MÖSSBAUER SPECTROSCOPY OF LYOPHILIZED RAT LIVER MICROSOMES

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Summary. The Mössbauer spectrum was obtained of lyophilized liver microsomes prepared from rats who had been treated with phenobarbital. The spectrum showed that there were two iron sites one of which was high-spin. This was confirmed from the electron spin resonance of the powder, which indicated that low-spin iron also is present. These results suggest that there may have been a conversion of some of the cytochrome P-450 (low spin) into the high spin P-420 form, or that there exists a low-high spin equilibrium in the lyophilized preparations.

Mössbauer spectroscopy provides information concerning the oxidation state, spin state and the configuration of the ligands around iron atoms. As part of a program to study the nature of iron in rat liver microsomal cytochrome P-450, the present studies were undertaken to evaluate the usefulness of this method for this problem. We report in these first experiments the resulting spectra obtained with lyophilized preparations of rat liver microsomes from animals treated with phenobarbital.

Experimental

The rats were treated with phenobarbital (80 mg/kg/day i.p.) for five days and washed microsomes prepared (1). The liver microsomes were analyzed by the spectrophotometric method of Omura and Sato (2) after suspension in water and then lyophilized. They contained 1.05 μm of cytochrome P-450 and 0.27 μm of cytochrome b_5/g of lyophilized microsomes.

The Mössbauer spectrometer used an electromagnetic drive in the acceleration mode with moving absorber geometry (3). The gamma radiation was detected with gas-flow proportional counter using 10% methane and 90% argon (4). The source was 30 mC ^{57}Co in a palladium matrix. About one-half gram samples of microsomes were pressed into pellets. A cryostat previously described by Travis and Spijkerman (5) was used for the measurements at liquid nitrogen temperature.

The electron spin resonance (esr) of the powder was measured at liquid nitrogen temperature using the Varian Model V-4500 spectrometer equipped with 100 kc. field modulation.

Results and Discussion

Typical Mössbauer spectra are shown in Fig. 1 at room and liquid nitrogen temperatures. The parameters after fitting the data for two Lorentzians are quadrupole splitting-0.64 $\pm$ 0.10 mm/sec and isomer shift-0.64 $\pm$ 0.03 mm/sec at room temperature for three samples and 0.57 $\pm$ 0.04 mm/sec and 0.58 $\pm$ 0.04 mm/sec, respectively, for two samples at liquid nitrogen temperature. These values are relative to sodium nitroprusside. The Mössbauer parameters of high-spin ferric complexes do not change much with temperature as is found in the spectra of the microsomes. Thus, these data indicate that the major portion of the iron in the microsomes is in the high-spin ferric form. Since the amount of cytochrome b_5 is small, the Mössbauer spectra result primarily from the cytochrome P-450. Using a constraint computer program, the data can be fitted to four Lorentzians suggesting that there are two iron sites present (See Fig. 1).

The esr study (Fig. 2) shows absorption near g values of 6.0, 4.3, 2.46, 2.26, 1.9 and a doublet(2.0). These are characteristic of spectra observed with high-spin (g values near 6.0, 4.3 and 2.0) and low-spin ferri hemoproteins (g values near 2.46, 2.26 and 1.9). The low-spin components in the spectrum cannot arise from the cytochrome b_5 since it has been shown that the esr signal from this component in frozen solutions is much smaller than the signals arising from the cytochrome P-450 (6). However, no results have been published for lyophilized samples of cytochrome b_5.

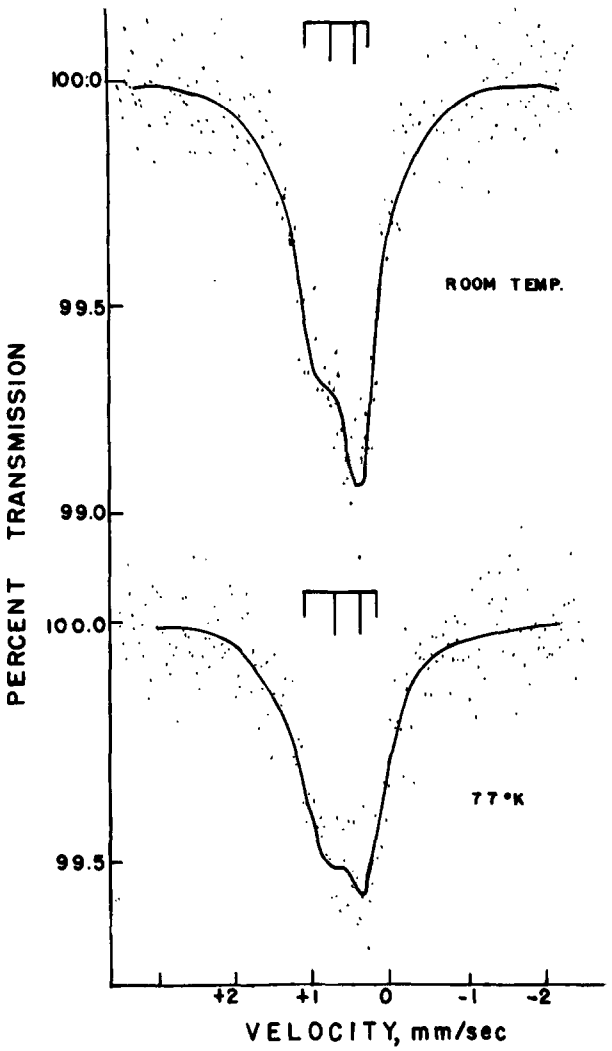

Fig. 1. Mössbauer spectra of 583 mg. of lyophilized rat liver microsomes. Top: Room temperature. Bottom: 77°K.

On the basis of esr studies, Mason and coworkers found that the rat liver microsomal heme iron that had been identified with cytochrome P-450 was in the low-spin ferri form (7). This observation has been confirmed by a number of other workers. On the other hand Hildebrandt, Remmer and Estabrook (8) found that both low- and high-spin forms of ferric iron exist in liver microsomes after induction with 3-methylcholanthrene. Our observation of a high-spin form may be due to a number of factors.

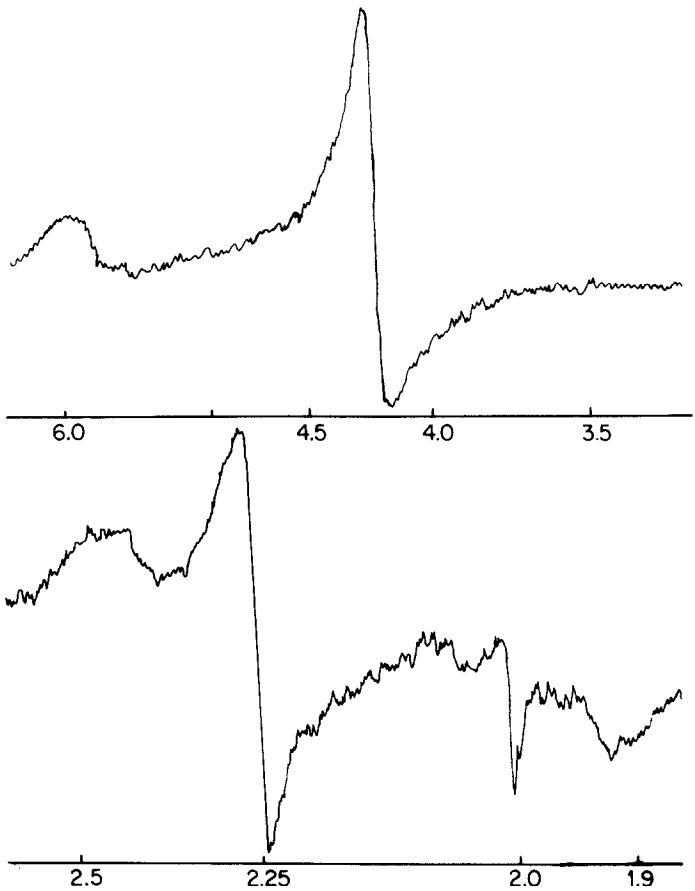

Fig. 2. Electron spin resonance of lyophilized rat liver microsomes.

It is possible that during lyophilization the iron is converted to the high-spin form. Although this seems to occur, it is unusual. Ferri-hemoproteins, which contain low-spin iron in solution, such as cytochrome c, retain the low-spin state after lyophilization (9). Further, high-spin ferri-hemoproteins, such as acid metmyoglobin, become predominately low-spin with some portion of the iron retained in the high-spin state upon drying (10). This latter portion is ascribed to the formation of hemin on drying, which is either free or bound to a denatured portion of the protein.

Murakami and Mason (11) found that the treatment of cytochrome P-450 with acid or p-chloromercurobenzoate leads to the formation of a high-spin

cytochrome P-420, which they call the ω-state. It is possible that a similar change is occurring in the microsomes upon drying. The presence of both spin states suggests that an equilbrium between low-spin and high-spin forms may be present or that two separate iron sites coexist.

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References

1. Holtzman, J. L., Gram, T. E., Gignon, P. L., and Gillette, J. R., Biochem. J. 110, 407 (1968).

2. Omura, T. and Sato, R., J. Biol. Chem. 239, 2379 (1964).

3. Ruegg, F. C., Spijkerman, J. J., and DeVoe, J. R., Rev. Sci. Instr. 36, 356 (1965). J. R. DeVoe, Ed., NBS Technical Note 276 (1966) p. 89.

4. DeVoe, J.R., Ed. NBS Technical Note 404 (1966) p. 128.

5. Travis, J. C., and Spijkermann, J.J., Mössbauer Effect Methodol. 4, 237 (1968).

6. Bois-Poltoratsky, R. and Ehrenberg, A., European J. Biochem. 2, 361 (1967).

7. Mason, H.S., North, J. C., and Vanneste, M., Fed. Proc. 24, 1172 (1965).

8. Hildebrandt, A., Remmer, H., and Estabrook R.W., Biochem. Biophys. Res. Commun. 30, 607 (1968).

9. Cooke, R. and Debrunner, P., J. Chem. Phys. 48, 4532 (1968); Lang, G., Herbert, D., and Yonetani, T., J. Chem. Phys. 49, 944 (1968).

10. Caughey, W. S., Fujimoto, W. Y., Bearden, A. J. and Moss, T. H., Biochemistry 5, 1255 (1966).

11. Murakami, K. and Mason, H. S., J. Biol. Chem. 242, 1102 (1967).