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Does cytochrome P-420 exist? Haem reductase activity in liver microsomes

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THERE is considerable interest in the haemoproteins of liver microsomes, especially because exposure to drugs such as barbiturates or carcinogens such as benzo(α)pyrene, causes a massive increase in drug metabolising enzyme activity and in cytochrome P-450.[1]

Omura and Sato[2] showed that the main haemoprotein of liver microsomes had an optical absorbtion peak at 450 nm after reduction and treatment with carbon monoxide, (hence P-450). This peak is shifted to 420 nm by denaturing processes such as solubilisation with deoxycholate.

Attempts have been made to study the turnover of the haemoproteins by labelling the haem moiety with δ amino laevulinic acid, and isolating particles showing the P-420 peak at various times thereafter.[3,4] The presence of the P-420 peak is frequently used as an indicator that degraded cytochrome P-450 is present in a preparation.[5]

We have found that microsomes avidly bind haem,[7] and now find that this added haem can show a typical P-420 absorbtion spectrum, on reduction and treatment with carbon monoxide.

The bound haem can be reduced by dithionite or by NADPH, and there seems hardly any limit to the amount of P-420 that can be produced in this way (Fig. 1).

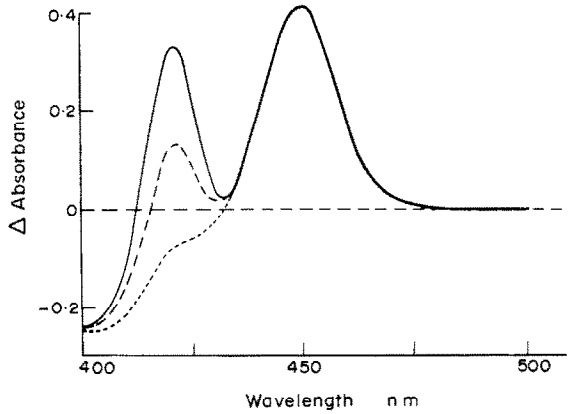

FIG. 1. Difference spectra of rat liver microsomes after addition of haem. Microsomes were prepared from rats pre-treated with phenobarbitone,[6] and suspended in 50 mM K phosphate buffer pH 7·4, at a concentration of 2 mg protein/ml. They were reduced with a few crystals of dithionite, and placed in cuvettes in both light paths of a double beam spectrophotometer, (Unicam SP 800). Carbon monoxide was added to the sample cuvette and the typical P-450 spectrum resulted (solid line 450 peak, followed by small dashed line to 400 nm (----). Haemin chloride was added to both cuvettes at a concentration of 2×10^{-6} M (dashed line, — —), and then 4×10^{-6} M (solid line, ———) and the difference spectra re-recorded. The peak that developed had a maximum at 420 nm, while the 450 peak was unaltered. Where NADPH reduction was used, NADP, 6×10^{-4} M, was added together with excess isocitrate, (5×10^{-3} M) and isocitrate dehydrogenase (0·2 units/ml).

When haem in phosphate buffer is reduced and a difference spectrum, plus vs minus carbon monoxide, is plotted, a broad peak at 406 nm is found. In the presence of albumin this peak becomes sharper and moves to 414 nm. In neither case is NADPH effective in reducing the haem.

When 2×10^{-6}M haem is added to a microsomal suspension containing 2 mg protein/ml, over 30 per cent of the haem becomes bound and can be precipitated by centrifugation. Only small amounts of the bound haem can be removed by washing the microsomes with buffer or albumin solution.

As the external haem concentration is raised, so increasing amounts of haem become bound, until the microsomal pellet becomes almost black with adsorbed haem.

It seems that some components of microsomes can bind large amounts of haem and that microsomes can catalyse the reduction of haem by NADPH. Presumably the NADPH–cytochrome *c* reductase of microsomes is in fact a haem–reductase.

In consequence it is not possible to distinguish between adsorbed haem, and degraded forms of haemoprotein, cytochrome P-450, on the basis of the P-420 measurement. It is possible that all P-420 preparations are in fact haem non-specifically bound to some fraction of microsomes. Clearly the spectrophotometric measurement held to define cytochrome P-420 cannot be used to imply that a particular haemoprotein is present.

These observations, together with our previous finding that the incorporation of δ amino laevulinic acid into microsomal haem is independent of protein synthesis,[7] should be kept in mind when interpreting the finding of a P-420 peak, and also when considering results of studies of haem turnover in microsomes.

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