EXISTENCE OF TWO DISTINCT HIPIP-TYPE IRON-SULFUR CENTERS IN MITOCHONDRIA

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Two distinct Hipip type iron-sulfur centers are present in pigeon heart mitochondria. These two can be distinguished by their EPR spectra which differ in the detailed line shape, field position and temperature dependence. These two seem to correspond to Center S-3, and an iron-sulfur protein purified by Ruzicka and Beinert. They exhibit different thermodynamic behavior and topographical location in the mitochondrial membrane.

A soluble iron-sulfur protein was isolated from the supernatant of sonicated beef heart mitochondria (1). This component is paramagnetic in the oxidized form, similar to bacterial high potential iron-sulfur proteins (Hipip) (2), but it exhibits a different EPR spectrum from Hipip with a rather symmetric resonance absorbance centered around g = 2.01. An iron-sulfur species responsible for a similar EPR absorbance was also concentrated in the succinate-ubiquinone reductase [Complex II (3)] (4).It was not possible at that time to ascertain whether or not these two Hipip species originated from a single component or whether there were two different Hipip species in the mitochondria. The uncertainty arose partly from the observation that the Hipip-type signal was reported not to be detectable in the succinate dehydrogenase (SDH) molecule which contained the same number of non-heme iron and acid labile sulfide per flavin as in Complex II (4). Recently a Hipip type iron-sulfur center was identified as being a constituent of the SDH molecule (Fe:S:flavin = 8:8:1), but it can only be detected in the unmodified, physiologically active SDH due to the extreme lability of the Hipip species after removal of the enzyme from the

membrane (5). The Hipip-type center in SDH was designated Center S-3 following Centers S-1 and S-2 (6), two ferredoxin type centers also found in the succinate dehydrogenase.

This communication provides direct evidence for the existence of at least two distinct Hipip type iron-sulfur centers in mitochondria, having different EPR characteristics and location in the mitochondrial membrane.

MATERIALS AND METHODS

Pigeon heart mitochondria and submitochondrial particles were prepared as described previously (7,8). EPR measurements were performed with a Varian E4 spectrometer. The temperature of the samples for EPR measurements was controlled using a variable temperature cryostat (Air Products Model LTD-3-110). Temperature was determined with a carbon resistor. The magnetic field was calibrated with the free radical signal of crystalline Diphenylpicryl-hydrazyl and commercial standard weak pitch.

RESULTS AND DISCUSSION

Fig. 1 is the temperature profile of Hipip-type EPR absorbance measured in both pigeon heart mitochondria and submitochondrial particles. Relative signal height was determined as a peak height from a low magnetic field base line. As presented in the top figure, Hipip-type resonance in mitochondria can be detected over a wide range of temperature, namely at all temperatures obtainable below 25°K (at a power setting of 10 mW). The temperature dependence of this signal in mitochondria indicates that more than one component contributes to the signal.

In contrast, in submitochondrial particles, a Hipip-type signal can be detected only at temperatures below 15°K with a sharp peak around 8°K. As will be described below, this component corresponds to Center S-3. Assuming that signals measured in pigeon heart mitochondria below 7.3°K arise only

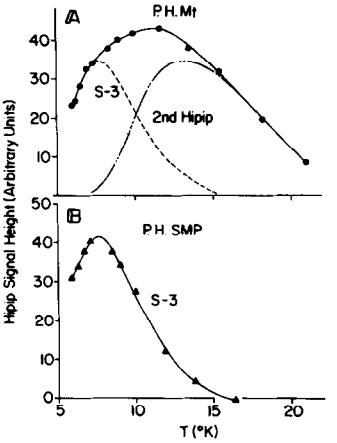

Figure 1: Temperature profile of the EPR signals arising from Hipip-type iron-sulfur centers, present in pigeon heart mitochondria and submitochondrial particles.

Pigeon heart mitochondria and submitochondrial particles were suspended in 0.20 M mannitol, 0.05 M sucrose and 30 mM morpholinopropane sulfonate buffer (pH 7.2) at protein concentrations of 36.8 mg/ml and 36.0 mg/ml, respectively. Ferricyanide at a final concentration of 3 mM was added to both suspensions to oxidize the iron-sulfur centers. EPR operating conditions were: magnetic field modulation, 100 kHz; modulation amplitude, 5 gauss; microwave power, 10 mW; microwave frequency, 9.14 GHz; time constant 0.3 sec; scanning rate, 500 gauss/min. Peak heights from the low field base line were plotted as a function of sample temperature.

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from Center S-3, Center S-3 contribution to the mitochondrial Hipip signals can be extrapolated as shown by the dotted line in Fig. 1 (top), analogous to the Center S-3 temperature profile in submitochondrial particles. Thus, the temperature dependence curve of mitochondrial Hipip-type signals was resolved into two species simply by subtraction of the dotted line from the overall curve. The two resolved curves indicate the presence of at least one additional Hipip-type iron-sulfur center in mitochondria which is detectable at much higher temperatures. EPR spectra of the second Hipip-type center can be obtained without interference from Center S-3 at temperatures above 15°K at 10 mW power setting. A comparison of the temperature profiles of Hipip signals in mitochondria and submitochondrial particles reveals that only the second Hipip species is removed from the mitochondrial membrane during particle preparation, but not the low temperature detectable Hipip center, Center S-3. Thus, EPR spectra of low temperature detectable Hipip species can be studied

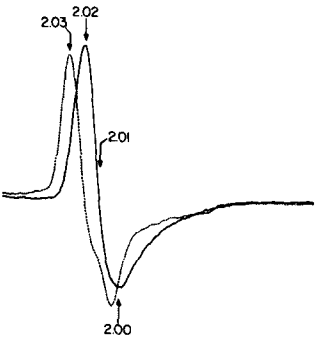

Figure 2: Typical EPR spectra of two distinct Hipip-type iron-sulfur centers. The Center S-3 (a Hipip-type center present in the succinate dehydrogenase) spectrum is represented by a solid line, which was obtained from pigeon heart submitochondrial particles. The spectrum of the second Hipip-type iron-sulfur center is represented by a dotted line, measured in pigeon heart mitochondria. Experimental conditions were the same as described in the legend of Fig. 1. EPR operating conditions were the same as in Fig. 1, except: scanning rate, 200 gauss/min; sample temperatures for spectrum shown by solid and dotted lines, 7°K and 15°K, respectively. The gain of the vertical scale used for the spectrum in the dotted line was 1.25 times higher than that for the solid line spectrum.

in the submitochondrial particle preparation without detectable interference from the second Hipip species at any temperature.

A Hipip spectrum obtained from submitochondrial particles is presented in Fig. 2 as a solid line. This Hipip-type absorption is centered at g = 2.01 with a peak to peak width of 25 gauss which is the same as the Center S-3 spectrum from the succinate-UQ reductase (5). An EPR spectrum of the second Hipip-type center obtained in pigeon heart mitochondria at 15°K (Fig. 2, spectrum in dotted line) is compared with that of Center S-3 obtained in submitochondrial particles. This spectrum is centered at g = 2.02 and the peak and trough positions are, respectively, approximately 12 and 5 gauss lower than those of Center S-3. A shoulder is discernible in the center of this latter signal. The EPR absorbance showing at a higher magnetic field seems to arise from copper in the cytochrome oxidase. The EPR line shape of the second Hipip species in pigeon heart mitochondria resembles that of the Hipip protein purified by Ruzicka and Beinert from the supernatant of sonicated beef heart mitochondria. In mitochondria at temperatures below

7°K, the EPR spectrum of the Hipip species approaches that of Center S-3.

The topological difference of these two Hipip-type iron-sulfur centers in mitochondrial inner membrane was examined using the membrane impermeable iron-chelator bathophenanthroline sulfonate [BPS](9) . The Center S-3 signal was highly quenched by BPS treatment of submitochondrial particles but not in mitochondria, while the second Hipip-type signal is over 75% quenched in BPS treated mitochondria (10). These results indicate that Center S-3 is located on the inside (matrix side) of the osmotic barrier of the mitochondrial inner membrane, while the second Hipip species, detectable only in whole mitochondria, is located on the outside (cytoplasmic side) of the mitochondrial inner membrane, similar to cytochrome c. These results clearly demonstrate that the Hipip-type iron-sulfur center in the succinate dehydrogenase, namely, Center S-3 (5), is a distinct entity from the Hipip species reported by Ruzicka and Beinert (1).

The midpoint potentials (pH 7.2) of Center S-3 and the second Hipip species measured in pigeon heart mitochondria are approximately +120 mV (11) and approximately +150 mV, respectively. These two centers also reveal different redox behavior in various metabolic states of the mitochondria (10). The physiological function of the second Hipip species and its exact location in the respiratory chain are as yet unknown.

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