EVIDENCE FOR THE PRESENCE OF AN IRON SULPHUR PROTEIN IN RAT KIDNEY CORTEX MICROSOMES

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Received July 10,1972

SUMMARY

An electron paramagnetic resonance (EPR) study of the microsomal fraction isolated from rat kidney cortex has provided evidence for the presence of cytochrome P-450 in the low-spin form (g = 2.42, 2.25 and 1.91) but also revealed the appearance of an EPR signal (g = 2.01, 1.93 and 1.88) of increasing intensity upon reduction with NADPH or dithionite. The characteristics of this signal lead us to suggest that it is ascribable to an iron-sulphur protein presumably involved in the electron transfer from NADPH to cytochrome P-450.

In many cytochrome P-450 containing monooxygenase systems, iron-sulphur proteins have been shown to be involved in the transfer of electrons from the reduced flavoprotein to the cytochrome P-450-substrate complex. Such iron-sulphur proteins have been isolated and characterized from bacteria (1) as well as from adrenal mitochondria (2) and their role in the monooxygenase process have partly been elucidated. Studies on microsomal monooxygenase systems have, however, up to now failed to give evidence for the involvment of such an intermediate electron carrier between the flavin and cytochrome components of the monooxygenase system. In the present study, EPR analysis of the microsomal fraction isolated from kidney cortex has revealed signals with the characteristics of an iron-sulphur protein, presumably functioning in the reduction of cytochrome P-450 by NADPH.

MATERIALS AND METHODS

Unstarved, male Sprague-Dawley rats (200-300 g) were killed by decapitation and the kidneys immediately removed and placed in ice-cold 0.25 M sucrose. The renal pelvis and medulla were removed and the cortex cut into

small pieces and homogenized in four volumes of 0.25 M sucrose. After sedimentation of cell debris, nuclei and mitochondria by centrifugation at 17,500 g for 15 min, the microsomal fraction was pelleted by centrifugation at 123,000 g for 60 min. The microsomal pellets were washed once with 50 mM tris-Cl buffer, pH 8.0, resedimented by centrifugation at 123,000 g for 30 min and suspended in 0.25 M sucrose at a protein concentration of 30-50 mg per ml. Samples were analyzed according to standard procedures. A typical preparation contained ca 0.15 nmoles of cytochrome P-450, 0.15 nmoles of cytochrome b_5, 0.10 nmoles of catalase, and 0.05 nmoles of hemoglobin per mg protein.

EPR (electron paramagnetic resonance) spectra were obtained as described in the figure captions. The g-values were determined from simultaneous measurements of the magnetic field and the microwave frequency by means of an AEG magnetic field meter and an HP frequency counter (5245L) and converter (5255A).

RESULTS

Fig. 1 shows the EPR spectra of rat kidney cortex microsomes. Clearly discernible (Fig. 1 A) are the spectral features of the low-spin form of cytochrome P-450 with g-values of 2.42, 2.25 and 1.91 (3,4).

As shown in Fig. 1 B, prominent signals appeared upon reduction with NADPH in the presence of carbon monoxide. The g-values were 2.17, 2.11, 2.01, 1.93, 1.88 and 1.86. The signals at g = 2.01, 1.93 and 1.88 are distinguishable from the other new signals by being comparatively difficult to saturate by the microwave power and thus show the characteristics of an iron-sulphur centre, suggesting that it is ascribable to an iron-sulphur protein present in the microsomal fraction. Similar signals were observed after reduction with dithionite, in which case we did not use carbon monoxide.

Iron-sulphur signals from microsomal preparations have earlier been shown to come form mitochondrial impurities (5). Our preparations contained succinate oxidase and NADH oxidase activity. From the method of preparation we conclude that the microsomal samples may be contaminated only by some mitochondrial subfractions. A comparison was therefore made between the microsomal preparation and submitochondrial particles prepared from the kidney cortex mitochondria by sonication, but in all other respects treated as the microsomes. While the submitochondrial samples were 17 times higher in NADH oxidase activity, the EPR signal in the g = 1.94 region upon reduction with NADH was only 0.6 of that in the microsomes. The succinate oxidase activity in the submitochondrial particles was low and about equal to that in the microsomal sample. The increase of EPR signals

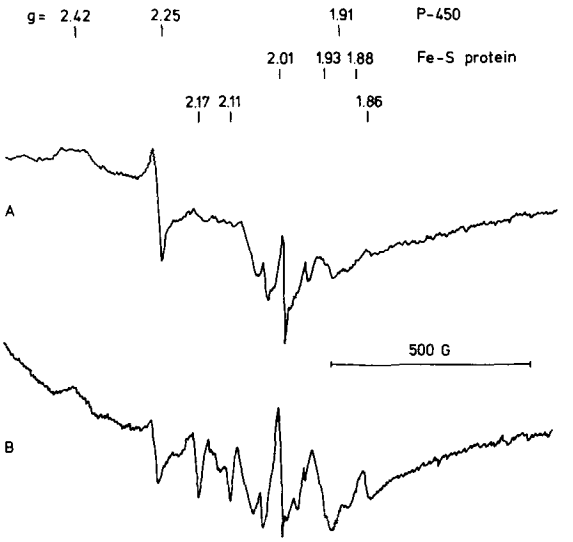

Fig. 1.

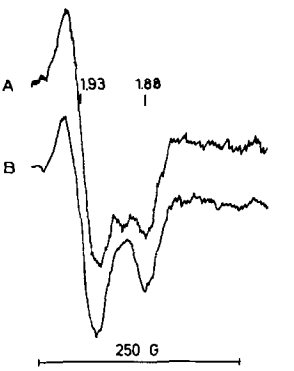

Fig. 2.

Fig. 1. EPR spectra from rat kidney cortex microsomes (42 mg protein/ml) at 90°K. A. Oxidized microsomes as prepared. B. Microsomes reduced with 15 mM NADPH after bubbling with carbon monoxide. Measurements were made on a Varian E-9 spectrometer with a large sample access cylindrical cavity and a Dewar insert for cold gas flow refrigeration. The sample tube had 6 mm inner diameter, and the sample volume was 1.2 ml. The microwave frequency was about 9.0 GHz, the power 50 mW, the modulation frequency 100 KHz, the modulation amplitude 8 G, the time constant 0.3 sec, and the scanning rate 250 G·min^{-1}. In each case 12 spectra were collected on a TMC CAT-1024 before recording.

Fig. 2. Details of EPR spectra from rat kidney cortex microsomes (50 mg protein/ml) at 77°K. A. Reduced with 6 mM NADPH. B. Reduced as A but in presence of 25 µM rotenone. Measurements were made on a Varian V-4502 spectrometer with a V-4531 multipurpose cavity and a liquid nitrogen cold finger. The sample tube had 3.5 mm inner diameter, and the sample volume was 0.4 ml. The microwave frequency was about 9.0 GHz, the power 30 mW, the modulation frequency 100 KHz, the modulation amplitude 15 G, the time constant 1 sec, and the scanning rate 100 G·min^{-1}

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at g = 1.93 upon addition of succinate were also about equal. The microsomal EPR signal due to iron-sulphur protein was, however, not fully developed until NADH or NADPH was added. The mitochondrial inhibitors rotenone and TTFA (Tenoyltrifluoroacetone) drastically decreased the mitochondrial iron-sulphur signals. When added to microsomes these inhibitors reduced the EPR intensity only where mitochondrial impurities were expected to contribute. This was especially striking for the spectral region shown in Fig. 2, where the reduced succinate dehydrogenase Fe-S intensity at g = 1.91 induces an increased resolution of the new signal at g = 1.88.

This signal at g = 1.88 is found to be linked with intensities at g = 1.93 and 2.01. The negative peak at g = 1.88 could possibly be confused with the negative parts of other signals which could occur in the same region. The microsomal signal is, however, clearly distinguished from that of mitochondrial NADH-ubiquinone reductase with g-values at 2.101, 1.886 and 1.864 (6), which only is detected at temperatures below 77°K, whereas the microsomal signal is well developed already at 77°K. It is also different from the absorption of the cytochrome b-c_1 Fe-S protein (7), which has the g-values 2.026, 1.90 and 1.809 (8), since there is no peak at g = 1.809 in the microsomes. We may thus conclude that there is a unique iron-sulphur centre in the microsomes characterized by the g-values 2.01, 1.93 and 1.88.

It is of interest to note that even when the iron-sulphur signal was well developed upon reduction with NADPH, there was still no or only a partial decrease in the intensity of the cytochrome P-450 low-spin signal.

Apart from the signals discussed also others were constantly observed upon reduction of microsomes from kidney cortex or from liver. Those at g = 2.17, 2.11 and 1.86 could be abolished by washing with EDTA and are thought to be due to contamination of Mn^{2+}.

DISCUSSION

Previous EPR analyses of cytochrome P-450 from Pseudomonas putida (9) and of untreated rabbit liver microsomes (10) have revealed cytochrome P-450 present in the low-spin form. The EPR signature for the low-spin form of this hemoprotein appears to be almost independent of the source and has been reported identical for aminal liver from different species (10). The same seems to hold true also for kidney cortex cytochrome P-450 which, in spite of exhibiting certain spectrophotometric and catalytic properties different from the liver hemoprotein (11), appears to have the same EPR characteristics.

The formation of the cytochrome P-450-Fe^{3+}-substrate complex is associated with a conversion of the low-spin form of the hemoprotein to high-spin (9,12). In the presence of reduced pyridine nucleotide and O_2, the reaction is then thought to proceed further via one-electron reduction to P-450-Fe^{2+}, formation of oxygenated P-450^{2+}, a second one-electron reduction of the oxygenated form, and the cleavage of the O-O bond concomitant with the product formation (13). In the bacterial (Pseudomonas putida) and adrenal mitochondrial monooxygenase systems so extensively studied, specific iron-sulphur proteins are required for the second reduction step in this sequence (13,14). Although it is too early to decide whether a similar reaction mechanism may apply also to a microsomal monooxygenase, the present observation of a NADPH-

reducible iron-sulphur protein in kidney cortex microsomes suggests that this may in fact be the case. Should it turn out to be true, it remains to understand why previous investigations (10) have failed to give evidence for the presence of a similar iron-sulphur protein in liver microsomes, unless one assumes that different cytochrome P-450 reductase systems operate in liver and kidney cortex microsomes. An alternative explanation would, however, be that the conditions for discovering this specific signal are more favourable in the kidney cortex microsomes than in the liver microsomes, possibly due to the several-fold lower concentration of cytochrome P-450 in the former fraction (11). It should be kept in mind that indirect evidence (15,16) has long suggested the involvment of an intermediate electron carrier between the flavin and cytochrome components also in the liver microsomal monooxygenase system.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (13X-2471 and 13X-542) and from the Swedish Natural Science Research Council (321-21).

REFERENCES

1. Cushman, D. W., Tsai, R. L. and Gunsalus, I. C., Biochem. Biophys. Res. Commun., 26, 577 (1967)
2. Suzuki, K. and Kimura, T., Biochem. Biophys. Res. Commun., 19, 340 (1965)
3. Mason, H. S., North, J. C. and Vanneste, M., Fed. Proc., 24, 1172 (1965)
4. Cammer, W., Schenkman, J. B. and Estabrook, R. W., Biochem. Biophys. Res. Commun., 23, 264 (1966)
5. Beinert, H. and Palmer, G., Adv. Enzymol., 27, 105 (1965)
6. Orme-Johnson, N. R., Orme-Johnson, W. H., Hansen, R. E., Beinert, H. and Hatefi, Y., Biochem. Biophys. Res. Commun., 44, 446 (1971)
7. Rieske, J. S., Hansen, R. E. and Zaugg, W. S., J. Biol. Chem., 239, 3017 (1964)
8. Albracht, S. P. J. and Slater, E. C., Biochim. Biophys. Acta, 245, 508 (1971)
9. Tsai, R., Yu, C. A., Gunsalus, I. C., Peisach, J., Blumberg, W., Orme-Johnson, W. H. and Beinert, H., Proc. Natl. Acad. Sci., 66, 1157 (1970)
10. Peisach, J. and Blumberg, W. E., Proc. Natl. Acad. Sci., 67, 172 (1970)
11. Ellin, Å., Jakobsson, S. V., Schenkman, J. B. and Orrenius, S., Arch. Biochem. Biophys., 150, 64 (1972)
12. Whysner, J. A., Ramseyer, J., Kazmi, G. M. and Harding, B. W., Biochem. Biophys. Res. Commun., 36, 795 (1969)
13. Huang, J. J. and Kimura, T., Biochem. Biophys. Res. Commun., 44, 1065 (1971)
14. Gunsalus, I. C., Tyson, C. A. and Lipscomb, J. D., In "International Symposium on Oxidases", Memphis, Tennessee, 1971
15. Ernster, L. and Orrenius, S., Fed. Proc., 24, 1190 (1965)
16. Dallner, G., Siekevitz, P. and Palade, G. E., J. Cell Biol., 30, 97 (1966)