The Contribution of Tunnelling to High Values of Kinetic Isotope Effect in Aliphatic Hydroxylation by a Cytochrome P-450 Model

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High values of kinetic isotope effect (KIE), up to 21.9 ± 1.9 at 20 °C, are obtained in cyclohexane oxidation by an iron tetramesitylporphyrin (TMPFeCl)–hypochlorite system; their very sharp dependence on temperature suggests tunnelling contribution to the C–H bond cleavage step.

Aliphatic hydroxylation catalysed by cytochrome P-450 exhibits high values of intramolecular kinetic isotope effect (KIE), k_H/k_D = 7—12.[1–5] High values of KIE (k_H/k_D = 7—13) were also observed for cytochrome P-450 models based on iron porphyrins.[3,6,7] Recently, we have observed unusually high values of KIE in cyclohexane hydroxylation by the iron porphyrins–hypochlorite system.[8,9] Herein, we present a more complete analysis of this phenomenon.

The KIE values were determined using competition kinetic experiments of cyclohexane and cyclopentane oxidation, which can yield relative rate constants with a high degree of precision.

The values of KIE depend on the structure of the porphyrin iron chloride (PFeCl) catalyst (Table 1) and correlate with redox potentials of the respective PFeCl. The highest values were obtained with iron porphyrins with donating substituents such as TMPFeCl and tetrakis(*p*-t-butylphenyl)porphyrin iron chloride. The temperature dependences of KIE were studied

Table 1. The observed isotope effects associated with the oxidation of cyclohexane by the PFeCl–NaOCl system at 20.0 ± 0.1 °C.[a]

Porphyrin	k_H/k_D
Tetramesitylporphyrin	21.9 ± 1.9
Tetrakis(*p*-t-butylphenyl)porphyrin	21.1 ± 1.6
Tetraphenylporphyrin	17.8 ± 2.3
Tetrakis(*p*-fluorophenyl)porphyrin	14.4 ± 0.7
Tetrakis(*o*-fluorophenyl)porphyrin	0.9 ± 0.9
Tetrakis(pentafluorophenyl)porphyrin	10.6 ± 0.4
Tetrakis(*o*-chlorophenyl)porphyrin	10.7 ± 0.4
Tetrakis(*o*-nitrophenyl)porphyrin	9.3 ± 0.3

[a] Typical procedure: porphyrin iron chloride (0.2 μmol) and cetyltrimethylammonium bromide (2 μmol) are added to a solution of 0.8 ml benzene and 0.2 ml of an equimolar mixture of cyclohexane (or [$^2H_{12}$]cyclohexane) and cyclopentane. After addition of 1 ml of 0.29 M NaOCl solution the mixture is stirred for 2 h, then GLC analysis of the aqueous and organic phases was performed for alcohols.

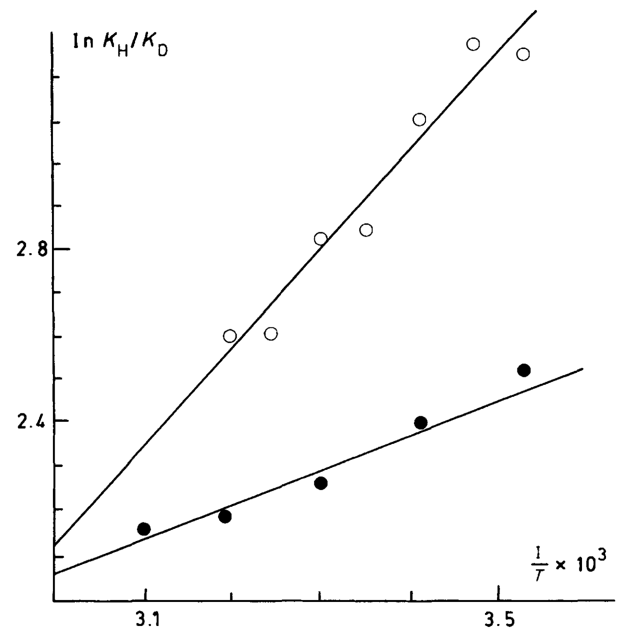

Figure 1. The KIE temperature dependences associated with cyclohexane oxidation; ○ = TMPFeCl–NaOCl system, ● = ToFPP-FeCl–NaOCl system.

for the cases of TMPFeCl and tetrakis(*o*-fluorophenyl)-porphyrin iron chloride(ToFPPFeCl) in benzene (Figure 1). The parameters of the Arrhenius equation $k_H/k_D = A_H/A_D \exp[(E_D - E_H)/RT]$ were calculated: $E_D - E_H$ equal to 4.5 ± 0.5 and 1.4 ± 0.4 kcal/mol (1 cal = 4.184 J), A_H/A_D equal to 0.01 and 0.90 for TMPFeCl and ToFPPFeCl, respectively. The parameters determined during cyclohexane oxidation by the TMPFeCl–NaOCl system differ greatly from the values $k_H/k_D \leqslant 9$, $A_H/A_D > 0.5$, and $E_D - E_H \leqslant 2$ kcal/mol,[10] characteristic of a semiclassical process. Recently, values different from the classical process were obtained during cyclohexane oxidation by CrO_3.[11] The unusually high values of k_H/k_D, and particularly the A_H/A_D Arrhenius parameter values suggested that during cyclohexane hydroxylation by the PFeCl–NaOCl system, an essential tunnelling contribution to hydrogen atom abstraction from the alkane occurs, as A_H/A_D values less than 0.5 are the most reliable values known from evidence for hydrogen tunnelling.[10] The highest tunnelling contribution was observed for thermoneutral reactions which had a higher energetic barrier, *i.e.*, hydrogen tunnelling is more probable than deuterium tunnelling through the high and narrow barrier.[10] Oxidation of cyclohexane by stronger oxidants/intermediates formed from iron porphyrins with electron withdrawing substituents was more exothermic and consequently the tunnelling contribution diminished or disappeared. Recently, it was shown that the reactions of hydrogen abstraction from alkanes in the gas phase also contributed to the tunnelling.[12]

The KIE of the cyclohexane oxidation depends on the solvent. Thus, with TMPFeCl as catalyst, KIE diminished in solvents more polar than benzene (k_H/k_D = 10.2 ± 0.6 and 10.0 ± 0.4 in benzonitrile and chlorobenzene respectively at 20 °C).

High values of KIE in the aliphatic C–H bond oxidation with participation of cytochrome P-450 and its models provided evidence for the mechanism of oxygen rebounding with a symmetrical transition state,[6] but did not take into account the tunnelling contribution.[5] The tunnelling contribution observed in this work associated with the cyclohexane hydroxylation by the PFeCl–NaOCl model system suggested that tunnelling can be observed in cytochrome P-450 participated reactions.

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