ON THE ROLE OF COENZYME Q IN ELECTRON TRANSPORT

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In previous reports from this laboratory, evidence has been presented that coenzyme Q is an essential component in the mitochondrial electron transfer chain. Succinate-O_2 or succinate-cytochrome c reductase activity is lost when Q* is extracted from the particles and restoration of activity is achieved by specific addition of Q to the extracted particles (Lester and Fleischer, 1959). It has also been shown that particle-bound Q undergoes cyclic oxidation-reduction in mitochondria and derivative particles (Hatefi, Lester, Crane and Widmer, 1959; Hatefi, 1959; Hatefi, 1959). Recently, Pumphrey and Redfearn (Pumphrey and Redfearn, 1959) have argued that the rate of reduction of particle-bound Q is not sufficiently rapid to account for the overall rate of oxidation of succinate by oxygen. In the present communication some new observations bearing on the role of coenzyme Q as a mitochondrial electron transport member will be described.

Particle-bound Q can be quantitatively extracted with cyclohexane from aqueous suspensions of particles which have been acidified with $HClO_4$ (final concentration 0.05 M) and then neutralized with phosphate buffer (final concentration 0.2 M). The use of $HClO_4$ (Hatefi, 1959) makes it possible to stop instantaneously any enzymic reaction and renders unnecessary the heating procedures used in earlier reports.

The rate of reduction of internal coenzyme Q of ETP was studied at 0° and compared with the overall rate of electron transport at the same

* Abbreviations--DPNH, reduced diphosphopyridine nucleotide; Tris, Tris(hydroxymethyl)aminomethane; Q and QH_2, oxidized and reduced coenzyme Q, respectively; ETP, electron transport particle; SDC, succinic dehydrogenase complex.

temperature. It was found that the rate of reduction of coenzyme Q with DPNH was the same as the rate of oxygen consumption (53% of the Q content of ETP was reduced one second after the addition of DPNH). At 0°, the rate of Q reduction by succinate was considerably lower, while the succinate-oxygen rate at 0° was essentially zero. The oxidation rate of QH_2 by molecular oxygen as catalyzed by a particle derived from mitochondria has been reported previously (Hatefi, 1959).

Aged and frozen suspensions of ETP or SDC gradually lose the capacity to reduce internal coenzyme Q with DPNH or succinate. These observations suggest: (i) that only rapid-flow techniques can be used to compare accurately the relative rates of reduction of coenzyme Q and oxygen; (ii) that in a particle suspension which is a mixture of fully active and inactive or less active particles, the overall rate of Q reduction is a composite of high and low rates and consequently is a minimal value. Since detailed experimental data have not been presented by Pumphrey and Redfearn (Pumphrey and Redfearn, 1959), it is not possible to decide where the discrepancy lies. However, if the particles they used to study Q reduction were not in the same state of activity as those used to measure overall oxygen uptake, then the comparison of relative rates is not fully justified.

Both succinate and DPNH reduce coenzyme Q in various particles such as ETP or SDC as prepared by the method of Crane and Glenn (Crane and Glenn, 1957) or Rabinowitz and de Bernard (Rabinowitz and de Bernard, 1957). However, in SDC prepared by the amyl and isobutyl alcohol procedures (Green, Mii and Kohout, 1955), bound coenzyme Q is reducible only by succinate (Hatefi, Lester, Crane, and Widmer, 1959). From the twin facts (i) that DPNH cannot be oxidized by O_2 or cytochrome c in particles in which coenzyme Q is not reducible by DPNH and (ii) that the rate of DPNH oxidation by O_2 never exceeds the rate of DPNH oxidation by Q, it would appear that coenzyme Q is also an essential component in the DPNH chain. These observations based on the perchlorate inactivation procedure are in full agreement with previous results obtained by terminating the reactions by heat denaturation of the

particles (Hatefi, Lester, Crane and Widmer, 1959).

The reduction of internal coenzyme Q is antimycin-insensitive (Table I, exp. 1). We have previously reported evidence of sensitivity when a heating procedure was used to stop the reaction. With the perchloric method which eliminates heating in the presence of antimycin, it can be clearly demonstrated in agreement with Pumphrey and Redfearn (Pumphrey and Redfearn, 1959) that the reduction of bound coenzyme Q is antimycin-insensitive while the oxidation of internal QH_2 is antimycin-sensitive (Table I, exp. 2). In this respect, the behavior of internal and external Q is identical (Hatefi, 1959).

Table I

Exp.	Additions	mμmoles of oxidized Q per mg. protein
1	none	4.8
"	succinate	1.35
"	" + Antimycin A	1.40
2	succinate (anaerobic)	1.86
"	" (aerobic)	4.76
"	" + Antimycin A (anaerobic)	1.43
"	" + " " (aerobic)	1.41

Conditions:

Exp. 1. 10 μmoles K-phosphate, pH 7.5; 23.5 mg. ETP protein; 2 μmoles KCN; 0.05 mg. Antimycin A; 50 μmoles Na-succinate; and 0.25 M sucrose to 2.0 ml. The experiment was carried out at 38°.

Exp. 2. 25 μmoles Tris-chloride, pH 7.5; 5 μmoles Na-succinate; 0.1 mg. Antimycin A; 22.1 mg. ETP protein; and 0.25 M sucrose to 2.0 ml. Anaerobic experiments were carried out in evacuated Thunberg tubes, and $HClO_4$ was added from side arm after 1 min. at 38°. Aerobic experiments were also carried out as above, but after the 1 min. incubation, air was allowed in the tubes, and the reactions were terminated by addition of $HClO_4$ after 30 seconds of aeration.

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Received August 10, 1959