PHOTOREACTIVATION SPECTRUM OF THE CO-INHIBITED TAUROCHENODEOXYCHOLATE 6β-HYDROXYLASE SYSTEM *

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The 6β-hydroxylation of chenodeoxycholic and taurochenodeoxycholic acid by cell free preparations of rat liver has been reported in a previous paper [1]. The enzymic activity was located in the microsomal fraction, and an active extract was prepared by suspension of the microsomes in 1.0 M phosphate buffer, pH 7.6 and subsequent centrifugation at 105,000 × *g*. The requirements for NADPH and O_2 indicated that the 6β-hydroxylase is a mixed function oxidase [2]. Carbon monoxide was found to inhibit the hydroxylase activity of the 1.0 M phosphate extract and the inhibition could be partially reversed by white light. This, along with the spectrophotometric detection of cytochrome P-450 in the extract [1], following reduction with Na dithionite and exposure to CO, suggested the participation of the P-450 in the 6β-hydroxylase system. In a continuation of our study we have determined the photoreactivation spectrum of the CO-inhibited hydroxylase system. This communication reports the results which support that P-450 functions as the oxygen activating enzyme in the 6β-hydroxylase system.

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The technique employed was adapted from that developed by Warburg for the study of the nature of the terminal oxidase in the respiratory chain [3], and it has been previously used to demonstrate the involvement of P-450 in several mixed function oxidases [4–7]. The method is based on the comparison of the optical spectrum of the P-450·CO complex with the action spectrum obtained by plotting the relative efficiencies of various monochromatic lights in reversing the CO inhibition versus the wavelength of light.

The incubations were carried out in Warburg flasks containing aliquots of the 1.0 M phosphate extract (approximately 10.0 mg protein) in a medium containing 5 mM $MgCl_2$, 1 mM nicotinamide, 0.5 mM glutathione, and a NADPH generating system (2 μmoles NADP, 25 μmoles glucose-6-phosphate, 1 Kornberg unit glucose 6-phosphate dehydrogenase) [8]. The final volume was adjusted to 5 ml with 0.1 M phosphate buffer pH 7.6. The flasks were gassed with the appropriate mixture for five minutes prior to the introduction of taurochenodeoxycholate-24-^{14}C (0.2 μmole, 1.1 × 10^5 dpm) from the side arm of the Warburg vessel. The contents were incubated for 30 min at 37° either in the dark or under a beam of monochromatic light of various wavelengths with approximately equal intensity. A description of the apparatus employed has been published earlier [9]. The reactions were stopped by the addition of 5 ml of 10% KOH, and 6β-hydroxylase activity was assayed

Table 1

Reversal of CO-inhibition by lights of various wavelengths. Incubation conditions are given in the text. The flasks were gassed with a mixture of 22% CO, 11% O_2, 67% N_2. Control incubations (without CO) were gassed with a mixture of 10% O_2, 90% N_2. V_{CO} is the rate of 6β-hydroxylation in the presence of CO. $n = V_{CO}/V_{O_2}$, where V_{O_2} is the rate of 6β-hydroxylation in the absence of CO. In experiment 1, the V_{O_2} was 1.35 mμmoles/min; in experiment 2, V_{O_2} was 1.39 mμmoles/min. $K = (CO)/O_2 \cdot (n/1-n)$. $\Delta K = K - K_d$, where K_d is the value of K obtained in the dark. The light sensitivity, $L = (1/i)(\Delta K/K_d)$, where i is the intensity of the light beam striking the flasks expressed in mole quanta/cm^{2}min. The method of measuring light intensity and the calculation for relative light sensitivity have been described previously [9].

	Wavelength (mμ)	V_{CO} (mμmoles/min)	n	K	ΔK	$10^{-6} L$	$L/L450$
Experiment 1	Dark	0.723	0.557	2.51			
	400	0.660	0.508	2.06	0	0	0
	412	0.667	0.513	2.11	0	0	0
	419	0.833	0.641	3.57	1.06	0.221	0.25
	426	0.867	0.666	3.99	1.48	0.317	0.36
	433	0.967	0.743	5.78	3.27	0.669	0.76
	441	0.980	0.754	6.13	3.62	0.712	0.81
	450	1.013	0.781	7.13	4.62	0.880	1.00
Experiment 2	450	1.030	0.739	5.66	3.62	0.857	1.00
	462	0.813	0.583	2.80	0.76	0.149	0.17
	475	0.763	0.548	2.42	0.38	0.099	0.12
	500	0.747	0.534	2.29	0.25	0.058	0.07
	Dark	0.703	0.505	2.04			

as described previously [1]. The results are shown in table 1.

The 6β-hydroxylation of taurochenodeoxycholate was about 50% inhibited in the dark at a CO/O_2 ratio of 2.0. Under irradiation with various wavelengths of light the inhibition was reversed in different degrees, light of 450 mμ being the most effective. No effect was observed by irradiation with light of 450 mμ wavelength in the absence of CO. The photoreactivation spectrum is shown in fig. 1. The similarity of the action spectrum to the absorption spectrum of cytochrome P-450·CO is evident, and indicates that cytochrome P-450 is the CO-inhibitable oxygen-activating catalyst of the 6β-hydroxylase system and that the photodissociation of its CO component is responsible for the light-reversal of the CO inhibition of the system.

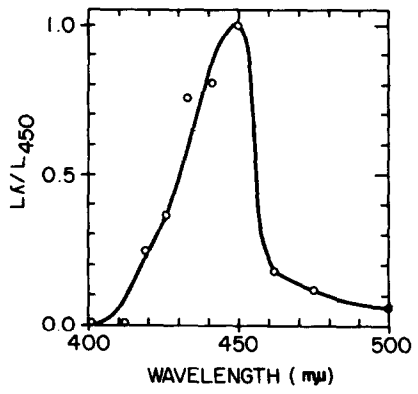

Fig. 1. Photoreactivation spectrum of the CO-inhibited 6β-hydroxylase activity. The relative light sensitivities, L/L_{450}, for each wavelength of light were obtained from table 1 and plotted as a function of the wavelength.

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