STUDIES OF THE DPNH-CYTOCHROME b SEGMENT OF THE RESPIRATORY CHAIN OF BAKER'S YEAST

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In the accompanying paper the preparation of phosphorylating submitochondrial particles from baker's yeast (Saccharomyces cerevisiae) was described. It was shown that the particles as well as the yeast mitochondria investigated in these studies did not catalyze oxidative phosphorylation at the DPNH-cytochrome b phosphorylation site. Since it was considered possible that this deficiency might be reflected in the composition of the electron transfer chain, an investigation of the DPNH-cytochrome b segment of mitochondria and of submitochondrial particles from baker's yeast was undertaken. This paper presents evidence that the DPNH-cytochrome b segment of S. cerevisiae lacks one or more components found in the corresponding segment of the mammalian cell respiratory chain.

Results and Discussion -- Addition of DPNH to phosphorylating submitochondrial particles from baker's yeast (1) causes essentially complete reduction of cytochromes a + a_3, c + c_1, and b as well as extensive bleaching in the

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spectral region between 450 and 500 mμ. Succinate reduces cytochromes a + a_3, c + c_1, and b to nearly the same extent as DPNH, but flavoprotein reduction is considerably less with succinate than with DPNH. The experiments in Figure 1 indicate that DPNH reduces the flavin moieties of both DPNH dehydrogenase and succinate dehydrogenase, whereas succinate does not reduce DPNH dehydrogenase. Similar results were obtained when reduction of flavin was measured fluorometrically.**

Previous studies (2,3) have shown that the electron transfer chain from DPNH to oxygen in submitochondrial particles from beef heart and various other mammalian tissues contains at least two functionally distinct groups which are sensitive to mercurials. One group is necessary for electron transfer between flavoprotein and cytochrome b and is slowly

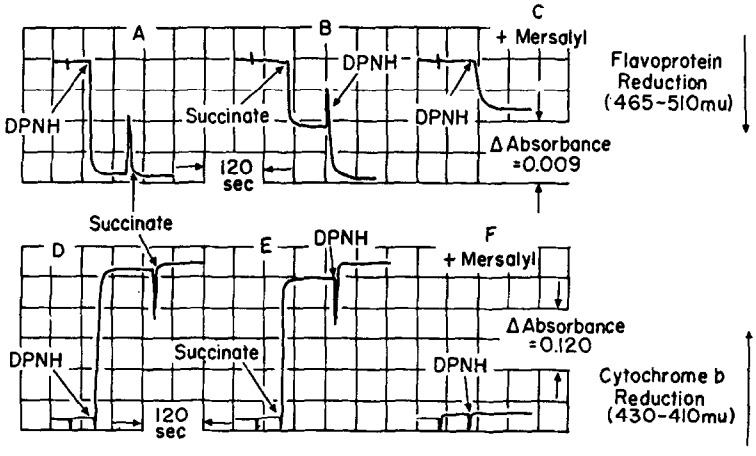

Fig. 1. Reduction of flavoprotein and cytochrome b in phosphorylating submitochondrial particles from yeast on addition of succinate (10 μmoles) or DPNH (1.5 μmoles) in the absence (A,B,D,E) or presence (C,F) of mersalyl (25 μmoles). The measurements were carried out (7) in an Aminco-Chance Dual Wavelength Spectrophotometer at the wavelengths indicated. All experiments were performed at 23° in a total volume of 2.5 ml containing 125 μmoles potassium phosphate buffer pH 7.4, 4.0 μmoles KCN and 7.7 mg particle protein. In the experiments with mersalyl the particles were incubated with the inhibitor for two minutes before the addition of DPNH.

** We are indebted to P. Hinkle for performing the fluorometric measurements.

inactivated by the mercurial mersalyl. The second group, detected (3) in beef heart submitochondrial particles pretreated with DPNH, is required for electron transfer from DPNH to flavoprotein and is rapidly reactive with mersalyl. In yeast particles, however, only one point of inhibition, namely between flavoprotein and cytochrome b, could be detected (Figs. 1C, F). The extent of inhibition of DPNH oxidase by mersalyl depended on the concentration of mersalyl, the ratio of mersalyl to particle protein and the type of buffer used. Reduction of the DPNH-flavoprotein by DPNH was not inhibited even when the particles were treated with DPNH prior to or during the exposure to mersalyl. Mackler (4) has observed that treatment of a purified preparation of DPNH dehydrogenase from yeast with mercurials does not inhibit the reduction of flavin by DPNH. The spectrophotometric results obtained with yeast phosphorylating particles were confirmed by determining the reduction of flavoprotein fluorometrically as well as by

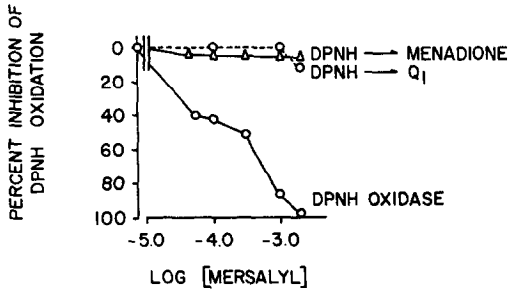

Fig. 2. Effect of mersalyl on DPNH oxidation with oxygen (O—O), coenzyme Q_1 (O---O) and menadione (Δ—Δ) as electron acceptors in phosphorylating submitochondrial particles of yeast. Aliquots of the particles (representing 460 µg protein) were incubated with the indicated concentrations of mersalyl for 60 minutes in a final volume of 1.5 ml in the presence of 150 µmoles potassium phosphate buffer pH 7.4. The samples were then placed into ice and assayed immediately. All reactions were assayed spectrophotometrically at 340 mµ in a Cary model 14 spectrophotometer equipped with a 0-0.1 slidewire. DPNH oxidase was measured in the presence of 100 µmoles potassium phosphate buffer pH 7.4, 0.06 µmole DPNH and 22 µg particle protein. The reference cuvette lacked DPNH. DPNH-Q_1 and DPNH-menadione reductase activities were assayed in the presence of 1.6 µmoles KCN with 0.07 µmole Q_1 or 0.4 µmole menadione as electron acceptors. The particle concentrations were 3.1 µg/ml and 12.4 µg/ml respectively. The measurements were carried out in a total volume of 1.0 ml at a temperature of 23°. The specific activities of DPNH oxidase, DPNH-Q_1 reductase and DPNH-menadione reductase in the controls were 0.87, 7.25 and 2.3 respectively. Specific activity is expressed as µmoles of DPNH oxidized per minute per mg protein.

assaying the activity of the particle bound DPNH dehydrogenase with CoQ_1 or menadione as artificial electron acceptors (Fig. 2). These experiments show that the mersalyl-sensitive group which is present in the DPNH-flavoprotein in mammalian mitochondria is either missing or masked in mitochondria from S. cerevisiae.

A further difference between the DPNH dehydrogenase region of mitochondria of S. cerevisiae and that of various mammalian cells was revealed by electron paramagnetic resonance (EPR) measurements. The EPR spectrum shows one prominent signal situated at about g = 2.03, which presumably represents copper associated with cytochrome oxidase (5). Reduction of the particles with dithionite, succinate or DPNH gave rise to signals at about g = 1.92, g = 1.94 and g = 2.03, all of which have been previously observed in submitochondrial particles or enzyme preparations from mammalian mitochondria (6). The signal at g = 1.94 is believed to represent a species of non-heme iron associated with both succinate dehydrogenase and DPNH dehydrogenase (6). It was found that in the submitochondrial particles from yeast, succinate induced a signal at g = 1.94 nearly identical in amplitude with that induced by DPNH or by DPNH plus succinate (Fig. 3B). In beef particles, however, the signal with DPNH was much greater than with succinate (Fig. 3A).

These observations suggested that in submitochondrial particles from S. cerevisiae the DPNH dehydrogenase region does not contribute to the g = 1.94 signal. An independent corroboration of this result was provided by EPR spectra of yeast particles in which electron transfer from the flavoprotein of DPNH dehydrogenase to cytochrome b had been inhibited by mersalyl. DPNH no longer elicited an EPR signal at g = 1.94, even though spectral (Fig. 1C) and fluorometric measurements indicated that the reduction of the flavin of DPNH dehydrogenase was not impaired.

In summary, the DPNH dehydrogenase region of yeast mitochondria employed in the present experiments differs from that of several mammalian

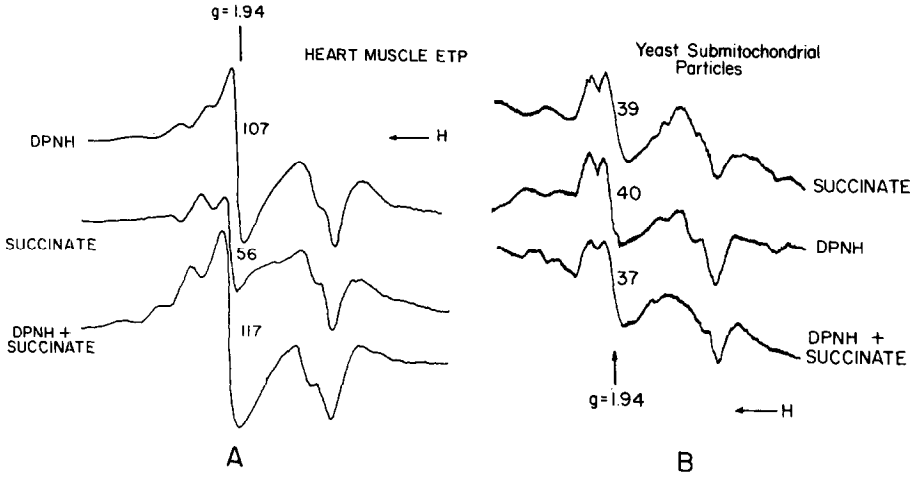

Fig. 3. EPR spectra of phosphorylating submitochondrial particles from yeast and of beef heart. Samples of yeast particles, representing 31 mg of protein in 0.45 ml of 0.1 M potassium phosphate buffer, pH 7.4, were incubated at 23° for about 5 minutes with either succinate (16 mM), DPNH (1.0 mM), or succinate (16 mM) and then after 5 minutes DPNH (1.0 mM). The samples were then frozen in liquid nitrogen and first derivative spectra were recorded with a Varian Model V4280A EPR spectrometer. Comparable samples of heart muscle ETP (8), 25 mg of protein in 0.5 ml of 0.1 M potassium phosphate buffer, were treated in an identical manner. The numbers next to the signal at g = 1.94 denote the peak to peak amplitude in arbitrary units. The microwave power was ~ 10 mWatts, the modulation amplitude ~ 2 gauss. The sensitivity chosen for ETP was 250, that for the yeast particles 400. The measurements were carried out at the temperature of liquid nitrogen.

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mitochondria by its lack of both a mercurial-sensitive group functionally situated between DPNH and flavoprotein as well as an EPR signal at g = 1.94. These results are of interest in connection with the lack of oxidative phosphorylation at this site and its insensitivity to rotenone.

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