BIOORGANIC CHEMISTRY 4, 307–316 (1975)

Chemical Model of the Enzyme System for Hydroxylating Cyclohexane

A. A. AKHREM, D. I. METELITSA, AND M. E. SKURKO

Institute of Bioorganic Chemistry, BSSR Academy of Sciences, Minsk, USSR

Received July 10, 1974

The kinetics of co-oxidation of cyclohexane and ferrous chloride by molecular oxygen in methanol in the temperature range 25–50°C has been studied. The total yield of the reaction products, cyclohexanol and cyclohexanone, equals about 25% of the reacted ferrous ion. A redox-active ligand, cysteine, added to the system $FeCl_2$–C_6H_{12}–O_2 increases product yield. The reaction involving $FeCl_2$ oxidation by oxygen in the presence of HCl is bimolecular. The effective value of the activation energy of reaction is 9.0 kcal/mol. In the absence of HCl the hydroxylating species is a complex $[Fe^{3+}O_2^-]$, and in the presence of HCl this complex comes together with $HO_2^{\cdot}$ radicals. A scheme for co-oxidation of $FeCl_2$ and cyclohexane is proposed. Similarities and contrasts between various hydroxylating species in enzymatic and nonenzymatic hydroxylations are discussed.

The hydrophobic energy being not high (*1*), the adsorption at the nonpolar site of an enzyme can, but only to a small extent, activate such nonpolar substrates as hydrocarbons. Therefore, enzymatic hydroxylation of hydrocarbons requires activation of molecular oxygen. Though the study of structure and properties of the enzyme systems which hydroxylate hydrocarbons, amino acids, and steroids has recently made great progress, the exact mechanism of molecular oxygen activation and the structure of the particle attacking the substrate remain unknown (*2*). The oxygen atom is transferred to the substrate by the terminal oxidase of monooxygenases, i.e., cytochrome P-450, containing the iron ion in the form of heme. Cytochrome P-450 is the most important component of monoxygenases of the liver, which hydroxylates a large variety of substrates. The iron ion in a high-spin form, Fe^{3+}, plays an important role in the function of phenylalanine hydroxylase, which ceases completely when the ion is bound with chelated ligands (*3*).

The mechanism of oxygen activation with enzyme systems could be elucidated with the help of simple models composed of a metal ion, molecular oxygen, and a hydroxylated substrate. This paper presents a kinetic study of such a model, made up of Fe^{2+} ion, molecular oxygen, and cyclohexane in a methanolic solution. The choice of a solvent was specified by our desire (i) to achieve higher concentrations of a substrate in a solution, on the one hand, and (ii) to ascertain the peculiarities of the O_2 activation in a nonaqueous medium, on the other. Cyclohexane was chosen as a substrate owing to the fact that upon its oxidation only two products, cyclohexanol and cyclohexanone, are formed. Furthermore, it is known that under incubation cyclohexane is hydroxylated by liver microsomes (*4*).

Printed in Great Britain

Our simple model is efficient enough as it produces in a noncyclic process comparatively large concentrations of products, up to 25% per reacted iron ion. When a redox-active ligand such as cysteine is added to the $FeCl_2$–O_2–C_6H_{12} system, the yield of the oxidation products increases.

EXPERIMENTAL

The experiments were carried out at 25–50°C in a thermostatically controlled oxidizing cell, into which $FeCl_2$ and C_6H_{12} dissolved in methanol were added along with various amounts of HCl. Oxygen was passed through the solution at a rate not less than 3.0 liters/hr to provide a kinetic regime of the reaction. Probes were taken at intervals and analyzed for the content of the ferric ion, Fe^{3+}, and cyclohexane oxidation products. Ferric ion concentrations were determined photocolorimetrically by a PhEK-56M instrument (USSR); the absorption spectrum of $FeCl_3$ in methanol ($\lambda_{max} = 370$ nm) was recorded by Specord (GDR).

Cyclohexanone and cyclohexanol were determined by gas–liquid chromatography on Chrom-31 (CSSR); the column (2.4 m long and 6.0 mm in diameter) contained 15% of polyethyleneglycol adipinate on Chromosorb W-DMCS. The temperature of the column was 110°C; the detector was flame-ionizing; and helium was used as carrier gas. Isoamyl alcohol was employed as an internal standard for quantitative determination of the oxidation products. The reaction was characterized by the initial rates of the $FeCl_2$ oxidation $W_0^{FeCl_2}$ and those of the cyclohexanol and cyclohexanone $W_0^{Pr} = W_0^{C_6H_{11}OH} + W_0^{C_6H_{10}O}$ formation, in M sec^{-1}. The kinetics of the ferrous ion consumption in methanol with and without cyclohexane at a constant rate of oxygen supply is described by a first-order equation (Fig. 1). This equation was used for calculating the pseudo-first-order rate constant of the $FeCl_2$ oxidation, $k = k^{eff}[O_2]$ sec^{-1}, where k^{eff} is the effective bimolecular rate constant for the reaction of $FeCl_2$ with O_2.

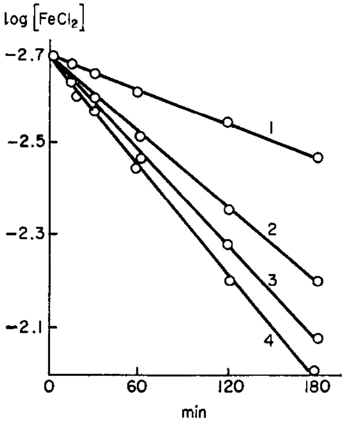


FIG. 1. The ferrous chloride consumption at $[FeCl_2]_0 = [HCl]_0 = 0.05$ M, $[C_6H_{12}]_0 = 1.80$ M; 1, 25°C; 2, 37°C; 3, 43°C; and 4, 50°C.

Effect of HCl on oxidation. The experiments were conducted at 50°C; the concentrations of $FeCl_2$ and C_6H_{12} were kept constant, but the initial concentrations of HCl were varied. In Fig. 2 the initial rates of $FeCl_2$ oxidation and accumulation of the cyclohexane oxidation products are plotted vs $[HCl]_0$. Addition of 0.10 *M* HCl enhances the $FeCl_2$ oxidation rate by a factor of more than two and the rate of product formation by more than three. Further increase in the HCl concentration does not cause further increase in both rates. It should be noted that, in the absence of HCl and of cyclohexane, the order of the $FeCl_2$ oxidation reaction in methanol varies; in this case the kinetics of the $FeCl_2$ consumption is described by second-order equations.

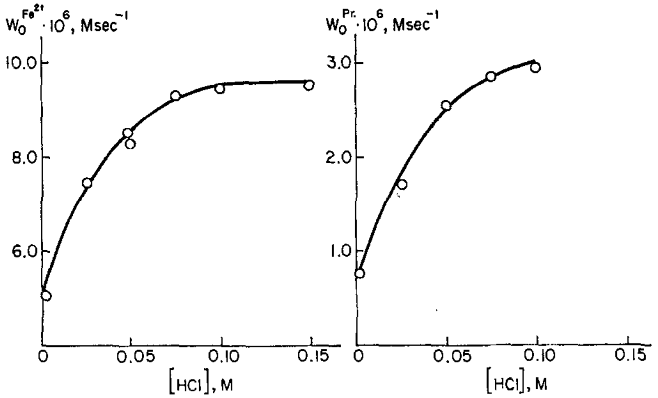


FIG. 2. The effect of $[HCl]_0$ on the coupled oxidation of cyclohexane and $FeCl_2$ at 50°C, $[FeCl_2]_0 = 0.050$ *M*, $[C_6H_{12}]_0 = 1.80$ *M*.

Effect of $[FeCl_2]$ *and* $[O_2]$ *on oxidation.* The effect of the initial $FeCl_2$ concentration on the initial oxidation rates is shown in Fig. 3. The initial rate of $FeCl_2$ consumption is found to be directly proportional to its initial concentration, W_0^{Pr} reaching its maximum with $[FeCl_2]_0$ increase. Experiments with oxygen and air showed that $W_0^{FeCl_2}$ is directly proportional to the partial pressure of oxygen.

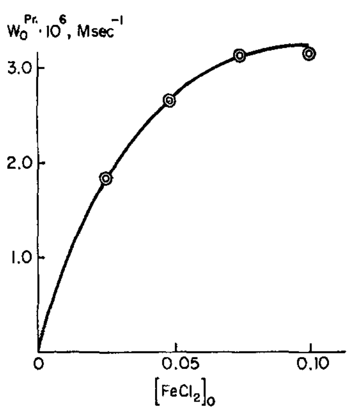


FIG. 3. The effect of $[FeCl_2]_0$ on the coupled oxidation of cyclohexane and $FeCl_2$ at 50°C, $[HCl]_0 = 0.05$ *M*, $[C_6H_{12}]_0 = 1.80$ *M*.

Effect of the initial concentration of cyclohexane. A series of experiments were carried out with different concentrations of cyclohexane at 50°C, $[FeCl_2]_0 = 0.05$ *M* and $[HCl]_0 = 0.05$ *M*. The results are listed in Table 1.

TABLE 1

OXIDATION OF $FeCl_2$ AND CYCLOHEXANE WITH OXYGEN AT 50°C

$[C_6H_{12}]_0$ (M)	$10^6 \cdot W_0^{FeCl_2}$ $(M\,sec^{-1})$	$10^6 \cdot W_0^{cyc\text{-}ol}$ $(M\,sec^{-1})$	$10^6 \cdot W_0^{cyc\text{-}one}$ $(M\,sec^{-1})$	$10^6 \cdot W_0^{pr}$ $(M\,sec^{-1})$
0	9.00	0	0	0
0.90	8.60	0.30	0.20	0.50
1.30	8.65	0.80	0.60	1.40
1.80	8.30	1.30	1.30	2.60

The experiments conducted at 25–43°C showed that the thermal path of the $FeCl_2$ oxidation rate constant, $k = k^{eff}[O_2]$, is the equivalent of an activation energy of 9.0 kcal/mol, while that of the initial rate of the reaction product formation is characterized by an activation energy of 6.5 kcal/mol (Fig. 4).

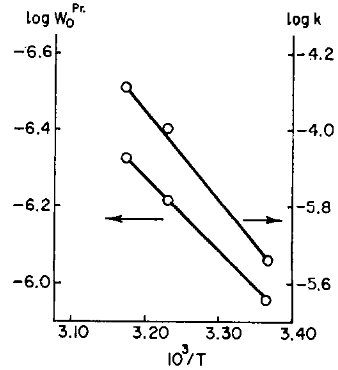


FIG. 4. The temperature dependence of the oxidation reaction at $[HCl]_0 = [FeCl_2]_0 = 0.05$ *M* and $[C_6H_{12}]_0 = 1.80$ *M*.

To reveal certain peculiarities of the mechanism of the $FeCl_2$ and cyclohexane coupled oxidation at 50°C, $[FeCl_2]_0 = [HCl]_0 = 0.05$ *M*, inhibitors of radical reactions α-naphthol and 2,4,6-tri*tert*-butylphenol at initial concentrations of 0.20 *M* were introduced into the reaction. Their introduction does not affect the rate of $FeCl_2$ oxidation but hinders the rate of reaction product formation (Fig. 5). Under the same conditions, ethylenediamine at a concentration of 0.050 *M* was introduced into the reaction, which gave an abrupt decrease in the reaction product formation rate (0.004 *M* total yield of cyclohexanol and cyclohexanone for 180 min).

Replacement of $[HCl]_0 = 0.05$ *M* by KCl of the same concentration at 50°C, $[FeCl_2]_0 = 0.05$ *M*, $[C_6H_{12}]_0 = 1.8$ *M*, does not affect the rate of $FeCl_2$ oxidation but causes a decrease in the reaction product formation rate from 2.66×10^{-6} to 1.1×10^{-6} $M\ sec^{-1}$.

The experiments on oxidation of $[FeCl_2]_0 = 0.05\ M$ and $[C_6H_{12}]_0 = 2.46\ M$ in the presence of $[HCl]_0 = 1.70\ M$ in acetone showed that for 120 min insignificant amounts of cyclohexane oxidation products are formed. Cysteine, at a concentration of 0.05 *M*, added to the system under the same conditions led to formation of $1.25 \times 10^{-3}\ M$ cyclohexanol and cyclohexanone for 30 min, i.e. cysteine stimulates oxidation of cyclohexane.

DISCUSSION

Coupled oxidation of $FeCl_2$ and cyclohexane with molecular oxygen is characterized by the following kinetic regularities: In methanol in the presence of HCl and cyclohexane the iron ions are oxidized with oxygen according to the bimolecular reaction, whereas in the absence of HCl and of cyclohexane the order of the reaction changes from the first to second order as to analytical concentration of ferric ions. Replacement of HCl by KCl practically does not affect the iron ion oxidation rate but decreases the rate of the cyclohexane oxidation product formation half as much. In all experiments cyclohexanol and cyclohexanone are formed simultaneously in almost equal proportions. Increase of the initial concentration of $FeCl_2$ results in the enhancement of the cyclohexane oxidation rate up to a definite limit (Fig. 3). Introduction of inhibitors of radical processes does not affect the oxidation of $FeCl_2$ but slows down the oxidation of cyclohexane (Fig. 5). Addition of cysteine to the reaction mixture causes an increase in the rate of the oxidation product formation; whereas introduction of ethylenediamine, which forms a complex with the iron ions, inhibits the oxidation of cyclohexane.

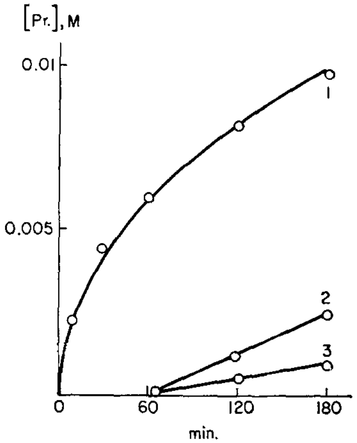


FIG. 5. The effect of inhibitors on the oxidation of cyclohexane at 50°C, $[FeCl_2]_0 = [HCl]_0 = 0.05\ M$, $[C_6H_{12}]_0 = 1.80\ M$; 1, without inhibitors; 2, in the presence of α-naphthol (0.02 *M*); 3, in the presence of 2,4,6-tri*tert*-butylphenol (0.02 *M*).

In view of our kinetic data, of numerous evidence in the literature on the oxidation of Fe^{2+} ion in aqueous solutions (*5*, *6*), and of the mechanism of the reaction $Fe^{2+} + H_2O_2$ (*6*, *7*), the following scheme for oxidation of $FeCl_2$ with oxygen in methanol can be proposed.

$$Fe^{2+} + Cl^- \overset{K}{\rightleftarrows} FeCl^+$$

$$Fe^{2+} + O_2 \xrightarrow{k_1'} [Fe^{3+}O_2^-] \qquad [1']$$

$$FeCl^+ + O_2 \xrightarrow{k_1''} [ClFe^{2+}O_2^-] \qquad [1'']$$

$$[Fe^{3+}O_2^-] + H^+ \xrightarrow{k_2'} Fe^{3+} + HO_2^{\cdot} \qquad [2']$$

$$[ClFe^{2+}O_2^-] + H^+ \xrightarrow{k_2''} FeCl^{2+} + HO_2^{\cdot} \qquad [2'']$$

Then the Fe ions and FeCl complexes start the reactions.

$$Fe^{3+}(FeCl^{2+}) + HO_2^{\cdot} \xrightarrow{k_3} Fe^{2+}(FeCl^+) + H^+ + O_2 \qquad [3]$$

$$Fe^{2+}(FeCl^+) + HO_2^{\cdot} \xrightarrow{k_4} Fe^{3+}(FeCl^{2+}) + HO_2^- \qquad [4]$$

$$HO_2^- + H^+ \rightleftarrows H_2O_2 \qquad [5]$$

$$Fe^{2+}(FeCl^+) + H_2O_2 \xrightarrow{k_6} Fe^{3+}(FeCl^{2+}) + HO^{\cdot} + HO^- \qquad [6]$$

The radicals $HO^{\cdot}$ formed in reaction [6] predominantly attack methanol (SH); using the reported values for the rate constants for the reaction of $HO^{\cdot}$ with methanol (*8*) and with Fe^{2+} ions (*9*), it can be shown that, under the conditions of the present studies, the reaction with methanol proceeds 260 times faster than with Fe^{2+}. In fact:

$$\frac{W(HO^{\cdot} + CH_3OH)}{W(HO^{\cdot} + Fe^{2+})} = \frac{k(HO^{\cdot} + CH_3OH)[CH_3OH]_0}{k(HO^{\cdot} + Fe^{2+})[Fe^{2+}]_0} = \frac{1.8 \times 10^8\, 19.7}{2.6 \times 10^8\, 0.05} = 260.$$

Since the rate constants for the reaction of $HO^{\cdot}$ with methanol and cyclohexane are equal, then

$$\frac{W(HO^{\cdot} + CH_3OH)}{W(HO^{\cdot} + C_6H_{12})} \approx \frac{[CH_3OH]_0}{[C_6H_{12}]_0} = \frac{19.7}{1.8} \approx 11$$

Thus the $HO^{\cdot}$ radicals preferentially react with the solvent:

$$HO^{\cdot} + SH \rightarrow H_2O + S^{\cdot} \qquad [7]$$

$$S^{\cdot} + O_2 \rightarrow SO_2^{\cdot} \qquad [8]$$

$$2SO_2^{\cdot} \rightarrow \text{recombination} \qquad [9]$$

In the absence of HCl, reaction [10] occurs:

$$[FeO_2^{2+}] + Fe^{2+} \rightarrow 2Fe^{3+} + O_2^{2-} \qquad [10]$$

Since in the presence of HCl, experiments exhibit first-order reaction as to the concentration of the iron ions, reaction [2] is rate limiting and the equation for the initial rate of the $FeCl_2$ oxidation will be written as

$$W_0^{FeCl_2} = -(d[FeCl_2]/dt) = f' k_1'[Fe^{2+}][O_2] + f'' k_1''[FeCl^+][O_2],$$

where f' and f'' are the stoichiometric coefficients for the reaction of O_2 with Fe^{2+} and $FeCl^+$, respectively.

Since

$$[FeCl^+] = \frac{K[Fe^{2+}]_0[Cl^-]_0}{1+K[Cl^-]_0} \quad \text{and} \quad [Fe^{2+}] = [Fe^{2+}]_0 - [FeCl^+] = \frac{[Fe^{2+}]_0}{1+K[Cl^-]_0},$$

we get

$$W_0^{FeCl_2} = \frac{f'k_1' + f''k_1''K[Cl^-]_0}{1+K[Cl^-]_0} \cdot [Fe^{2+}]_0[O_2]_0 = k^{eff}[Fe^{2+}]_0[O_2]_0,$$

where

$$k^{eff} = \frac{f'k_1' + f''k_1''K[Cl^-]_0}{1+K[Cl^-]_0}.$$

Thus, in full agreement with experimental data, the first order of the reaction in Fe^{2+} is obtained, the rate constant being an effective value dependent on the concentration of Cl^- and independent of the H^+ concentration, which agrees with the experiment as well; i.e., replacement of HCl by KCl does not affect the rate of the $FeCl_2$ oxidation.

It is easily seen that, at large concentrations of Cl^-, $k^{eff} \rightarrow f''k_1''$. k_1'' at 50°C can be calculated from the experimental data accurate to the stoichiometric coefficient f'':

$$k_1'' \approx \frac{W_0^{FeCl_2}}{[FeCl_2]_0[O_2]_0} = \frac{9.5 \times 10^{-6}}{0.05 \times 8.0 \times 10^{-3}} = 0.024\ M^{-1}\ \text{sec}^{-1}$$

It should be noted that the rate constant of the $FeCl_2$ oxidation in the acidic aqueous medium at 50°C is essentially higher and equal to 1.6 M^{-1} sec^{-1} according to Ref. (*10*).

It should also be noted that reactions [3] and [4] in our scheme are competitive: The rate constants of these reactions are similar and equal at room temperature, $k(HO_2^{\cdot} + Fe^{3+}) = 7.3 \times 10^5$ and $k(HO_2^{\cdot} + Fe^{2+}) = 9.1 \times 10^5\ M^{-1}\ \text{sec}^{-1}$ (*11*, *12*). The radical $HO_2^{\cdot}$ possesses amphoteric properties and can be both an oxidant and a reductant providing regeneration of the Fe^{2+} or $FeCl^+$ ions.

When discussing a scheme for cyclohexane oxidation it should be taken into account that (i) increase in the cyclohexane concentration does not affect the rate of the $FeCl_2$ oxidation (Table 1); (ii) inhibitors of the radical processes slow down oxidation, and finally, (iii) cyclohexanol and cyclohexanone are formed simultaneously. It is essential that, in the absence of HCl, cyclohexane is oxidized not only in methanol, but in an aprotic solvent, i.e. acetone, as well. This means that the complex $[Fe^{3+}O_2^-]$ can be an attacking particle, for otherwise oxidation would not be observed in aprotic media where the radicals $HO_2^{\cdot}$ are not formed, whereas the ion-radical $\dot{O}_2^-$ is known to possess no hydroxylating ability. Antioxidative action of α-naphthol and 2,4,6-tri*tert*-butylphenol testifies to the fact that the attacking particle is a radical.

In view of the foregoing it can be assumed that $[Fe^{3+}O_2^-]$ is present in the radical form $FeOO^{\cdot}$, being a kind of an inorganic analog of the peroxyradicals $RO_2^{\cdot}(HO_2^{\cdot})$. There can be proposed alternative schemes of cyclohexane oxidation. Scheme "A" includes the reactions

$$C_6H_{12} + \dot{O}OFe \rightarrow \dot{C}_6H_{11} + HOOFe \qquad [11]$$

Then $\dot{C}_6H_{11}$ is converted via the known chain mechanism into:

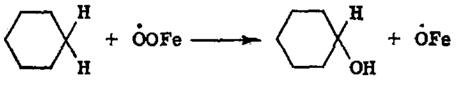
$$\dot{C}_6H_{11} + O_2 \rightarrow C_6H_{11}OO^{\cdot} \xrightarrow{C_6H_{12}} C_6H_{11}OOH$$

$$C_6H_{11}OOH + Fe^{2+} \rightarrow C_6H_{11}O^{\cdot} + Fe(OH)^{2+}$$

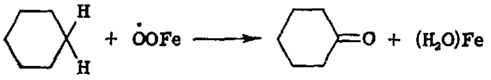
$$C_6H_{11}O^{\cdot} + C_6H_{11}OH \rightarrow C_6H_{11}OH + \dot{C}_6H_{11}$$

$$C_6H_{11}OOH \xrightarrow{H^+} C_6H_{10}O + H_3O^+$$

It is assumed that an oxygen atom transfers to the substrate following scheme "B".



[12]



[13]

Reactions [12] and [13] can proceed in several stages, some of which can be acid calalyzed. In this case the effect of the H^+ ions on the rate of reaction product formation will be taken into account. It should be noted that for the radical $C_6H_{11}OO^{\cdot}$ (*13*), a reaction was supposed similar to reaction [12].

It is difficult enough to choose between schemes "A" and "B" on the basis of the kinetic data alone, as both of them agree with kinetic laws.

It can be shown that in protonic media the hydroxylating role of $HO_2^{\cdot}$ radical cannot but be accounted for. In fact, reaction

$$C_6H_{12} + HO_2^{\cdot} \rightarrow \dot{C}_6H_{11} + H_2O_2$$

is the exothermic one since its thermal effect $q = Q_{C_6H_{11}-H} - Q_{HOO-H} = 87.0–89.5 = -2.5$ kcal/mol. On the basis of the Semyonov–Polany equation (*14*) for exothermic reactions $E_{act} = 11.5 - 0.25|q| \approx 10$ kcal/mol. If a preexponential multiplier for the reaction of $HO_2^{\cdot}$ with C_6H_{12} is taken to equal $10^6\ M^{-1}\ sec^{-1}$ in analogy to the reaction of cyclohexylperoxyradicals with C_6H_{12} (*15*), then from $k(HO_2^{\cdot} + C_6H_{12}) = 10^6 \times \exp(-10\,000/RT)\ M^{-1}\ sec^{-1}$ we shall obtain 0.005 $M^{-1}\ sec^{-1}$ for 27°C and 0.17 $M^{-1}\ sec^{-1}$ for 50°C. These are essentially large values for the reaction rates to testify towards the possibility of the reaction of $HO_2^{\cdot}$ with C_6H_{12}. Effective activation energy of 6.5 kcal/mol found in this work coincides well with its values for the reaction of cyclohexane with cyclohexyl-peroxy-radicals, which are 8.5 kcal/mol in Ref. (*15*) and 7.0 kcal/mol in Ref. (*16*).

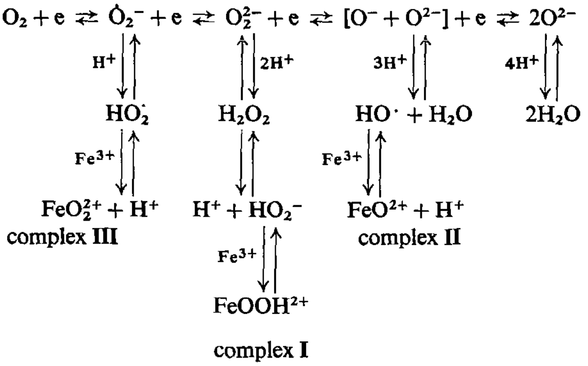
The stimulating role of cysteine in oxidizing cyclohexane involves regeneration of Fe^{2+}-ions according to the reaction:

$$RSH + Fe^{3+} \rightarrow RS^{\cdot} + H^+ + Fe^{2+} \qquad [14]$$

Reaction [14] is one of the most important reactions in a general scheme of the catalytic oxidation of organic compounds (*17*). It is of interest that thiol compounds are the fifth coordinating ligand of the iron ion in cytochrome P-450 (*18*) and their role in the reduction of cytochrome is very significant (*19*). That the rate of cyclohexane oxidation reaches its limit with increase of $[FeCl_2]_0$ can be explained by the growing role of reaction [10] (Fig. 3). In the experiments at a concentration of $[FeCl_2]_0 > 0.05\ M$, transition from the reaction of the first order with respect to Fe^{2+} to that of the fractional and then to that of the second order is observed.

The simple model system in question allows hydroxylation of cyclohexane, which is hardly oxidized with oxygen, to be performed under mild reaction conditions. The number and the nature of hydroxylating species differ with the reaction medium. Hydroxylating agents could be either the complexes $[Fe^{3+}O_2^-]$ which transfer oxygen to the substrate via the "oxenoid" mechanism (*20*) or the radicals $HO_2^{\cdot}$ which react following a conventional chain scheme.

A detailed analysis of the hydroxylation mechanisms (*6*) shows that, depending on reaction conditions, different species may act as hydroxylating agents: (i) The radical $HO^{\cdot}$ (Fenton reagent and radiation-chemical hydroxylation), (ii) the radical $HO_2^{\cdot}$ (coupled oxidation of aromatic compounds with alcohols and hydrocarbons), and (iii) complexes of the iron-containing enzymes with oxygen. It is a frequent practice to contrast our mechanism with another, though there may be a close connection between them. Here, we try to demonstrate this connection by successive consideration of the oxygen molecule reduction and the fate of intermediate particles in this reduction in the presence of the iron ions. The scheme below shows gradual reduction of the O_2 molecule and formation of all possible hydroxylating species:



According to George (*21*), the oxidizing ability of oxygen differs depending on at which stage the reaction ceases. If a reaction proceeds to the iron radical $\dot{O}_2^-$ level, and the latter cannot react further, then oxygen is a weak oxidant. A higher degree of oxidation is reached when $\dot{O}_2^-$ combines with a proton and $HO_2^{\cdot}$ is formed. As it follows from the scheme, in the presence of iron ions, the $HO_2^{\cdot}$ and $HO^{\cdot}$ radicals formed at different stages and the O_2 molecule reduction can be stabilized with the iron ions as a result of which there appear complexes **I**, **II** and **III**, well known in enzymology.

Thus, depending on the conditions of the O_2 reduction, the $HO^{\cdot}$ and $HO_2^{\cdot}$ radicals or their stabilized modifications, complexes **I**, **II** and **III** may be hydroxylating agents. As is shown here, the character of the hydroxylating particle depends primarily on the presence of H^+ ions: in aprotic media a perferryl ion (complex III) acts as a hydroxylating particle, whereas in protonic media this role is played by the radicals $HO_2^{\cdot}$ and $HO^{\cdot}$. The character of the perferryl ion attack on the substrate can change and be either radical, electrophilic, or even nucleophilic one, depending on the ligand bound with the iron ion (*22*).

REFERENCES

1. G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).
2. A. A. Akhrem, D. I. Metelitsa, and M. E. Skurko, *Usp. Khim.* (Russ.), **44**, 868 (1975).
3. D. B. Fischer, R. Kirkwood, and S. Kaufman, *J. Biol. Chem.*, **247**, 5161 (1972).
4. A. Kramer, H. Staudinger, and V. Ulrich, *Chem. Biol. Interact.*, **8**, 11 (1974).
5. F. E. Hufman and N. Davidson, *J. Amer. Chem. Soc.*, **78**, 4836 (1956).
6. D. I. Metelitsa, *Usp. Khim.* (Russ.), **40**, 1175 (1971).
7. N. Uri, *Chem. Rev.*, **50**, 375 (1952).
8. M. Anbar, D. Meyerstein, and P. Neta, *J. Chem. Soc. B*, 742 (1966).
9. H. A. Schwarz, *J. Phys. Chem.*, **66**, 255 (1962).
10. B. P. Matzeewski and D. P. Feldman, *Kinet. Katal.* (Russ.), **14**, 921 (1973).
11. J. P. Keene, *Radiat. Res.*, **22**, 1 (1964).
12. G. G. Jayson, B. J. Parsons, and A. J. Swallow, *J. Chem. Soc. Faraday Trans.*, **1**, 236 (1973).
13. I. V. Berezin, E. T. Denisov, and N. M. Emanuel, "Oxidation of Cyclohexane," Pergamon Press, New York, 1966.
14. N. M. Semyonov, "On Some Problems of Chemical Kinetics and Reactivity" (Russ.), Edition of the USSR Academy of Science, pp. 41–43, Moscow, 1958.
15. L. Bateman and G. Gee, *Trans. Faraday Soc.*, **47**, 155 (1951).
16. J. C. Robb and M. Shanin, *J. Inst. Petrol*, **44**, 283 (1958).
17. E. T. Denisov, V. D. Komissarov, and D. I. Metelitsa, *Discuss. Faraday Soc.*, **46**, 130 (1968).
18. V. Ulrich, H. H. Ruf, and H. Mimoun, *Biochem. J.*, 9P (1972).
19. J. C. Gunsalus, J. D. Lipcomb, V. Marshall, H. Frauenfelder, E. Münck, and E. Greenbaum, *Biochem. J.*, 5P (1972).
20. G. A. Hamilton, J. R. Giacin, T. M. Hellman, M. E. Shook, and J. M. Weller, *Ann. N.Y. Acad. Sci.*, **212**, 5 (1973).
21. P. George, "Oxidases and Related Redox Systems," p. 4, Wiley, New York, 1965.
22. G. A. Hamilton, *Advan. Enzymol.*, **32**, 55 (1969).