COMMUNICATIONS

Cytochrome P-450 as a Microsomal Peroxidase in Steroid Hydroxylations

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Cholesterol 20 α -hydroperoxide was found to undergo an enzymic rearrangement to 20 α ,22R-dihydroxycholesterol and 20 α ,21-dihydroxycholesterol by a cytochrome P-450 preparation and has been proposed as an initial intermediate in pregnenolone biosynthesis (van Lier & Smith, 1970a,b,c). Hydroperoxides have also been identified as intermediates in the NADPH-dependent hydroxylation of fluorene and tetralin by a 10 000g liver supernatant fraction (Chen & Lin, 1968, 1969). In the following, cytochrome P-450 seems to be responsible for the decomposition of steroid and other organic hydroperoxides to hydroxy derivatives by microsomal fractions.

The peroxidase activity of rat liver and adrenocortical microsomal fractions was measured with NNN'-N'-tetramethyl-p-phenylenediamine as hydrogen donor (O'Brien & Hrycay, 1971). The hydroperoxides that could act as substrates were, in order of effectiveness, progesterone 17 α -hydroperoxide, allopregnanolone 17 α -hydroperoxide, linoleic acid hydroperoxide, cumene hydroperoxide, cholesterol 7 β -hydroperoxide and cholesterol 25-hydroperoxide. Purified cytochrome b₅ had low peroxidase activity, whereas a cytochrome P-450 preparation was very active. Liver microsomal fraction from phenobarbital-injected rats with a threefold higher specific cytochrome P-450 content was much more active. Further evidence for cytochrome P-450 being a microsomal peroxidase was the inhibition by type I and type II ligands. Detergents, alcohols and ketones, p-hydroxymercuribenzoate and trypsin also inhibited peroxidase activity, probably as a result of cytochrome P-420 formation from cytochrome P-450.

The rate of NADPH or NADH oxidation by microsomal fractions was markedly enhanced by these hydroperoxides. The reaction rate was first-order with respect to protein concentration with K_m values for NADPH and NADH less than 10 μ M and an apparent K_m 0.4 mM for cumene hydroperoxide. Evidence for the involvement of the flavoenzyme NAD(P)H oxidoreductases was the complete inactivation by 0.05 mM p-chloromercuribenzoate and protection by NADH or NADPH respectively. Removal of cytochrome b₅ by incubation of microsomal fraction with trypsin did not affect the NADH-hydroperoxide activity. Cytochrome P-450 seemed to be involved in both activities, since type I and type II ligands or agents

converting cytochrome P-450 into cytochrome P-420 produced inactivation whereas phenobarbital administration *in vivo* caused a marked stimulation. Although liver and adrenocortical microsomal fractions had similar activity, intact or sonicated adrenocortical mitochondria, which have similar cytochrome P-450 contents, were only slightly active. Liver mitochondria had no activity.

The hydroxy products formed in these microsomal reactions with progesterone 17 α -hydroperoxide included intermediates probably involved in testosterone, oestrone or cortisol biosynthesis. The above results suggest that cytochrome P-450 can act as a peroxidase for steroid hydroperoxides and that these hydroperoxides, like oxygen, can also oxidize reduced cytochrome P-450 to cytochrome P-450.

This work was supported by the National Research Council of Canada. We thank Dr J. L. van Lier (University of Sherbrooke) for preparing cholesterol 7 β -hydroperoxide and 25-hydroperoxide.

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Plasma 11-Hydroxy Corticosteroid Concentrations in Stressed Adult Rats after Under-nutrition in Early Life

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Elevation of plasma 11-hydroxy corticosteroid concentrations in response to stress in adult rats differs between animals handled or left undisturbed in infancy (Levine, 1962). It has been proposed (Levine & Mullins, 1966) that this is a result of experimentally produced steroid variations in infancy that permanently modify the regulatory mechanisms of the hypothalamic-pituitary-adrenal system. It is possible that nutritional status during infancy may also have lasting effects on this system, since plasma 11-hydroxy corticosteroid concentrations are elevated in malnourished children (Alleyne & Young, 1967).