STUDIES ON THE ELECTRON TRANSPORT SYSTEM. XXXII-Reduction of Coenzyme Q by DPNH

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Much has been learned about the electron transport system by fractionating the chain into smaller segments and studying the catalytic function and the composition of each fragment. A major limitation in this approach has been the lack of appropriate oxidoreduction substrates to interact at specific points all along the chain. Artificial redox compounds such as phenazine methosulfate, potassium ferricyanide, 2,6-dichlorophenol indophenol and methylene blue have been successfully used as electron acceptors for the flavoprotein dehydrogenases of the electron transport system, and as a result it has been possible to isolate succinic dehydrogenase and DPNH** dehydrogenase in highly purified state. Of course, the undesirable feature of these unnatural electron acceptors is obvious. The only natural redox substrate that has been conveniently used in this type of study is cytochrome c, (cyt. c), which in turn has made possible the isolation and purification of cytochrome oxidase on the one hand and DPNH and succinic cyt. c reductases on the other. As other components of the electron transport system become available in purified form, appropriate enzyme systems for interaction with these carriers can

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** Abbreviations: DPNH, reduced diphosphopyridine nucleotide; pCMS, p-Chloromercuriphenyl sulfonate; Q_1, Q_2 and Q_{10}, Coenzymes Q_1, Q_2 and Q_{10} respectively (cf. Lester et al., 1959); Q_0, 2,3-dimethoxy-5-methyl benzoquinone; MeQ, 2,3-dimethoxy-5,6-dimethyl benzoquinone.

be sought, isolated and characterized. It has already been shown, for example, that purified cyt. c_1 is rapidly oxidized by molecular oxygen in presence of catalytic amounts of cyt. c and cytochrome oxidase and that no oxidation takes place in absence of cyt. c (Hatefi, 1959).

Another segment of the electron transport chain which has now become amenable to direct study is the enzyme system operative between DPNH and Coenzyme Q (cf. Doeg et al., 1960, for succinic-Coenzyme Q reductase). It has been found that among the isoprenologues of Coenzyme Q, that with only one isoprene unit in position 5 of the ring (Coenzyme Q_1) can rapidly interact with the electron transport system in the expected manner. This reaction can be conveniently studied in the spectrophotometer by following the disappearance of DPNH at 340 mμ. Table I shows the rate of reduction of Q_1 as catalyzed by several types of mitochondrial enzymes. For comparison, the rates of DPNH oxidation by molecular oxygen or by cyt. c are also given. It is seen that as the particles are further purified with respect to DPNH oxidase or DPNH-cyt. c reductase activity, the rate of reduction of Q_1 by DPNH increases. The rate of DPNH oxidation catalyzed by DPNH-cyt. c reductase enzyme (Hatefi et al., 1960) is very high and nearly the same with either cyt. c or Q_1 as the terminal electron acceptor.

Not all the Coenzyme Q type compounds are reduced as rapidly as Q_1 (Table II). Coenzyme Q_2, which forms a fine suspension in water, is reduced at a rate about one-fourth that of Q_1. However, Q_{10} which is utterly insoluble in water cannot be reduced under the conditions of the assay. The reduction of Q type compounds by mitochondrial particles does not depend on solubility properties alone; a considerable degree of structural specificity is apparently involved. This is evident from the feeble reducibility of Q_0 and MeQ, both of which are completely water soluble but lack the isoprene side chain.

It has been shown earlier that reduction of Coenzyme Q by DPNH is inhibited by Amytal (Hatefi et al., 1959) while the oxidation is inhibited by antimycin A (Hatefi, 1959; Pumphrey and Redfearn, 1959; Green et al., 1959). These two inhibitors as well as pCMS, which also inhibits DPNH-cyt. c reductase

Table I

Reduction of Coenzyme Q_1 by DPNH

ENZYME	ELECTRON ACCEPTOR	ACTIVITY
Mitochondria	Oxygen	1.1
"	Q_1	1.68
" + deoxycholate + cyt. c*	Oxygen	4.3
" + deoxycholate	Q_1	2.6
ETP_H**	Oxygen	2.58
"	Q_1	6.42
S-1***	cyt. c	12.3
"	Q_1	9.4
DPNH-cyt. c reductase	cyt. c	42.0
"	Q_1	35.0

* - Mitochondria were treated with 0.3 mg. K-deoxycholate/mg. protein and 0.05 mg. cyt. c was added to the assay medium.

** - cf. Linnane and Ziegler, 1958.

*** - An intermediate-stage particle in preparation of DPNH-cyt. c reductase, which also has succinic-cyt. c reductase activity (cf. Hatefi et al., 1960).

activity (Hatefi et al., 1960), behave as expected in the reduction of Q_1 by DPNH (cf. Table III). Amytal and pCMS inhibit the reduction while antimycin A does not. Recently Ernster et al., (1960), have described a soluble DPNH-TPNH diaphorase which is capable of reducing inter alia Q_2 as well as Q_0. This enzyme is completely insensitive to Amytal but is inhibited at very low levels of dicoumarol (50% at 1×10^{-8} M dicoumarol). As seen in Table III, dicoumarol also inhibits the reduction of Q_1 and Q_2 catalyzed by the Amytal-sensitive mitochondrial system. However, much higher concentrations of dicoumarol are required to inhibit the latter system.

Table II

Reducibility of Coenzyme Q Analogues

Exp.	ENZYME	Q ANALOGUE	ACTIVITY
1	DPNH-cyt. c reductase	Q_1	32.2
	"	Q_2	8.1
	"	Q_{10}	0.0
2	ETP_H	Q_1	6.4
	"	Q_2	1.54
3	DPNH-cyt. c reductase	Q_1	29.6
	"	Q_0	< 3.4
	"	MeQ	< 2.6

The addition of fine suspensions of lipid to the reaction medium aids the interaction between Coenzyme Q and the mitochondrial enzymes. In the oxidation of reduced Q_{10} by a submitochondrial enzyme, the requirement for lipid was found to be absolute (Hatefi, 1959). DPNH-Q_1 reductase activity is also stimulated by lipid. In absence of added lipid (asolectin), the rate falls to about 50% of the maximal rate which obtains in the complete system.

Methods

Coenzyme Q reductase assay conditions -- The reaction mixture (in a 1 ml quartz cuvette) consisted of 20 μmoles K-phosphate, pH 8.0, 2 μmoles NaN_3, 0.12 μmole DPNH, 0.05 μmole Q dissolved in 5 μl ethanol (Q_0 and MeQ were dissolved in water), 0.15 mg. asolectin suspension (cf. Hatefi et al., 1960) and water to a final volume of 1 ml. The blank cuvette contained the same mixture except that half as much DPNH was present. At zero time enzyme was added to the experimental cuvette and DPNH disappearance followed every 15 seconds at 340 mμ in a DU Beckman spectrophotometer. Where mitochondria and ETP_H were used, the particles were pre-treated with antimycin A to inhibit

Table III

Effect of Inhibitors on the Reduction of Coenzyme Q by DPNH

Exp.	Inhibitor	Q Homologue	Activity
1	None	Q_1	30.8
	Antimycin A (1×10^{-7}M)	"	28.4
2	None	"	30.2
	Amytal (3×10^{-3}M)	"	< 2.6
	pCMS (3.8×10^{-4}M)	"	1.7
3	None	"	32.2
"	Dicoumarol (1×10^{-4}M)	"	15.5
"	" (2.5×10^{-4}M)	"	6.4
"	None	Q_2	8.14
"	Dicoumarol (1×10^{-4}M)	"	1.3
"	" (2.5×10^{-4}M)	"	0.0

The enzyme used in these studies was DPNH-cyt. c reductase (Hatefi et al., 1960).

oxidation of DPNH by molecular oxygen. The latter activity was measured as above except that Q and NaN_3 were omitted from the reaction medium. The assay conditions for measurement of cyt. c reductase activity have been described elsewhere (Hatefi et al., 1960). All reactions were carried out at 38° and specific activities are reported as μmoles substrate (one electron) oxidized or reduced/min./mg. enzyme protein. The Coenzyme Q compounds were gifts of Dr. K. Folkers, Merck, Sharp and Dohme Research Laboratories.

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