Chemistry of Natural Compounds and Bioorganic Chemistry

The $Fe^{IV}_2(\mu\text{-}O)_2$ cluster and bridge O_2 activation at the active center of methane monooxygenase

A. A. Shteinman

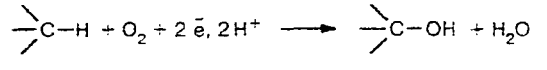
Institute of Chemical Physics in Chernogolovka, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: 007 (096) 515 3588. E-mail: ast@icp.ac.ru

The newest experimental data concerning soluble forms of methane monooxygenase (MMO) were analyzed taking into account the bridge mechanism of O_2 activation by this enzyme proposed earlier. The results confirm that the scheme suggested and the structures of the key intermediates are valid and show a basic difference between the mechanisms of activation for heme (cytochrome P-450) and non-heme (MMO) monooxygenases. The X-ray diffraction analysis of MMO allowed us to develop a more detailed scheme that reflects the dynamics of O_2 activation and the role of ligands in this process.

Key words: O_2 activation, mechanism; iron clusters; methane monooxygenase.

The mechanism of methane and dioxygen activation by the iron-containing non-heme center of methane monooxygenase (MMO) from methanotrophic bacteria is of interest for researchers due to the unique selective oxidation of methane by this enzyme.[1–5] The structures of the two most studied soluble forms of MMO from *Methylococcus capsulatus* (Bath) and *Methylosinus trichosporium* OB3b have been reliably determined.[6–8] The enzyme consists of three protein components (**A**, **B**, and **C**), the main of which is hydroxylase (**A**), which contains a binuclear iron cluster and is responsible for dioxygen activation and methane oxidation. Similarly to other monooxygenases, MMO requires reductase (**C**), which contains flavin and Fe—S cofactors and transfers electrons from NADH (reduced nicotinamide dinucleotide) to hydroxylase. There is also protein **B** (with a small molecular weight), which contains no cofactor and performs a regulatory function: when it is absent, hydroxylation is either inefficient or does not occur at all. The transfer of electrons from reductase to the Fe-center results in the reductive activation of O_2, which leads to the formation of an active oxidant capable of attacking a strong C—H bond. The overall process (Scheme 1) is conjugated oxidation completed in the formation of two products: alcohol and water.

Scheme 1

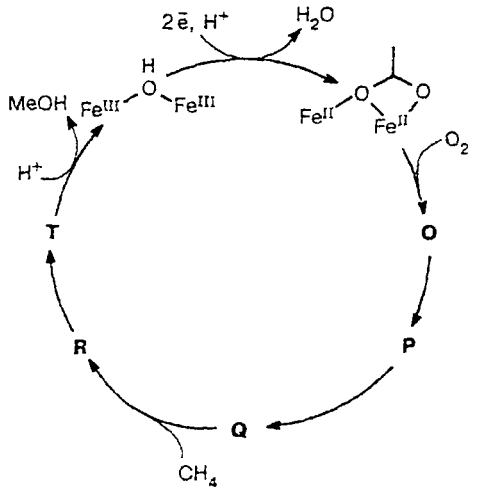


This route of dioxygen activation is the same for all monooxygenases, but the nature of the intermediates

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 9, pp. 1676—1682, September, 1997.

1066-5285/97/4609-1599 $18.00 © 1997 Plenum Publishing Corporation

Scheme 2



and the individual features of this process depend on the structure of a given active center and need to be revealed in each particular case.

The sequence of catalytic stages has been established for MMO (Scheme 2). The molecular structures of the initial (ferri-) and reduced (ferro-) MMO were determined[7] from the X-ray diffraction data for crystals of the corresponding forms. The interaction of ferro-MMO with O_2 results in the formation of several intermediates:[1,2] **O**, **P**, **Q**, **R**, and **T**, which were characterized by spectral and kinetic methods for studying fast reactions (since they accumulate in noticeable concentrations before decay). The reaction with O_2 is accompanied by the disappearance (k = 22 s^{-1}, 4 °C) of the characteristic ESR signal of the ferro-form (g = 16) corresponding to the appearance of intermediate **P**. The rate of this process is independent of the concentration of O_2 in a wide range. This points to the very fast and, in fact, irreversible formation of a precursor of intermediate **P**, oxygen complex **O**, which retains the ESR signal with g = 16 and has no other distinctions from the ferro-form. The Mössbauer spectrum of intermediate **P** (δ = 0.66 mm s^{-1}, ΔE_Q = 1.55 mm s^{-1}) indicates that the iron in this intermediate exists in the ferri-state, but possesses unusual properties. A band at 905 cm^{-1}, which is typical of peroxides, was observed by resonance Raman spectroscopy. Compound **P** spontaneously transforms (k = 1.2 s^{-1}, 4 °C) into yellow intermediate **Q** (λ_{max} = 330, 430 nm). The rate of formation of compound **Q** is independent of the addition of methane, and the rate of its disappearance is proportional to the concentration of CH_4. From this it was concluded that **Q** is an intermediate interacting with methane. In the absence of a substrate, compound **Q** can accumulate to a concentration sufficient for spectral studies. The Mössbauer spectrum of intermediate **Q** (δ = 0.17 mm s^{-1}, ΔE_Q = 0.53 mm s^{-1}) indicates that the iron in this intermediate exists in the high-spin Fe^{IV}-state. The disappearance of complex **Q** in the reaction with methane (k = 16 L $mmol^{-1}$ s^{-1}) is detected by a decrease in the intensity of its characteristic absorption. The published data[1] contain arguments in favor of the fact that compound **Q** reacts with methane by the elimination of H to form radical intermediate **R**. However, as shown below, other data favor the inclusion of a nonradical intermediate at this stage. The use of an alternative substrate (nitrobenzene) for studying the reactivity of compound **Q** makes it possible to use the stop-flow technique to observe the formation of colored *m*- and *p*-nitrophenols. Thus, the formation of a complex between the product and MMO (T) was shown. The decomposition of this complex to the free product and ferri-MMO is the rate-limiting stage of the whole process.

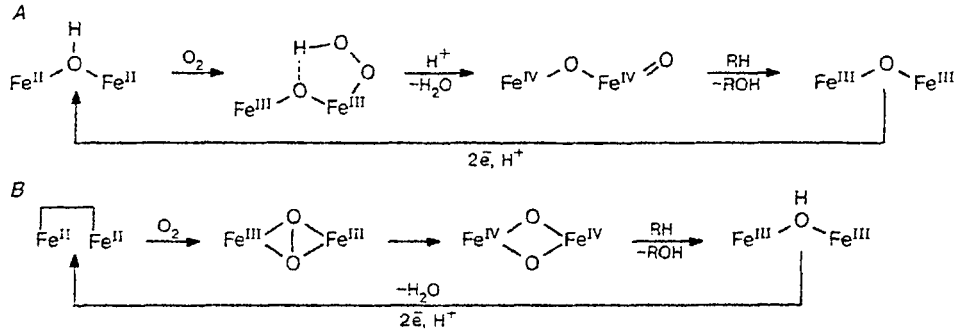
Based on numerous studies of a soluble form of MMO from various cultures of microorganisms, it has been shown that the iron-containing active center of hydroxylase is a binuclear complex, in which Fe atoms are bound by oxo and carboxylate bridges.[1—3,6—8] Two types of O_2 activation are possible, in principle, at this center: activation due to the terminal coordination of oxygen to one Fe atom or activation as a result of bridge coordination of oxygen between two Fe atoms, *i.e.*, terminal and bridge activation of dioxygen, respectively. Since only terminal activation of dioxygen is possible for hydrocarbon oxidation by iron-porphyrin complexes, which model heme monooxygenase (cytochrome P-450), and terminal binding of oxygen by the binuclear Fe center of hemerythrin is well studied, it has been considered for a long time that the mechanisms of dioxygen activation and hydrocarbon oxidation by MMO resemble the mechanism proposed for cytochrome P-450, *i.e.*, they involve terminal activation of O_2. It has been assumed that the intermediate hydroxyhemerythrin-like peroxide is cleaved with the participation of a proton to yield water and to form an active ferryl intermediate that is capable of attacking the C—H bond (Scheme 3, *A*).

Recently we suggested[9] a basically new, bridge mechanism for activation of dioxygen by MMO that takes into account the binuclear structure of its active center and agrees better with the known experimental data (Scheme 3, *B*). This mechanism has been accepted by many researchers and, as shown below, is in accordance with recent experimental results.

This work is aimed at analyzing recent achievements in this intensely developed field that confirm that the bridge mechanism is valid and suggest a more detailed scheme based on the data of X-ray structural studies of two oxidized and one reduced form of MMO. This scheme demonstrates clearly the dynamics of O_2 activation and the role of ligands in this process.

The main point of the bridge mechanism[9] is the following. In the case of a harder (according to Pearson's classification) non-heme center of MMO, the terminal stabilization of an O atom at one Fe atom in the form of ferryl (Fe=O), as in iron-porphyrin models of cytochrome P-450, seems improbable. In this case, the

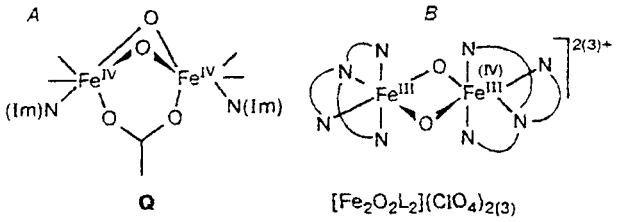
Scheme 3



bridge stabilization of an O atom by its binding with two Fe atoms is considerably more favorable. This concept has found strong experimental support:[10] it has been established that both Fe^{IV} atoms are indiscernible in the Mössbauer spectra of the active intermediate **Q** (see Scheme 2) detected in MMO from *Methylosinus trichosporium* by the stop-flow method and rapid freezing. The Mössbauer spectra of MMO from *Methylococcus capsulatus* indicate that the Fe atoms are nonequivalent,[11] which, however, can be related to a change in the ligand surroundings. These studies[10,11] also observed intermediate **P**, which is a peroxide precursor of intermediate **Q** (see Scheme 2).

The concept of bridged oxo intermediate **Q** logically resulted in a bridged structure for a peroxide precursor formed by the coordination of O_2 between two Fe atoms, contrary to the concept on a terminal peroxide intermediate similar to hydroxyhemerythrin. Examples for the bridge activation of O_2 with the formation of μ-1,2-peroxide are well known for model iron(III) complexes.[12—14]

Scheme 4



Q $[Fe_2O_2L_2](ClO_4)_{2(3)}$

L = 5-Me_3, 6-Me_3TPA
(TPA is tris(2-pyridylmethyl)amine)

In the case of the bridge mechanism, the high-valent $Fe^{IV}_2(\mu\text{-}O)_2$ cluster (Scheme 4, *A*), in which both O atoms are generated from an O_2 molecule, was suggested[4,9] as an alternative to ferryl. Since at that time clusters with two O-bridges were unknown for binuclear iron complexes, we attempted to synthesize these complexes. However, soon the report[15] of the first bis(μ-oxo)diiron(III) cluster (see Scheme 4, *B*) appeared. Somewhat later,[16] a similar structure of the $Fe^{III}Fe^{IV}(\mu\text{-}O)_2$ cluster was established for an intermediate isolated at low temperature in a model chemical system. This intermediate was capable of oxidizing weak C—H bonds. The latter result is expected, and it can be assumed that the postulated $Fe^{IV}_2(\mu\text{-}O)_2$ cluster should be more active, because two-electron transfer involving the insertion of the O atom into the C—H bond, which is thermodynamically more favorable than any one-electron process of abstraction of the H atom, becomes possible for this cluster. In fact, the reaction of $Fe^{III}Fe^{IV}(\mu\text{-}O)_2$ cluster with ethylbenzene is 3,000,000 times slower than that of intermediate **Q** with methane: the second-order rate constants are equal to $5 \cdot 10^{-6}$ and 16 L $mmol^{-1}$ s^{-1}, respectively. When $Fe^{III}Fe^{IV}(\mu\text{-}^{18}O)_2$ reacts with the tertiary C—H bond of isopropylbenzene in acetonitrile at −40 °C, ^{18}O is incorporated into the alcohol formed with 88% efficiency.[17] For the hydroxylation of the secondary C—H bond by this species, the kinetic isotope effect (KIE) is equal to 20. However, the study of the reaction mechanism showed that, as should be expected, $Fe^{III}Fe^{IV}(\mu\text{-}O)_2$ behaves as a one-electron oxidant abstracting H atom and generating a hydrocarbon radical at the first limiting stage; the reaction of this radical with a second equivalent of the oxidant gives alcohol and olefin. The formation of alcohol resembles the function of MMO, and the formation of olefin models the function of a desaturase, for example, stearoyl-Δ^9-desaturase,[18,19] which also contains an active Fe—O—Fe center, has spectral characteristics similar to those of MMO, and is capable of catalyzing the dehydrogenation of saturated hydrocarbon chains. Although the $Fe^{III}Fe^{IV}(\mu\text{-}O)_2$ intermediate from the chemical model system has one electron more than intermediate **Q** of the MMO enzyme, the Mössbauer parameters of both intermediates are very similar,[16] which is probably not a coincidence.

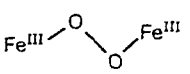
The lower limit for the constant of antiferromagnetic interaction coupling of two paramagnetic Fe atoms in intermediate **Q** was estimated[10] as 60 cm^{-1}, which is outside the range of values known for binuclear μ-oxo bridged iron complexes (90—120 cm^{-1}), but is close to

values of this parameter for bis(μ-oxo)diiron(III)[15] (61 cm^{-1}) and bis(μ-oxo)dimanganese(IV)[20] (44 cm^{-1}) clusters. In the light of the hypothesis of the $Fe^{IV}_2(\mu\text{-}O)_2$ intermediate of MMO as an alternative to ferryl, it is of interest that no resonance Raman band corresponding to stretching of the Fe^{IV}=O bond is detected for compound **Q**.[21] By and large these observations agree with the suggested structure of active intermediate **Q** as the $Fe^{IV}_2(\mu\text{-}O)_2$ cluster.

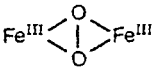
The transfer of an O atom from the $Fe^{IV}_2(\mu\text{-}O)_2$ center accompanied by its insertion into a C—H bond should occur as a synchronous two-electron process and involve an intermediate[4,9] similar to that with pentacoordinated carbon that has been suggested recently for the reactions of ferryl with alkanes.[22] In the case of a methane molecule, which has the smallest size, additional nucleophilic assistance by a second bridged O atom is possible due to the formation of a hydrogen bond. This assumption could explain the strong proton tunneling effect observed for MMO only in methane hydroxylation.[23] In this case, for methane homologs, the increasing sizes of the molecules could prevent the formation of a hydrogen bond with the second O atom due to the steric hindrances created by the ligands at the Fe atoms. The electronic and steric requirements for the interaction between the active $Fe^{IV}_2(\mu\text{-}O)_2$ intermediate and the C—H bond explain the fact that the rate of oxidation of methane and lower alkanes is greater than that of higher alkanes and that the attack of primary, secondary, and tertiary C—H bonds observed in the oxidation of lower alkanes is almost nonselective.[4,9] Similar tendencies were observed for a chemical model system using binuclear iron complexes. For this system, a two-electron mechanism of the transfer of an O atom with insertion at the C—H bond of alkanes has been proved recently.[24] A detailed study of this system suggests that it involves a bis-μ-oxo bridged intermediate.[25] A similar intermediate probably participates in the oxidation of methane in the purely inorganic model of MMO based on binuclear iron oxo complexes immobilized on a zeolite matrix.[26]

In order to explain the differences in the KIE values calculated from the ratio of the rate constants of the consumption of intermediate **Q** in its reactions with CH_4 and CD_4 (k^H/k^D = 50—100) and from the data of analysis of the products of this reaction (k^H/k^D = 19), the authors of Ref. 23 assumed an equilibrium between the bis-μ-oxo form and a more reactive form of intermediate **Q** (**Q′**), which can shift to the latter in the reversible interaction with methane. Ferryl or, more probably, an oxene complex in which the O atom is bound to two Fe^{III} centers, may be this reactive form. In this case, the greater isotope effect corresponds to the reaction of methane with **Q**, and the smaller one corresponds to that with **Q′**. At the same time, the concept that the reaction CH_4 + **Q′** occurs *via* an intermediate with pentacoordinated carbon[22] allows one to explain[27] the different values of KIE obtained[23] for the ratio of the reaction rate constants at the C—H and C—D bonds in different molecules (CH_4/CD_4) and within the same molecule (CHD_3, CH_2D_2, or CD_3H).

The bridged structure of peroxide intermediate **P** in MMO is confirmed by the existence of a sharp and symmetric doublet in the Mössbauer spectrum of this intermediate, which indicates binding of O_2 to both Fe atoms.[11] The formation of the active $Fe^{IV}_2(\mu\text{-}O)_2$ intermediate due to the rearrangement of the bridged peroxide makes it possible to avoid the participation of a proton and release of a water molecule at this stage. This is one of the important advantages of the bridge mechanism, which takes place due to the binuclear structure of the active center and is characterized by the fact that the cleavage of the O—O bond is facilitated by the participation of two Fe atoms. The bridged peroxide intermediate can have the structure of a μ-1,2-peroxide corresponding to the "end" (end-on) coordination of O_2 to two Fe atoms or a $\mu\text{-}\eta^2\text{:}\eta^2$-peroxide corresponding to the "side" (side-on) coordination of O_2. Evidently, the side-on $\mu\text{-}\eta^2\text{:}\eta^2$-peroxide, which is as much as possible ready for further transformation into the $Fe^{IV}_2(\mu\text{-}O)_2$ structure, is more preferable as a precursor of active intermediate **Q**.[9] The rearrangement of the side-on $\mu\text{-}\eta^2\text{:}\eta^2$-peroxide into the bis-μ-oxodimetallo-intermediate has been recently demonstrated[28] for the first time for the corresponding copper complexes. An interesting feature of this transformation is its reversibility depending on the solvating nature of the solvent. By analogy, one can propose a possible regulatory mechanism for the effect of protein **B** on the hydroxylase active site due to the variation of its surroundings (adjacent residues of amino acids) through several conformational changes in the polypeptide framework that occur after binding of protein **B**. Favorable surroundings could strongly increase the rate of transformation of the side-on $\mu\text{-}\eta^2\text{:}\eta^2$-peroxide into the active bis-μ-oxo intermediate.



end-on



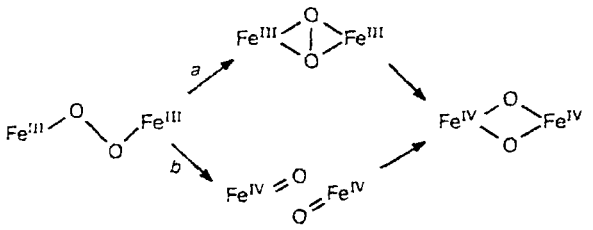
side-on

The high activation entropy (147 J mol^{-1} deg^{-1}) determined[21] for the transformation of intermediate **P** into **Q** probably agrees better with the end-on μ-1,2-structure for peroxide intermediate **P** (see below). On the other hand, if the transformation of the side-on $\mu\text{-}\eta^2\text{:}\eta^2$-peroxide into the active bis-μ-oxo-intermediate is related to strong desolvation, this also can increase the activation entropy.

The model μ-1,2-peroxide iron complex,[14] whose Mössbauer spectral parameters are very similar to the corresponding parameters of peroxide intermediate **P**, has been synthesized recently. This can be considered to be a proof[14] of a similar structure of intermediate **P**. However, the coordination surroundings of iron in MMO and in the model indicated are different, and the coincidence of the Mössbauer spectra may be random.

Accepting the μ-1,2-structure for peroxide intermediate **P**,[14,21] it is necessary to analyze the mechanism of its transformation into intermediate **Q**. Two routes are most probable (Scheme 5, *a* and *b*). Since the route involving the high-energy diferryl intermediate[13] is less favorable, the transformation **P** → **Q** should evidently include the μ-η^2:η^2-peroxide as a still shorter-lived side-on intermediate formed from end-on intermediate **P**. The intertransformation of the side-on and end-on peroxides has been demonstrated recently[29] for binuclear copper(II) complexes. The end-on μ-1,2-peroxide is formed more rapidly, but then rearranges to the final product, side-on μ-η^2:η^2-peroxide. In the case of μ-1,2-peroxides, the M...M distance changes by up to 4.4 Å, while it cannot be longer than 3.7 Å for the μ-η^2:η^2-structure. The rearrangement of the initially formed end-on peroxide to the side-on one is postulated for the oxygen-binding Cu_2-center of hemocyanine[29] and a close analog of MMO, ribonucleotide reductase.

Scheme 5



An X-ray diffraction study of the hydroxylase component of MMO from *Methylococcus capsulatus* revealed[7] two types of organization of its active sites with different distances between the Fe atoms, which are transformed into each other when the temperature is varied between −160 and +4 °C. The study of hydroxylase from *Methylosinus trichosporium* by X-ray absorption spectroscopy (XAS) and then by X-ray diffraction analysis also showed the existence of two forms in a ratio of 60 : 40 with the distances between the Fe atoms (presumably in equilibrium with each other) equal to 3.0 and 3.4 Å.[8] The existence of this equilibrium reflects the capability of the binuclear center of MMO to adapt to several structures formed during O_2 activation.

Direct experimental evidence for the bis-μ-oxo bridged structure of active intermediate **Q** has been obtained[30] recently using EXAFS spectroscopy. Analysis of the spectra indicate that the structure of **Q** contains one short (1.77 Å) and one long (2.05 Å) Fe—O bond per each Fe atom, and the Fe...Fe distance is unusually short (2.46 Å). This value of the Fe...Fe distance agrees with the fact that the active center contains a carboxylate bridge along with two oxygen bridges, which is in accordance with the structure predicted previously.[4,9] The observation of two different distances to the bridged O atoms and asymmetry of Fe—O—Fe allowed one to consider the bis-μ-oxo bridged structure to be a dimer of two ferryl species.[30]

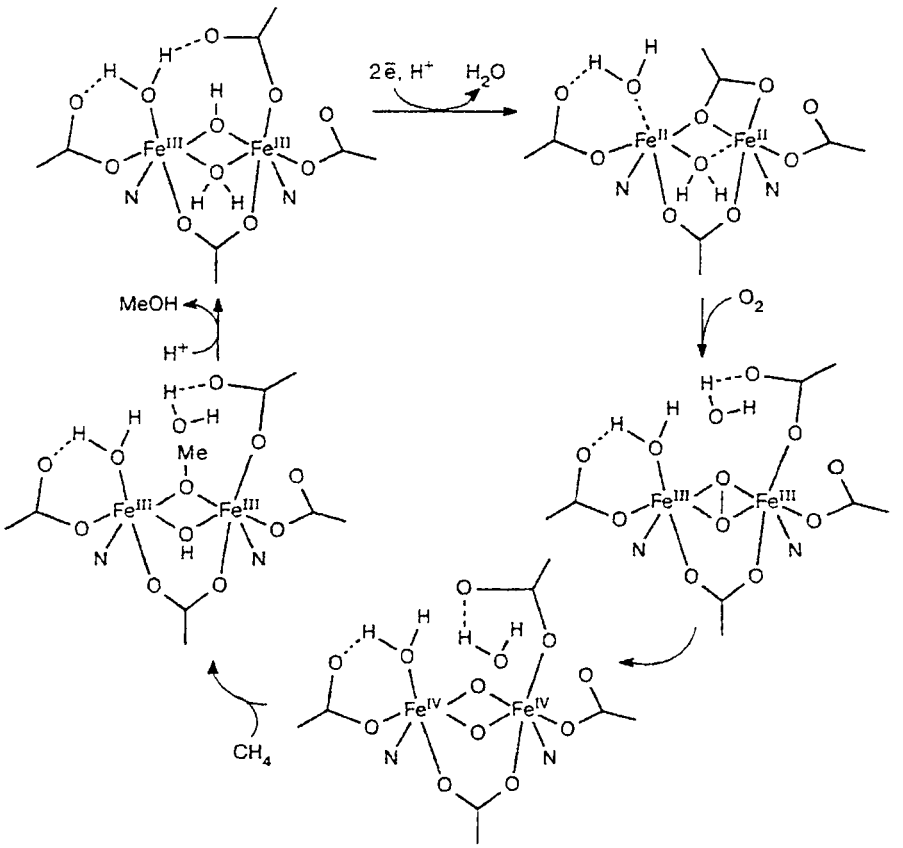
The results of studies of MMO performed over the past two years confirm reliably the bridge mechanism of O_2 activation in its binuclear active center and make it possible to describe in detail (Scheme 6) the previously suggested[4,9] scheme of the catalytic cycle of MMO. The most convincing evidence for the bridge mechanism was obtained from X-ray diffraction studies[6,7] of the initial Fe^{III}-form of MMO and its reduced Fe^{II}-form. The analysis of these structures shows that another product of the monooxygenase reaction (H_2O) leaves from the active center at the stage of reduction in accordance with the bridge mechanism (see Scheme 2, *B*). In the case of the terminal mechanism (see Scheme 2, *A*), which occurs in cytochrome P-450 and its iron-porphyrin models, an H_2O molecule is released at the stage of the formation of an active intermediate.

The detailed version of the catalytic cycle of MMO (see Scheme 6), which reflects the dynamics of O_2 activation, allows one to understand the role of carboxylate and water shifts[31] during the transformations postulated. When reduced hydroxylase reacts with O_2, the removal of the monooxygen-bridged carboxylate and a water molecule is accompanied by the simultaneous formation of a hydrogen bond between them and releasing four coordination sites necessary for side-on binding of O_2. At subsequent stages, $-COO^-$ must occupy the bridge position again to displace the reaction products (methanol and water) from the active center with the assistance of protons.

The above-mentioned process of shifting bridged ligands, carboxylate, and water can occur not synchronously, but in stages. The O_2 activation probably includes the initial formation of a μ-1,2-peroxide (end-on, see Scheme 5), because releasing rotational degrees of freedom of carboxylate and water at the next stage of the **P** → **Q** transformation could explain the high activation entropy found for this stage.[21] However, as indicated previously, the high values of the activation entropy also can be related to desolvation at the stage of cleavage of the O—O bond. The proton transfer transforms methoxyl into coordinated MeOH, which can be expelled to the hydrophobic medium surrounding the active center of MMO under the action of a more polar water molecule fixed near the active site by the H bond with carboxylate. Then this carboxylate with the assistance of a proton displaces another product of the monooxygenase reaction (H_2O) at the stage of reduction.

Thus, the bridge mechanism of O_2 activation by the binuclear center of MMO agrees well with the recent experimental results. EXAFS spectroscopy of intermediate **Q** [30] confirmed the structure of the three-bridged $Fe^{IV}_2(\mu\text{-}O)_2(\mu\text{-}COO)$ cluster suggested previously for this intermediate. The data of X-ray diffraction analysis of MMO[6,7] and information on the kinetics of the

Scheme 6



Note. N is the imidazole fragment of histidine;  is the carboxylate group of glutamic acid.

reaction of active intermediate **Q** with methane[23] made it possible to consider in detail the scheme of the catalytic cycle of MMO, to show the dynamics of O_2 and methane activation, to suggest a mechanism for the release of products from the active center, and to demonstrate the role of ligands in these transformations.

The intense development of studies in the field of MMO in recent years allows one to believe that the suggested bridge mechanism of dioxygen activation and selective methane oxidation by this enzyme will be completely proved and refined in the near future and adequate chemical models of the process will be developed.

The author is grateful to Academician A. E. Shilov for interest in the work and helpful remarks and to Professors L. Que and D. M. Kurtz for copies of their manuscripts kindly presented before publication.

This work was financially supported by the Russian Foundation for Basic Research (Project Nos. 94-03-08529 and 97-03-32253) and INTAS (Grant 93-315).

References

1. B. J. Wallar and J. D. Lipscomb, *Chem. Rev.*, 1996, **96**, 2625.
2. K. E. Liu and S. J. Lippard, *Adv. Inorg. Chem.*, 1995, **42**, 263.
3. K. K. Anderson and A. Graslund, *Adv. Inorg. Chem.*, 1995, **43**, 359.
4. A. A. Shteinman, *Izv. Akad. Nauk, Ser. Khim.*, 1995, 1011 [*Russ. Chem. Bull.*, 1995, **44**, 975 (Engl. Transl.)].
5. D. M. Kurtz, *J. Biol. Inorg. Chem.*, 1997, **2**, 159.
6. A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, and P. Nordlund, *Nature*, 1993, **366**, 537.
7. A. C. Rosenzweig, P. Nordlund, P. M. Takahara, C. A. Frederick, and S. J. Lippard, *Chem. and Biol.*, 1995, **2**, 409.
8. L. Shu, Y. Liu, J. D. Lipscomb, and L. Que, *J. Biol. Inorg. Chem.*, 1996, **1**, 297; N. Elango, R. Radhakrishnan, W. A. Froland, B. J. Wallar, C. A. Earhart, J. D. Lipscomb, and D. H. Ohlendorf, *Protein Sci.*, 1997, **6**, 556.
9. A. A. Shteinman, *FEBS Lett.*, 1995, **362**, 5.
10. S.-K.Lee, B. G. Fox, W. A. Froland, J. D. Lipscomb, and E. Munck, *J. Am. Chem. Soc.*, 1993, **115**, 6450.

11. K. E. Liu, D. Wang, B. H. Huynh, D. E. Edmondson, A. Salifoglou, and S. J. Lippard, *J. Am. Chem. Soc.*, 1994, **116**, 7465.
12. N. Ookubo, H. Sugimoto, T. Nagayama, H. Masuda, T. Sato, K. Tanaka, Y. Maeda, H. Okawa, Y. Hayashi, A. Uehara, and M. Suzuki, *J. Am. Chem. Soc.*, 1996, **118**, 701.
13. Y. Dong, S. Yan, V. G. Young, and L. Que, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 618.
14. K. Kim and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 4914.
15. Y. Zang, Y. Dong, and L. Que, *J. Am. Chem. Soc.*, 1995, **117**, 1169.
16. Y. Dong, H. Fujii, M. P. Hendrich, R. A. Leising, G. Pan, C. R. Randall, E. C. Wilkinson, Y. Zang, L. Que, B. G. Fox, K. Kauffmann, and E. Munck, *J. Am. Chem. Soc.*, 1995, **117**, 2778; L. Que and Y. Dong, *Acc. Chem. Res.*, 1996, **29**, 190.
17. C. Kim, Y. Dong, and L. Que, *J. Am. Chem. Soc.*, 1997, **119**, in press.
18. B. G. Fox, J. Shanklin, J. Ai, T. M. Loehr, and J. Sanders-Loehr, *Biochemistry*, 1994, **33**, 12776.
19. J. Shanklin, E. Whittle, and B. G. Fox, *Biochemistry*, 1994, **33**, 12787.
20. K. R. Reddy, M. V. Rajasekharan, S. Padhye, F. Dahan, and J.-P. Tuchagues, *Inorg. Chem.*, 1994, **33**, 428.
21. K. E. Liu, A. M. Valentine, D. Wang, B. H. Huynh, D. E. Edmondson, A. Salifoglou, and S. J. Lippard, *J. Am. Chem. Soc.*, 1995, **117**, 10174.
22. A. F. Shestakov and A. E. Shilov, *J. Mol. Catal., A*, 1996, **105**, 1; *Zh. Obshch. Khim.*, 1995, **65**, 622 [*Russ. J. Gen. Chem.*, 1995, **65** (Engl. Transl.)].
23. J. C. Nesheim and J. D. Lipscomb, *Biochemistry*, 1996, **35**, 10240.
24. V. S. Kulikova, O. N. Gritsenko, and A. A. Shteinman, *Mendeleev Commun.*, 1996, 119.
25. O. N. Gritsenko, G. N. Nesterenko, V. S. Kulikova, and A. A. Shteinman, *Kinet. Katal.*, 1997, in press [*Kinet. Catal.*, 1997, in press (Engl. Transl.)].
26. N. S. Ovanesyan, V. I. Sobolev, K. A. Dubkov, G. I. Panov, and A. A. Shteinman, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 1583 [*Russ. Chem. Bull.*, 1996, **45**, 1509 (Engl. Transl.)].
27. E. I. Karasevich, A. F. Shestakov, and A. E. Shilov, *Kinet. Katal.*, 1997, in press [*Kinet. Catal.*, 1997, in press (Engl. Transl.)].
28. J. A. Halfen, S. Mahapatra, E. C. Wilkinson, S. Kaderli, V. G. Young, L. Que, A. D. Zuberbuhler, and W. B. Tolman, *Science*, 1996, **271**, 1397.
29. B. Jung, K. D. Karlin, and A. D. Zuberbuhler, *J. Am. Chem. Soc.*, 1996, **118**, 3763.
30. L. Shu, J. C. Nesheim, K. Kauffmann, E. Münck, J. D. Lipscomb, and L. Que, *Science*, 1997, **275**, 515.
31. A. C. Rosenzweig, C. A. Frederick, and S. J. Lippard, *Microbial Growth on C1 Compounds: Proceedings of the 8th International Symposium on Microbial Growth on C1 Compounds*, Eds. M. E. Lindstrom and F. R. Tabita, Kluwer Acad. Publishers, Dordrecht, 1996, 141.

Received October 29, 1996;
in revised form February 28, 1997