Studies of an Electron Transport Particle from *Candida utilis*[1]

We previously isolated an electron transport particle (ETP) from *Saccharomyces cerevisiae* and showed that oxidation of DPNH by the preparations is not inhibited by seconal or rotenone (1, 2) in contrast to heart muscle preparations in which DPNH oxidation is highly sensitive to the inhibitors (3). In addition, the preparations contain only flavin adenine dinucleotide (FAD), and the mammalian preparations contain both FAD and flavin mononucleotide (FMN) (1, 4). Finally, the soluble DPNH dehydrogenase isolated from the yeast ETP does not contain iron and labile sulfide groups (5, 6) as does the similarly prepared soluble DPNH dehydrogenase from beef heart (6, 7).

The present communication describes the properties of an ETP from another yeast, *Candida utilis* (*Torulopsis utilis*), and presents data showing that the preparation is seconal- and rotenone-sensitive and contains FMN in addition to FAD, thus resembling the mammalian preparation rather than the ETP of *S. cerevisiae*. Furthermore, the ETP from *C. utilis* contains coenzyme Q_7 rather than Q_6 (*S. cerevisiae*) or Q_{10} (heart muscle), and in much lower amounts than in the preparations of ETP from heart or *S. cerevisiae*.

Electron transport particles from *S. cerevisiae* and *C. utilis* were prepared as described previously by Mahler *et al.* (2). Assays for enzymic activity were performed either spectrophotometrically or by use of an oxygen polarograph as described previously (1, 8). Protein, iron, copper, coenzyme Q, cytochromes, flavin, and labile sulfide were determined by methods described elsewhere (1, 6).

Preparations of ETP from *C. utilis* catalyzed the oxidation of 1.5–1.8 μmoles of DPNH and approximately 0.5 μmole of succinate per minute per milligram of enzyme protein and did not consistently show the marked stimulation of activity when cytochrome *c* was added to the assays that is shown by preparations of ETP from *S. cerevisiae* (1, 2). As shown in Table I, preparations of ETP (*C. utilis*) of the highest activity contain cytochromes *a*, a_3, *b*, *c*, and c_1, nonheme iron, labile sulfide, copper, flavin, coenzyme Q, and approximately 30% lipid (dry weight). The preparations of ETP from *C. utilis* differ in composition from those of *S. cerevisiae* in that they contain lower amounts of flavin and coenzyme Q. Further studies of the flavins by chromatographic procedures and by studies of reactivation of specific apoenzymes performed as described previously (4) demonstrated that FMN and FAD were present in the *C. utilis* ETP in nearly equal amounts in contrast to an ETP from *S. cerevisiae* which has been shown to contain only FAD (1). In other experiments, coenzyme Q extracted both from intact cells and from preparations of ETP of *C. utilis* was studied by thin-layer chromatography on silica gel in a solvent system of 1% ether in benzene, and the preparation was identified as coenzyme Q_7, thus differing from preparations from *S. cerevisiae* and heart muscle which contain coenzyme Q_6 and coenzyme Q_{10}, respectively. No coenzyme Q_9 was found in the *C. utilis* preparations as previously described by Crane (10), but this may be due to the small amount of coenzyme Q_9 which was reported to be present. In preparations of ETP from *C. utilis* of lower activity (1–1.2 μmoles DPNH oxidized per minute per milligram protein), the nonheme iron and labile sulfide content was nearly double the concentrations found in the more active preparations.

Preparations of ETP from *C. utilis* were inhibited over 95% (succinic and DPNH oxidase activities) by antimycin A (1 μg/ml) as are ETP preparations from heart (11) and *S. cerevisiae* (1). The DPNH oxidase and DPNH coenzyme Q reductase activities of preparations of *C. utilis* ETP were inhibited 90 and 75%, respectively, by seconal (2 mM), and by rotenone (2 μM), compounds which inhibit preparations of heart ETP (3) but not ETP from *S. cerevisiae* (2) at low concentrations. In addition, seconal (4 mM) inhibited the succinic oxidase and succinic cytochrome *c* reductase activities of the *C. utilis* ETP 80–90% (the corresponding activities of heart ETP are not inhibited); however, rotenone (10 μM) did not inhibit the succinic oxidase activity. The inhibition of succinic oxidase activity by seconal was usually not immediate, and increased to maximal values progressively over a 2- to 3-minute period of incubation at 38°. Furthermore, the inhibition was present only when the enzyme was assayed in phosphate buffer. When assays were performed in Tris buffer or at temperatures under 30°, no inhibition of succinic oxidase activity by seconal was observed.

The ETP from both *S. cerevisiae* and *C. utilis* have been examined by low-temperature EPR spectroscopy. Both preparations show the copper signal of cytochrome oxidase in the oxidized form and the "$g = 1.94$" resonance of nonheme iron proteins on reduction, either enzymically with DPNH or succinate or chemically with dithionite (12). As shown in Fig. 1, the main qualitative differences in the EPR spectra of the reduced

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TABLE I

COMPARISON OF THE COMPOSITION[a] OF ETP FROM *C. utilis* AND *S. cerevisiae*[b]

Preparation	Total flavin	Coenzyme Q	Nonheme iron	Labile S	Copper	Cytochromes			
						$c + c_1$	b	$a + a_3$	Total
ETP of *C. utilis*	0.33	0.27	2.0	2.4	1.5	0.39	0.46	0.99	1.8
ETP[b] of *S. cerevisiae*	0.95	1.6	2.6	—	1.0	0.61	0.77	0.78	2.2

[a] All values are the average of data for three or more preparations and are expressed as mμmoles/mg of protein except as noted in the Table.

[b] Values previously reported by Mackler *et al.* (9).

ETP from the two organisms is the location of the shoulder on the principal high-field resonance. In *S. cerevisiae* the shoulder appears unusual and may represent the middle component of an anisotropic *g*-tensor. Both DPNH and succinate (though to a lesser extent) elicit this signal, which suggests that it is related to a component of the enzyme common to the pathways of electron transport for both substrates. With *C. utilis*, however, the shoulder appears to be due to a second species of iron associated with the succinic dehydrogenase portion of the ETP, for whereas reduction with DPNH produces the whole signal, succinate gives only that component responsible for the shoulder.

In summary, ETP prepared from *C. utilis* appears to differ from ETP from *S. cerevisiae* and resembles heart muscle ETP by containing a second nonheme iron component which is reduced by DPNH but not by succinate, and which may therefore be associated with the DPNH dehydrogenase portion of the system as suggested by the EPR studies described above. In addition, the *C. utilis* preparations contain FMN in addition to FAD, and the DPNH oxidase activity is inhibited by low concentrations of seconal and rotenone. The similarity in structure of the heart muscle preparations and the ETP from *C. utilis* suggests the possibility that the sensitivity of DPNH oxidase activity to seconal and rotenone and the substrate level phosphorylation associated with DPNH oxidation in heart muscle may be related to the FMN component and/or to the nonheme iron component which are not present in the ETP of *S. cerevisiae*. Finally, the ETP from *C. utilis* differs from similar preparations from heart in that the succinic oxidase activity of the preparations under certain conditions is highly sensitive to inhibition by seconal.

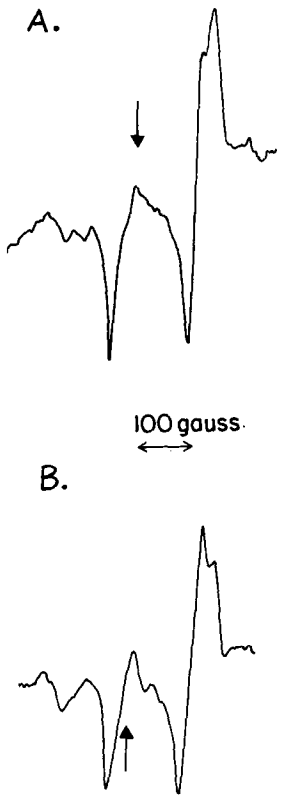

FIG. 1. Electron paramagnetic resonance spectra of ETP from *S. cerevisiae* (A) and *C. utilis* (B) reduced with excess DPNH. Protein concentration was 20 mg/ml. Modulation amplitude, 12 gauss; microwave power, 27 mW; scan speed, 400 gauss/minute; time constant, 1 second; Temperature 68°K; Varian V-4500, X Band Spectrometer. The vertical arrows denote $g = 2$; the magnetic field increases from left to right.

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REFERENCES

1. MACKLER, B., COLLIPP, P. J., DUNCAN, H. M., RAO, N. A., AND HUENNEKENS, F. M., *J. Biol. Chem.* **237**, 2968 (1962).
2. MAHLER, H. R., MACKLER, B., GRANDCHAMP, S., AND SLONIMSKI, P. P., *Biochemistry* **3**, 668 (1964).
3. COWGER, M. L., LABBE, R. F., AND MACKLER, B., *Arch. Biochem. Biophys.* **96**, 583 (1962).
4. RAO, N. A., FELTON, S. P., HUENNEKENS, F. M., AND MACKLER, B., *J. Biol. Chem.* **238**, 449 (1963).
5. DUNCAN, H. M., AND MACKLER, B., *Biochemistry* **5**, 45 (1966).
6. MACKLER, B., *in* "Non-Heme Iron Proteins: Role in Energy Conversion" (A. San Pietro, ed.), p. 421. Antioch Press, Yellow Springs, Ohio (1965).
7. MACKLER, B., *Biochim. Biophys. Acta* **50**, 141 (1961).
8. SHARP, C. W., Doctoral dissertation, Indiana University (1966).
9. MACKLER, B., DOUGLAS, H. C., WILL, S., HAWTHORNE, D. C., AND MAHLER, H. R., *Biochemistry* **4**, 2016 (1965).
10. CRANE, F. L., *Ciba Found. Symp. Quinones in Electron Transport* p. 414. Little, Brown, Boston (1961).
11. MACKLER, B., AND GREEN, D. E., *Biochim. Biophys. Acta* **21**, 6 (1956).
12. BEINERT, H., HEININ, W., AND PALMER, G., *Brookhaven Symp. Biol.* **15**, 229 (1962).

CHARLES W. SHARP[2]
BRUCE MACKLER[3]
H. C. DOUGLAS

Departments of Pediatrics, Microbiology, and Genetics
University of Washington
Seattle, Washington

GRAHAM PALMER[3]

Biophysics Research Division
University of Michigan
Ann Arbor, Michigan

S. P. FELTON

Children's Orthopedic Hospital
Seattle, Washington
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