Mode of Mammalian Oxygenation at Primary Carbon Atoms: Steric Course of Hydroxylation of C-1 Chiral Octanes by Rat Liver Microsomes

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Summary It is shown that mammalian microsomal cytochrome P_{450} converted C-1 isotopically labelled (1*R*)- and (1*S*)-octane into octan-1-ol with retention, whereby the incoming hydroxy-group assumes the same orientation as the displaced hydrogen.

IN previous articles[1,2] we reported on the steric mode of hydroxylation at primary carbon atoms by *Pseudomonas oleovorans*, strain TF4-1L. This study was carried out using (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H; 1-^{14}C]octane and was based on the premise that hydroxylation proceeds with a normal kinetic isotope effect ($K_H > k_D > k_T$). It was assumed that hydroxylation at the chiral termini of octanes will yield mixtures of C-1 tritiated chiral octan-1-ols in different proportions, R-C-^{3}H,^{2}H(OH) > R-C-^{3}H,^{1}H(OH).† We showed that hydroxylation does indeed proceed with a normal hydrogen isotope effect, since the major products of enzymic hydroxylation (70—80%) were octanols obtained by oxygenation at the achiral termini of the octanes. As expected, hydroxylation at the chiral termini gave mixtures of (1*R*)- and (1*S*)-C-1 tritiated octanols in different proportions irrespective of the chirality of the substrate. From the composition of the mixtures of C-1 tritiated chiral octan-1-ols, it was deduced that oxygenation proceeds with retention, *i.e.* the incoming hydroxy-group assumes the orientation of the displaced hydrogen (deuterium) atom.[1,2]

Since hydroxylation reactions are very important routes of hydrocarbon catabolism in animals,[3,4] we considered it necessary to define the mode of oxygenation at primary carbon atoms by mammalian tissues, *i.e.* rat livers. The problem is of added significance because the alkane hydroxylases of *Ps. oleovorans* and rat liver microsomes are distinctly different. Although both are sideroproteins, rat liver microsomal P_{450} is a b-type cytochrome[5] with iron-protoporphyrin IX at the active site,[6] while the prosthetic group of the bacterial enzyme contains non-heme iron.[7] We therefore undertook to determine whether these very different enzymes catalyse identical reactions in the same steric mode. The results of our investigations are the subject of this communication.

The previously described substrates[1,2] (1*R*)-[1-^{3}H,^{2}H,^{1}H; 1-^{14}C]octane {spec. act. *ca.* 45 μCi ^{3}H μl^{-1} (7·3 mCi ^{3}H mmol^{-1}), [^{3}H : ^{14}C] ratio 8·96 : 1} and (1*S*)-[1-^{3}H,^{2}H,^{1}H; 1-^{14}C]octane {spec. act. *ca.* 45 μCi μl^{-1} (7·3 mCi ^{3}H mmol^{-1}), [^{3}H : ^{14}C] ratio 8·47 : 1}, were used. Chiral octanes admixed with [1-^{14}C]octane were incubated with microsomes prepared from uninduced, unstarved albino male rats;[8] usually *ca.* 0·02 μCi of [^{3}H]octan-1-ol (*ca.* 0·05% ^{3}H recovered as octan-1-ol, admixed with [^{14}C]octan-1-ol) per incubation was obtained. The [^{3}H : ^{14}C] ratios of the octanols were determined on the derived *p*-tolylurethanes. The extent of oxygenation at the chiral termini of the octanes was determined by oxidation of the mixtures of alcohols with Jones' reagent to octanoic acids (counted as the *p*-toluidides). From the changes in [^{3}H : ^{14}C] ratio on oxidation (octanols → octanoic acids), the amount of C-1 tritium in the octanols was determined. The mixture of alcohols obtained from incubation of (1*R*)- and (1*S*)-octane and synthetic (1*RS*)-[1-^{3}H; 1-^{14}C]octanol‡ was equilibrated for 24 h in the system horse liver alcohol dehydrogenase–NAD/NADH–

† Since analysis of the composition of the mixture of biosynthesized octanols is based on the determination of the amount of tritium lost during enzymic equilibration, the small (additional) amount of R-C-^{2}H,^{1}H(OH) formed through C–^{3}H bond cleavage may be disregarded.

‡ We have proved that identical amounts of tritium are lost on equilibration for 24 h of (1*RS*)-[1-^{3}H,^{1}H; 1-^{14}C]octanol and (1*RS*)-[1-^{3}H,^{2}H; 1-^{14}C]octanol.

TABLE. Hydroxylation of (1*R*)- and (1*S*)-octanes by rat liver microsomes.

Expt.	Chirality of octane	% ^{3}H at C-1 of octanols	% of (1*R*)-octanol[a]	C-1 chirality of the major octanol	Stereochemistry of of hydroxylation
1	(1*R*)	25	24±2	1*S*	Retention
	(1*S*)	29	85±6	1*R*	Retention
2	(1*R*)	32	26±2	1*S*	Retention
	(1*S*)	29	63±4	1*R*	Retention

[a]The average deviation in the [^{3}H : ^{14}C] ratios from successive crystallizations of the acid and alcohol derivatives is ±0·05 The resulting relative error[10] in the calculated percentage of (1*R*)-octanol is ±7% (*e.g.* 24±2%).

diaphorase.[1,2,9] The amount of (1*R*)-octanol in the mixture was calculated from the total amount of tritium lost on equilibration, corrected for the amount of tritium lost simultaneously from (1*S*)-octanol. The relative fraction of tritium lost from (1*S*)-octanol was defined on the basis of the tritium lost from (1*RS*)-[1-^{3}H; 1-^{14}C]octanol.[1,2,9]

The hydroxylation of (1*R*)-octane produced mixtures of (1*R*)- and (1*S*)-tritiated octan-1-ols. Kinetic configurational assay of these mixtures[1,2,9] indicated that 24—26% of the octanol in the mixtures was (1*R*)-octanol and, hence, the major product of hydroxylation of the chiral methyl terminus of (1*R*)-octane is (1*S*)-octanol (Table). In contrast, hydroxylation of (1*S*)-octane gave octanols containing mostly (63—85%) (1*R*)-octanol (Table). These results are consistent with the view that the hydroxylation proceeds with retention, in which a hydrogen atom, and to a lesser extent a deuterium atom, is displaced.

Hence, hydroxylation of octane to octan-1-ol by *Ps. oleovorans* and by rat liver microsomes proceeds with net retention.

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