziegler *et al.*[14]

whether the cyt. *c* reductase activity might not also be an artifact, since it is not present in the lipoflavoprotein or the Straub diaphorase, which both contain FAD only. Such a situation necessitates a re-evaluation of the concepts of electron transport pathways in mitochondria.

CONCLUDING REMARKS

The lipoproteins which I have described in the foregoing show many interesting properties. Their role in electron transport is not yet fully understood, but the observation, that three of the main components of the terminal oxidation sequence, diaphorase, cyt. c_1 and CoQ are closely associated with lipoproteins is very suggestive.

Apart from the role in electron transport which must be attributed to the lipoproteins, a role in the mechanism of oxidative phosphorylation has to be envisaged. Recently Clark *et al.*[22] (and other authors) have indicated that compounds having the quinone structure may play a decisive role in phosphorylation. Furthermore, Löw[23] has produced evidence of the direct participation of mitochondrial flavins in the ATPase and ATP–inorganic phosphate exchange reactions, which have been thought to be "shadows" of oxidative phosphorylation.

This evidence must now be considered in the light of the investigations of Grabe[24], which indicate that phosphorylative processes may be favoured by a milieu where access of water to the phosphate compounds, presumably formed as intermediates, is restricted. The lipoproteins could obviously provide such a water-free environment, and the fact that quinone-like compounds (CoQ and flavins) have been found associated with lipoproteins, makes the assumption that the lipoproteins play a role in oxidative phosphorylation even more attractive.

Acknowledgement—I wish to express my most sincere gratitude to Dr. David Green, in whose laboratory I had an excellent opportunity to become familiar with these exciting problems, and who also encouraged me to discuss them at this meeting.

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