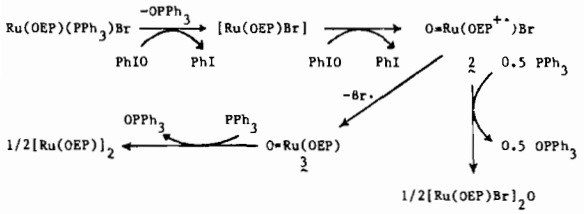

Scheme.

An inactive green complex, isolated at the end of the oxidations, and also formed by decomposition of *2* in solution, is believed to be a O=Ru(OEP) species, *3*, since it reacts quantitatively with PPh_3(1:1) to give the phosphine oxide and $[Ru(OEP)]_2$ [8]. Species *3*, which is rapidly converted by trace amounts of base into $[Ru(OEP)(OH)]_2O$ [7, 8], may contain an axial water ligand in which case it would resemble O=Ru(bipyridine)$_2$(py), which is known to oxidize PPh_3 by an oxygen atom transfer mechanism [11].

Spectroscopic studies are in progress in attempts to characterize more fully the putative oxo species *2* and *3*.

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Q17

Unusual Spin Interactions in the 24 Heme Hydroxylamine Oxidoreductase and Diheme Cytochrome *c* 554 from *Nitrosomonas*

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Nitrosomonas oxidizes NH_3 to HNO_2 with NH_2-OH as an intermediate. Oxidation of NH_2OH appears to involve two multiheme cytochromes: hydroxylamine oxidoreductase (HAO) [1] and cytochrome *c* 554 [2]. Hemes of HAO have midpoint potentials varying from +100 mV to −350 mV [3]. HAO can accept electrons from NH_2OH and pass them to cyt *c* 554 (midpoint potential −50 mV, 2).

HAO, with an $\alpha_3\beta_3$ subunit structure, contains 7 *c*-type hemes and one unique heme P460 per $\alpha\beta$ dimer. The CO-binding heme P460 is essential for the NH_2OH dehydrogenase activity and is specifically destroyed by H_2O_2. EPR studies of HAO reveal several classes of low spin (s = ½) hemes [4]. Two species, accounting for half of the hemes, have been assigned g-values by reductive EPR titration; g = 3.06, 2.14, 1.35 and g = 2.98, 2.24, 1.44 [5]. Only four other EPR signals appear in the oxidized spectrum (g = 3.38, 2.70, 1.85 and 1.66). These resonances titrate coordinately but are not typical of magnetically isolated heme spectra. The apparent g-values of these 4 resonances are frequency dependent suggesting that they arise from spin-interactions of the hemes. Frequency dependence of the type observed has not been previously reported. The Mössbauer spectrum of ferric HAO contains a quadrupole doublet at 4.2 K in addition to the expected broad magnetically split spectrum, typical of s = ½ hemes. This doublet, which corresponds to at least one and probably two irons per $\alpha\beta$-dimer, has parameters (ΔE_Q = 2.1 mm/s and δ_{Fe} = 0.24 mm/s) which are typical of either low spin ferric heme with fast electronic spin relaxation or a pair of spin-coupled hemes [6]. We speculate that this doublet may be associated with the four frequency dependent EPR resonances. Heme P460 is not a component of the latter species since selective destruction of P460 by H_2O_2 fails to alter the EPR spectrum of the oxidized HAO. Thus heme P460 of native HAO is EPR silent.

Cytochrome c554 at pH 7 has an unusual 10 K EPR spectrum (g = 4.18, 3.85) similar to intermediate spin (s = 3/2) complexes. At pH 4 the EPR spectrum consists of one high spin (g = 6.0, 2.0 and one low spin (g = 2.93, 2.25, 1.52) component. At pH 2 a single high spin component (g = 6.0, 2.0) is present, whereas two low spin forms are observed at pH 10.5. Optical spectra of oxidized cyt *c* 554 at 20 °C are consistent with high spin heme at pH 4 and low spin heme at pH 10.5. Reduced cyt *c* 554 reacts with O_2 and binds CO at pH 4: the CO spectrum has two Soret maxima indicating a different interaction with each heme. ^{1}H-NMR spectra at room temperature show contact shifted heme methylene resonances in both the low spin (10–30 ppm) and high spin (60–100 ppm) Fe^{3+} spectral regions at all pH values between 4.5 and 9. Contact shifted resonances similar to those reported for s = 3/2 model heme complexes are not observed at this temperature. We conclude that the unusual low temperature EPR spectrum at

pH 7 results either from a spin conversion or interaction between high and low spin hemes. EPR, NMR and optical spectra show that this is a different type of heme-heme interaction than observed with diheme cyt c'.

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Q18

The Electron Transfer Reactivity of Cytochrome P-450-CAM. Molecular Oxygen Dependent Multiple Turnovers Using Chemical Reductants

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Cytochrome P-450, unlike most other cytochromes, does not function merely as an electron carrier but is also an enzyme capable of catalyzing oxygenation reactions. This heme-containing monooxygenase activates molecular oxygen for insertion of one oxygen atom into organic substrates with concomitant reduction of the other oxygen atom to water. Bacterial P-450, isolated from camphor-grown *Pseudomonas putida* (P-450-CAM)[†], utilizes molecular oxygen and NADH to hydroxylate camphor at the exo-5 position and initiate camphor degradation [1]. Because the hemoprotein itself cannot react directly with NADH, electrons are transferred from NADH to P-450-CAM via first a FAD-containing flavoprotein (putidaredoxin reductase, fp) and then an iron-sulfur protein (putidaredoxin, Pd).

[†]Abbreviations: P-450-CAM, the camphor hydroxylating P-450 isolated from *Pseudomonas putida* grown on camphor; fp, the flavoprotein (putidaredoxin reductase) that accepts electrons from NADH; Pd, the iron-sulfur protein (putidaredoxin) that accepts electrons from fp and delivers them to P-450-CAM; PMS, 5-methylphenazinium methyl sulfate (phenazene methosulfate).

TABLE I. Product Formation.[a]

A. *As a Function of P-450-CAM Concentration*

	P-450-CAM Conc. (μM)	*Exo*-5-hydroxy-camphor formed[c] (nmol)
With Putidaredoxin ($3\ \mu M$)	0	0.01 ± 0.01(2)
	0.5	23.6 ± 1.0(2)
	1.0	49.8 ± 1.5(6)
	2.0	97.0 ± 2.5(2)
Without Putidaredoxin	0	0.02 ± 0.01(2)
	0.5	1.3 ± 0.1(2)
	1.0	2.7 ± 0.2(3)
	2.0	5.1 ± 0.2(3)

B. *As a Function of Time*

	Incubation (min)	*Exo*-5-hydroxy-camphor formed[c] (Mol/Mol of P-450-CAM)
With Putidaredoxin ($3\ \mu M$)	1	28.8(1)
	2	49.8 ± 1.5(6)
	5	101.7(1)
	10	188.7 ± 5.0(2)
Without Putidaredoxin	1	1.5 ± 0.1(2)
	2	2.7 ± 0.2(3)
	5	5.8 ± 0.1(2)
	10	9.8 ± 0.3(2)

C. *Control Experiments*

	Exo-5-hydroxycamphor formed[c] (nmol)	
	Without Pd[d]	With 5 μM Pd[e]
1. No P-450	0.02 ± 0.01(2)	0.01 ± 0.01(2)
2. No NADH	0.01 ± 0.01(2)	0.01 ± 0.01(2)
3. No PMS	0.02 ± 0.01(3)	0.01 ± 0.01(3)
4. Boiled P-450[f]	0.03 ± 0.02(2)	–
5. Myoglobin (no P-450)[g]	None	–

[a]Incubations were done using optimized conditions unless otherwise indicated. Optimized conditions: 1 μM P-450-CAM, 600 μM d-camphor, 3 mM NADH, and 50 μM PMS, in 20 mM phosphate buffer (pH 7.40, and 100 mM KCl), 2 ml total volume, with gentle oxygen bubbling. In the presence of Pd (3–5 μM), 5 mM NADH was used. [b]Two minute incubations. [c]The number in parentheses is the number of trials of a particular experiment. [d]Ten minute incubations without oxygen bubbling using optimized conditions except as follows: 1.6 mM NADH, 250 μM PMS. [e]Same conditions as described in footnote d, plus oxygen bubbling. [f]P-450-CAM was boiled for 10 minutes prior to use. [g]1 μM myoglobin.