A MICROSOMAL IRON-SULFUR CENTER IN RAT LIVER*

J.C. Salerno and W.J. Ingledew

Johnson Research Foundation
University of Pennsylvania
Philadelphia, Pa. 19174, U.S.A.

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A hitherto unreported ESR signal, with features at g = 1.93 and g = 1.88, has been observed in the microsomal fraction of rat liver. This new species is paramagnetic in the reduced form, and has a midpoint potential of approximately -270 mV. The field positions of the signals, their midpoint potential and their temperature dependence are unlike those of any known mitochondrial non-heme iron component. The failure of previous attempts to observe an iron-sulfur center in rat liver microsomes may be due to the partial overlap of the high field component of the cytochrome P-450 signal and the signals of the non-heme iron. Resolution of these components has been achieved by the combination of EPR spectroscopy and redox potentiometry.

Iron-sulfur proteins have been shown to be involved in the transfer of electrons from flavoprotein to the cytochrome P-450-substrate complex in several P-450 containing systems (1). Such iron-sulfur proteins have been characterized in bacteria, in adrenal cortex mitochondrial microsomes (2), and in rat kidney cortex microsomes (3). In the latter, an iron-sulfur protein with an ESR spectrum having features at g = 2.01, 1.93 and 1.88 has been implicated in reduction of cytochrome P-450 by NADPH. In the present study an iron-sulfur type signal with a midpoint potential in the region of minus 270 mV is observed in the microsomal fraction of livers from Buffalo rats fed a normal rat diet. Features were observed at g = 1.93 and 1.88. The role of this non-heme iron protein in microsomal function has not yet been elucidated.

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METHODS - Unstarved male Buffalo rats (200-250 g) were killed by decapitation and the livers quickly removed. Microsomes were prepared by a modification of the method described by Remmer et al. (4) using a 225 mM mannitol, 75 mM sucrose, 100 μM EDTA medium at pH 7.2. At the same time mitochondria were prepared by the method of LaNoue (5) for the purpose of comparison.

Since Morris hepatomas have been found to contain somewhat lower levels of P-450 than normal liver (6), microsomes from Morris hepatoma 9633 were examined to provide clearer spectra of the non-heme iron component. Microsomes were prepared as above, but an additional centrifugation at 12,000 g for 20 minutes was inserted to remove any slowly sedimenting mitochondria which may be present in the tumors.

Redox titrations were performed by the method of Dutton et al. (7) as applied to ESR by Wilson et al. (8). ESR measurements were performed on a Varian E-4 microwave spectrometer. Low temperatures were attained by a liquid helium flow through an Air Products transfer line. Fine control was provided by ohmic heating; temperature was measured by a calibrated carbon resistor.

RESULTS - EPR spectra of rat liver microsomes under oxidized and reduced conditions are shown in Fig. 1. In the oxidized form the dominant features are those of P-450 type cytochromes, at g = 2.42, g = 2.25 and g = 1.90 (10). P-450 type cytochromes are paramagnetic only in the oxidized form; therefore upon reduction these signals will be lost. This can be seen in the reduced sample. The spectrum of the reduced sample shows, in the region of g = 1.93, a new signal overlapped with the remainder of the P-450 signal. This new species is paramagnetic in the reduced form.

The g = 2.42 and g = 2.25 cytochrome P-450 signals titrate simultaneously and the g = 2.25 signal can be seen plotted against E_h in Fig. 2. Two n=1 components are suggested by these titrations of the

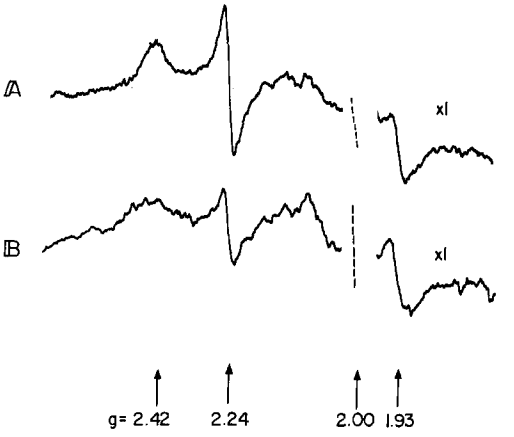

Figure 1: ESR spectra of rat liver microsomes. Temperature 20°K, power = 20 mW; modulation amplitude = 10 gauss; time const. = 1 sec; scan rate = 250 gauss/min; microwave frequency = 9.134 GHz.
A) Redox potential - 100 mV
B) Redox potential - 395 mV

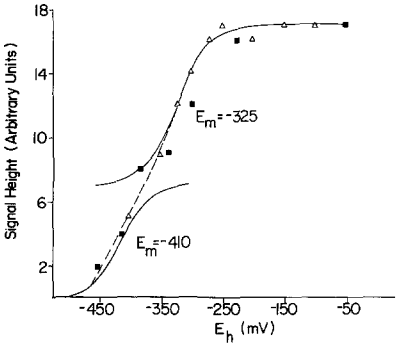

Figure 2: E_h vs. signal height of g = 2.24 signal in rat liver microsome samples. ESR conditions as in Fig. 1.

cytochrome; these have midpoint potentials at -325 mV and -410 mV (pH 7.2). The existence of two components with different midpoint potentials is consistent with previous observations using spectrophotometric techniques (11). As there may be at least two cytochromes of this type present in liver, cytochromes P-450 and P-446 (12), further experiments are needed to clarify the relationship of the two cytochromes to the two different

E_m's. It has been noted that the low potential cytochrome component is selectively depleted in Hepatoma 9633 preparations. This allowed the full reduction of the cytochrome content (not possible in the presence of the low potential component), and the observation of the non-heme iron signal free from overlap by the cytochrome spectrum.

In Fig. 3 spectra from a reduced Morris hepatoma (9633) microsomal preparation are displayed against spectra of reduced hepatoma mitochondrial preparations. Both microsomal and mitochondrial preparations show a g = 1.93 absorption at 14°K (in mitochondria this signal is due to center N-2, a highly temperature-sensitive component (9,13). The temperature profile of this signal in the two systems is very different, indicating that they are due to different components. Further evidence for the absence of mitochondrial contaminants in the microsomal preparations

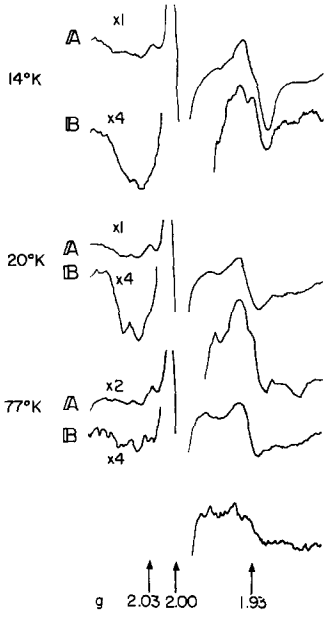

Figure 3: ESR spectra of microsomes and mitochondria from Morris hepatoma 9633. ESR conditions as in Fig. 1, except as noted; E_h = minus 450 mV.
A) Mitochondria (40 mg/ml protein)
B) Microsomes - the microsomal spectra taken at 20°K is an average of 16 scans. (40 mg/ml protein).

comes from the absence of the mitochondrial g = 2.06 and g = 2.03 signals (9,13) in the microsomal spectra.

The microsomal spectrum taken at 20°K and shown in Fig. 3 is the output of a computer of average transients. This was done to improve resolution; features at g = 1.93 and g = 1.88 can be seen. We have been unable to determine whether the non-heme iron center possesses a g = 2.01 absorption due to interference from free radical signals at g = 2.00, originating from the redox dyes.

Fig. 4 shows two theoretical n = 1 lines, one for a cytochrome, paramagnetic in the oxidized form, and one for a ferredoxin-type iron-sulfur center, paramagnetic in the reduced form, and their sum. The plots correspond to components with midpoint potentials at -270 mV (non-heme iron) and -325 mV (cytochrome). The points are from a redox titration of the g = 1.93 region; it can be seen that the behavior of the g = 1.93 region is very different during a redox titration than the g = 2.25 region shown in Fig. 2. The fit of the points to the theoretical curve is fairly good. The points are taken from redox titrations of rat liver and hepatoma micro-

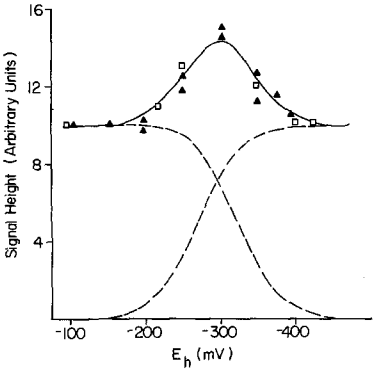

Figure 4: Dotted lines - theoretical n=1 curves for oxidation-reduction of two components, one paramagnetic in the reduced form, the other paramagnetic in the oxidized form; E_m = -270 and -325 mV. Solid line = sum of the two curves described above as a model for oxidation-reduction of P-450 and non-heme iron signals overlapped at g = 1.93.

somal samples; the spectra were taken at 20°K. The theoretical line for the cytochrome 'P-450' change is confirmed by the redox behavior of its other EPR resonances at g = 2.25 and g = 2.42 (Fig. 2). The lowest potential points for the liver microsomal preparations are not shown because of changes in the low potential P-cytochrome which would further complicate the curve. The hepatoma microsomes do not contain the lower potential cytochrome.

DISCUSSION - Iron-sulfur centers detected by EPR and found in microsomal fractions have hitherto been shown to arise from mitochondrial contamination (14). We believe that this possibility has been ruled out in the present case by the absence of signals in the g = 2.03 (Rieske, S-1, S-2, N-1a and N-1b) and the g = 2.06 region (N-2) (9,13) in the microsomal preparations, and by the obvious differences in lineshape and temperature dependence of the mitochondrial and microsomal spectra. The resolution of an iron-sulfur center has been facilitated by the application of redox potentiometry to the study of microsomes. There is no known mitochondrial component which has an EPR spectrum and temperature profile similar to that reported for the microsomal non-heme iron center. It has been estimated from double integration of the spectra under non-saturated conditions, that the non-heme iron component is present at a concentration of approximately one-tenth that of cytochrome P-450.

Some years ago it was proposed (see 15), on the basis of inhibitor studies, that a component X was functioning between flavin and cytochrome P-450. However, when X could not be identified and a purified reconstituted system which contained no non-heme iron was shown to be able to carry out reduction of P-450, component X was forgotten. This iron-sulfur center could be component X; alternatively, it could be in an enzyme complex associated with the microsomal preparation.

The present studies need to be expanded to ascertain the function

of the non-heme iron center and to relate the two different midpoint potentials of the P-450 type cytochromes to spectroscopic data and data from drug induction experiments.

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