Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 312 (2003) 143–148
BBRC
www.elsevier.com/locate/ybbrc

Effector proteins from $P450_{cam}$ and methane monooxygenase: lessons in tuning nature's powerful reagents☆

Brian J. Brazeau, Bradley J. Wallar,[1] and John D. Lipscomb*

Department of Biochemistry, Molecular Biology, and Biophysics, and the Center for Metals in Biocatalysis, University of Minnesota, Minneapolis, MN 55455, USA

Received 25 September 2003

Abstract

Effector proteins alter the kinetic or catalytic course of many oxygenase reactions. One of the first oxygenase effectors to be described was putidaredoxin, which serves to gate electron transfer into oxy-$P450_{cam}$. In the nonheme, methane monooxygenase (MMO) system, the B-component (MMOB) serves a distinct effector function by gating substrate and oxygen into the active site of the hydroxylase component (MMOH). Here the binding parameters and binding surfaces of the MMOB–MMOH complex are determined by site-specific labeling, fluorescence titrations, chemical cross-linking, and MALDI-TOF peptide identification. Based on these data, a model for the bimolecular complex is described and a hypothesis for the structural basis for the effector function is elaborated. The bearing on the putidaredoxin effector function is discussed.

Keywords: Methane monooxygenase; $P450_{cam}$; Effector; Kinetics; Fluorescence; Cross-linking; Regulation; Component docking

In the late 1960s, the cytochrome $P450_{cam}$ system was shown to consist of three protein components through pioneering work in the laboratory of I. C. Gunsalus (Gunny) [1]. The P450 containing component was clearly the site of oxygenase chemistry, but an NADH and FAD dependent reductase and the Fe_2S_2 cluster containing protein putidaredoxin were also required to observe sustained turnover. Two or three components were also shown to be essential for the function of mammalian P450s principally through work in the laboratories of M. J. Coon, D. Y. Cooper, O. Rosenthal, and R. W. Estabrook [2–4]. The presence of multiple components in these systems fostered consideration of the interaction of the proteins and the roles that such interaction might have on the progress and/or regulation of catalysis. It was soon recognized that component interactions in oxygenase systems play sophisticated roles that transcend delivery of reducing equivalents.

Early in 1970, the Gunsalus laboratory embarked on a study to define the intermediates in the catalytic cycle of $P450_{cam}$ using kinetic approaches [5,6]. Study of the binding reaction of O_2 to one-electron reduced $P450_{cam}$ revealed an oxy-complex that decayed slowly in the absence of putidaredoxin and could thus be trapped and characterized. The discovery of this complex allowed the recognition of a fundamental level of regulation in this system. The electron required to form the oxy-complex could derive from many sources, but that required to breakdown the oxy-complex to yield the reactive form of P450 could only be efficiently transferred from reduced putidaredoxin [5,7]. Gunny applied the term "effector" to describe this interaction to differentiate it from nonspecific electron transfer. Later we showed that the requirement for reduced putidaredoxin was not absolute, such that oxidized putidaredoxin, rubredoxin, apo-rubredoxin, cyt. b_5, apo-cyt. b_5, and lipoic acid could fulfill this role, albeit poorly [7,8]. The activity elicited by the apo-proteins suggested that a critical protein–protein interaction independent of electron

☆ *Abbreviations used:* MMO, methane monooxygenase; MMOH, hydroxylase component of MMO; MMOB, B component of MMO; MMOR, reductase component of MMO; EDC, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide; BADAN, 6-bromoacetyl-2-dimethylaminonaphthalene.

* Corresponding author. Fax: 1-612-624-5121.
E-mail address: Lipsc001@umn.edu (J.D. Lipscomb).
[1] Present address: Department of Chemistry, Grand Valley State University, Allendale, MI 49401, USA.

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2003.09.242

transfer was at the root of the effect. It was clear that the effects of putidaredoxin on the product forming second electron transfer reaction could be saturated, implying that a complex was formed. Also, chemical and enzymatic modifications of the putidaredoxin were used to show that specific residues, notably the C-terminal Trp of the protein, greatly influenced the stability of the complex [9]. Although these experiments revealed the effector role of putidaredoxin, the rationale for regulating the reaction in this way was not apparent.

In recent years, the work of the corresponding author has turned to non-heme oxygenase systems that catalyze oxygen insertion reactions into unactivated C-H bonds equivalent to the hallmark chemistry of P450. These systems, in particular methane monooxygenase (MMO), are also composed of several protein components [10,11]. Looking back on it now, our early work with Gunny prepared us to seek functions beyond what might seem to be obvious roles for the MMO components. Indeed, the MMO system does employ an effector protein (MMOB) with some of the properties of putidaredoxin, but without an electron transfer role, thereby allowing these two aspects of the oxygenase reaction to be clearly resolved [10,12]. In contrast to the $P450_{cam}$ system, the rationale for the effector role of MMOB is beginning to emerge and some new aspects of this intriguing interaction are reported here.

Materials and methods

MMO components were isolated from *Methylosinus trichosporium* OB3b as previously described [10,13]. Steady state assays were performed using methane, furan, or nitrobenzene as substrates as previously described [10,13,14]. All of the mutations were made in the MMOB gene located in plasmid pBWJ400 and were performed using the QuikChange system from Stratagene (La Jolla, CA) according to the instructions from the manufacturer. Cross-linking of MMOB to MMOH and separation of the cross-linked MMOB–MMOHα-subunit were performed as previously described [12]. In-gel proteolytic digests were preformed using the method of Shevchenko et al. [15] and MALDI-TOF mass spec was performed at the University of Minnesota. Fluorescence spectra were obtained using an ISS K2 Fluorometer. BADAN labeling was performed by incubating the A62C mutant of MMOB with a 6-fold excess of BADAN for 2.5 h at 4 °C followed by quenching with cysteine and removal of the excess reagent by Sephadex G25 gel chromatography.

Results

Binding characteristics

The MMO system consists of a reductase (MMOR), a cofactorless effector (MMOB), and an oxygenase (MMOH) containing a bis μ-hydroxo-bridged diiron cluster at the active site [10,16]. Together these components catalyze the following mixed function oxidation reaction:

$$NADH + H^+ + CH_4 + O_2 \rightarrow NAD^+ + CH_3OH + H_2O$$

MMOR contains both an FAD and a Fe_2S_2 cluster, thereby combining the electron transfer roles of the $P450_{cam}$ reductase and putidaredoxin in a single protein. MMOH is a dimeric protomer with each protomer containing α, β, and γ subunits. The crystal structure of MMOH has been solved and shows that the diiron cluster is buried 12 Å below the surface in a cavity with no connection to the bulk solvent [17,18]. Previous cross-linking studies employing EDC have shown that MMOB and MMOR bind to the MMOH α and β subunits, respectively [12]. Steady state kinetic studies showed that the rate of catalysis increases as the MMOB:MMOH ratio increases until it saturates at about a 1:1 (active sites) ratio [10,12]. At higher ratios, the rate decreases, presumably due to formation of inhibitory complexes [12].

To quantitatively evaluate the affinity and properties of the MMOB–MMOH complex, site-directed mutants of MMOB have been generated. One of these was based on the predicted secondary structure of MMOB that placed residue A62 at the surface. This position was later confirmed by the solution structure of MMOB obtained using NMR approaches [19]. Mutation of A62 to cysteine (A62C) results in a protein that elicits the full activity of MMOH and causes no change in the MMOB:MMOH ratio required to maximize the turnover rate. Because MMOB contains no other cysteines, the A62C mutation allows specific spin and

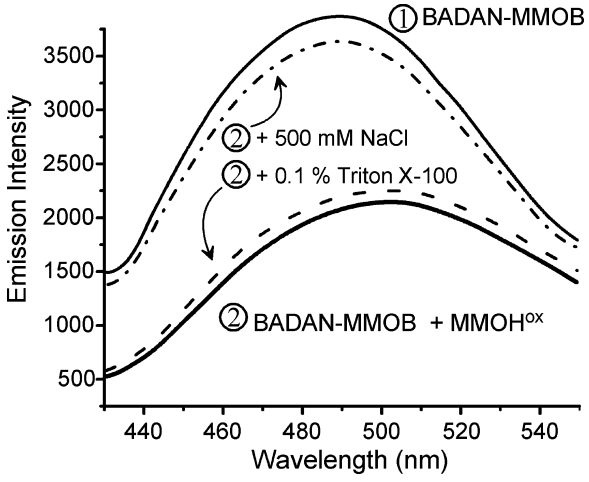

Fig. 1. Fluorescence emission spectra of A62C–BADAN labeled MMOB. The top spectrum (light solid line) represents 200 nM BADAN–A62C MMOB in 50 mM Mops, pH 7.0, at 25 °C. The bottom spectrum (heavy solid line) is the spectrum after the subsequent addition of 400 nM MMOH (active sites). After this initial quenching, NaCl was added to the final concentrations of 500 mM (dash-dot line). The loss of quenching suggests breakdown of the complex. Addition of Triton X-100 to 0.1% to the original complex caused little change in the spectrum (dashed line), suggesting the complex remains intact. Excitation wavelength, 394 nm.

fluorescence labels to be covalently incorporated using maleimide or haloacetyl adducts of probes. Labeling of A62C with BADAN fluorescent probe again causes no catalytic changes. Fig. 1 shows that the fluorescence emission spectrum of BADAN–MMOB is partially quenched and red shifted when MMOH is added, providing a direct demonstration of complex formation.

The nature of the interactions that stabilize the complex was probed by adding salts or detergents and observing the effects on the fluorescence spectrum. As shown in Fig. 1, addition of 500 mM NaCl to the preformed complex results in nearly the full restoration of the fluorescence spectrum of the uncomplexed BADAN–MMOB, while addition of detergents causes no effect. The results are consistent with a complex stabilized by critical ionic linkages.

Titration of the BADAN–MMOB with MMOH (Fig. 2) shows that the observed dissociation constant depends strongly on the oxidation state of the diiron cluster. We have shown that the redox potential of MMOH shifts −132 mV when MMOB is present, indicating that binding and redox energies are coupled in this system as in $P450_{cam}$ [20]. Thus, the MMOB complex transmits effects of some sort to the buried diiron cluster.

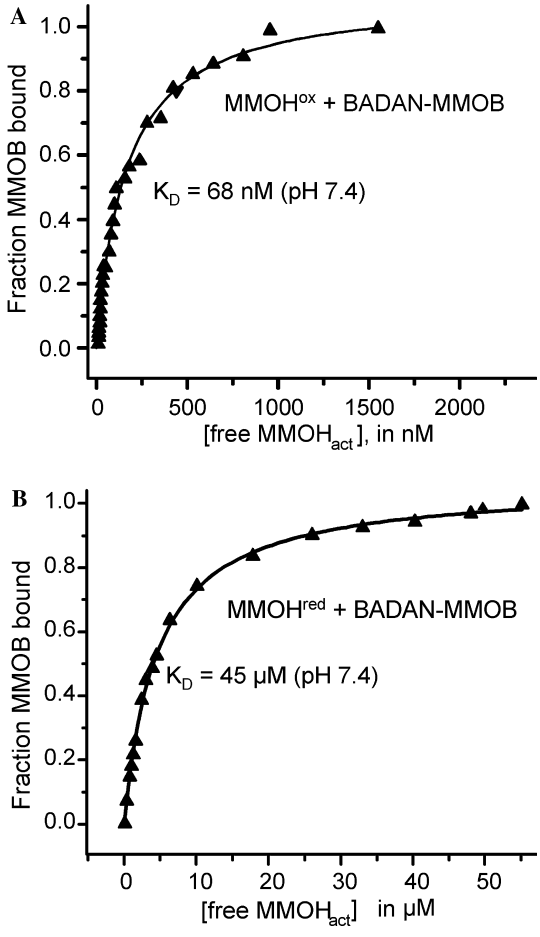

Fig. 2. Fluorescence titrations of MMOH binding to BADAN–A62C MMOB. 200 nM BADAN–A62C MMOB in 50 mM Mops was titrated with (A) oxidized MMOH or (B) fully reduced MMOH at 25 °C. Free MMOH was calculated from using the fraction bound assuming a 1:1 complex (per MMOH protomer) is formed. Excitation wavelength, 394 nm.

Structure of the complex

The symmetry of the dimeric protomer of MMOH creates a "canyon" between the two halves of the molecule that may provide a component binding surface. We have begun a cross-linking study that allows the binding surfaces to be described. After cross-linking MMOH and MMOB with EDC, the cross-linked MMOB–MMOH α-subunit unit was isolated by SDS–PAGE and digested with trypsin or chymotrypsin. Then the molecular weights of the resulting peptides were determined by MALDI-TOF MS for comparison to non-cross-linked samples. Normally, this technique does not allow direct detection of cross-linked peptides; rather they are detected as peptides missing from the mass spectrum after cross-linking. An example is shown in Fig. 3 for one of the MMOB peptides. The molecular mass identifies this chymotryptic peptide as that shown in the figure. Following cross-linking, the peptide disappears, suggesting that it is cross-linked to MMOH. EDC cross-links solvent exposed Glu or Asp residues in ionic linkages with Lys. Thus, the peptide shown in Fig. 3 has six potential cross-link sites (arrows) including five grouped acidic amino acids (carboxylate rich sequence 3 (CRS3)). There are two other CRS sequences in MMOB. The single Lys in the peptide of Fig. 3 can be eliminated as a possible cross-link site because it is also present in an MMOB tryptic peptide that does not disappear after cross-linking, thus one or more of the acidic residues provides the cross-link site.

The detailed analysis of the cross-linked peptides will be presented elsewhere. However, the data collected to date can be summarized as follows: (i) Five peptides on MMOH and five peptides on MMOB are involved in

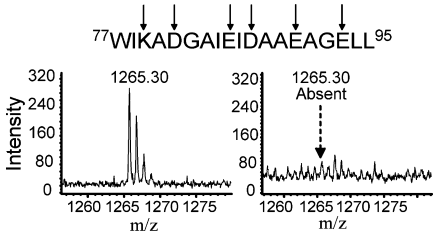

Fig. 3. MALDI-TOF spectra of a daughter ion from a chymotryptic digestion of MMOB alone (left) and after cross-linking to MMOH with EDC (right). The spectra shown are the sum of 100 different spectra. The mass of the ion identifies it as that shown above the spectra based on masses expected from theoretical chymotryptic digestion of MMOB. Arrows represent potential cross-link sites.

cross-links. (ii) All of the MMOB peptides appear on the same MMOB surface, identifying the binding surface on this component. (iii) All of the MMOH peptides occur on the same surface facing toward the canyon, identifying the binding surface on this component. (iv) Two of the three CRS sequences of MMOB are involved in cross-linking, consistent with charge stabilization of the interface described above. (v) The peptides cross-linked on MMOH form a footprint the size of MMOB, suggesting a docking orientation.

A model for the interaction surface

The structure of MMOB shows that it has an elongated, well-folded core region, and long disordered regions on the C- and N-termini. Our results show that both the N- and C-terminal regions contain at least one of the cross-linked residues. By aligning the known binding surface of MMOB with that of MMOH and then simultaneously aligning MMOB CRS regions with strongly positive regions on MMOH-α subunit, a binding orientation can be found in which all of the cross-linked peptides line up across a subunit boundary. In this orientation, the oval shape of MMOB and the elongated "footprint" on MMOH align, at least seven specific charge interactions are simultaneously aligned, and the termini of MMOB are directed toward a cross-linked MMOH peptide well removed from the others. This model for the MMOB–MMOHα interaction and some of the charge pairs are shown in Fig. 4.

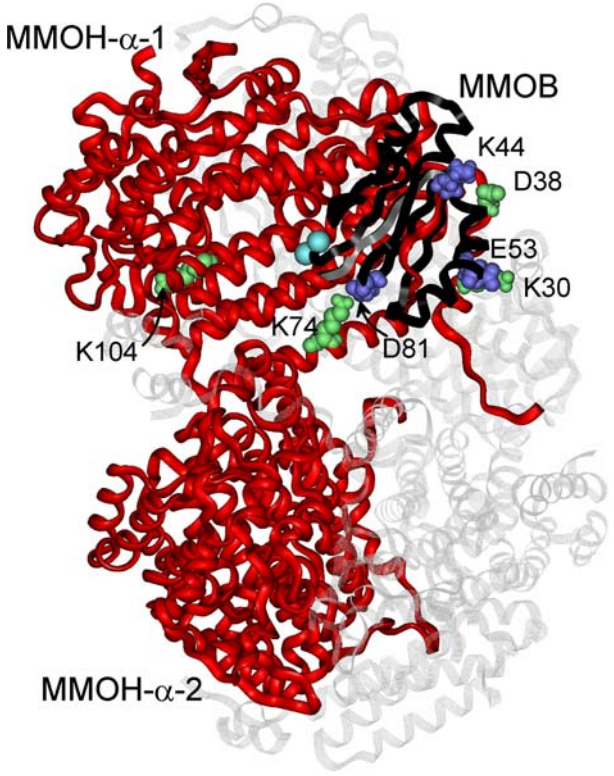

Fig. 4. Model for the structure of the complex formed between MMOB (black) and MMOH (red). The diiron cluster is shown in aqua. Interacting residues from MMOB are K44, E53, and D81. Four additional interacting pairs are omitted for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

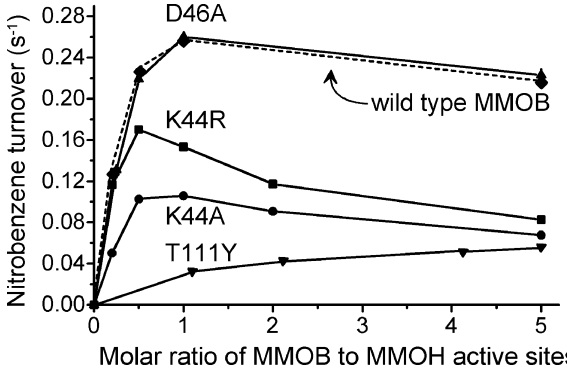

Fig. 5. The effects of MMOB mutants on MMO steady-state kinetics. The rate of nitrobenzene turnover is plotted as a function of the ratio of MMOB mutant to MMOH active sites at 25 °C and pH 7.6. The MMOB mutant concentration was varied from 0 to 10 μM. Other concentrations: 1 μM MMOH active sites, 1 μM MMOR, 100 μM NADH, and 1.6 mM nitrobenzene.

The model can be used to predict that MMOB K44 is a key residue because it is the only positive residue in the midst of a largely negative region, and it appears to be positioned adjacent to negative residue MMOH D38. In contrast, nearby residue MMOB D46 (part of CRS2) does not have a binding partner in the model. Both residues were changed and the effects on the steady state kinetics assessed as shown in Fig. 5. It is seen that only the modifications at K44 cause a significant change in the maximum steady state velocity, consistent with the model proposed. Among the K44 mutants, the K44R mutant, which conserves the positive charge, least affects the maximum velocity.

Discussion

The studies described here show that MMOB forms a specific complex with MMOH-α on the MMOH surface directly over the buried active site. This suggests a general mechanism by which MMOB affects the structure and catalytic properties of the MMOH diiron cluster. The connections between the proposed juxtaposition of components and the effector roles of MMOB will now be discussed.

MMOB is known to have many effects on MMOH and MMO catalysis [10,12,14,21–23] including a 150-fold increase in V_{max}, acceleration of the reaction with O_2 by 1000-fold, and alteration of the electronic and spectroscopic properties of the MMOH diiron cluster. Based on an NMR evaluation of the MMOB binding surface

[24], which correlates well with that presented here, we have altered many of the key interface residues [25]. Individual MMOB mutants have been found that affect each step of the catalytic cycle. One mutant that altered the rates of two steps was N107G/S109A/S110A/T111A (the Quad mutant). Significantly, these steps were the reaction of substrate with the reactive oxo-$(Fe(IV))_2$ reaction cycle intermediate, termed compound Q, and the product release step. Based in part on this observation, we proposed a novel role for the effector function of MMOB [26]. When MMOB binds, it appears to create a "hole" into the occluded active site of MMOH to allow molecules the size of methane and O_2 to enter, in effect, converting MMOH into a molecular sieve for methane.

The molecular sieve hypothesis accounts for many rather vexing observations that have been made over the years for MMO. For example, it suggests how methanotrophs can select methane as their only grow substrate from a sea of potentially more easily oxidized hydrocarbons. Also, it can account for our observation that the deuterium kinetic isotope effect (KIE) for the methane reaction with compound Q is about 50 [27] while no isotope effect is observed for the reaction with ethane [28]. If it is assumed that the methane reaction is limited by bond breaking while the ethane reaction is limited by entry into the active site, no isotope effect would be expected for the latter.

This hypothesis was tested using the quad mutant, which substitutes small hydrophobic residues for large hydrophilic residues at the binding surface. The reduction in residue size at the interface allowed larger substrates to bind more easily and led to an increase in the rate of ethane turnover as well as the observation of a KIE for the ethane reaction [28]. The key residue in the Quad mutation has been identified as T111 by site directed mutagenesis [29]. As shown in Fig. 5, substitution of a larger residue, T111Y, slows down steady state turnover, but more significantly, shifts the amount of MMOB required to saturate the MMOH interaction to a significantly higher ratio (at least 10:1). Apparently the binding surface is disrupted.

The discussion above suggests why the effector function of MMOB is critical to the role of MMO in the metabolism of the methanotroph. MMOB allows the organism to specifically oxidize methane. Thus, the large bond energy of methane can be exploited while avoiding oxidation of irrelevant substrates with weaker bonds.

The current study provides the first insight into how the effector function occurs. The model presented in Fig. 4 is based on an alignment of cross-linked peptides. Remarkably, although no information from the MMOB surface modification studies was used in developing this model, the residues of the Quad mutant are found immediately over the buried active site of MMOH. Residue T111 appears to extend between two MMOH helicies that support the cluster and also form the walls of the active site. Thus, it is reasonable that the placement of these specific MMOB residues disrupts the interaction between MMOH helicies and creates the critically sized hole into the active site.

What lessons about putidaredoxin can we learn from MMOB? The most important known effector functions of putidaredoxin is to facilitate transfer of the second electron required by the reaction stoichiometry [5,6]. This leads to the formation of the reactive π cation radical species that is equivalent electronically and chemically to MMO compound Q. Like MMOH, $P450_{cam}$ has a buried active site. However, the binding site of putidaredoxin does not appear to be directly over the active site of $P450_{cam}$, and accordingly, the binding of camphor and O_2 are not greatly affected by the binding of putidaredoxin. This suggests that the effector role of putidaredoxin and MMOB are different. Nevertheless, they both serve to accelerate key aspects of the catalytic cycle that result in the hydroxylation of specific substrates before the reactive species can break down to release toxic activated oxygen species.

Effectors are now recognized as rather common components of oxygenase systems. However, at the time putidaredoxin was first recognized as an effector, there was no precedent for this function in the oxygenase family, and it seemed unlikely to the corresponding author at the time that a role beyond electron transfer in an iron-sulfur protein was possible. This example frames a very relevant question for all of us whose role it is to recognize new things: How does one learn to accept the result of an investigation that is contrary to the obvious? In the current instance, Gunny listened to the evidence once, dubbed putidaredoxin an effector, and we were launched on a new track that has touched many areas of science and continues to yield fruitful insight to the present day. If one asked Gunny how he did it, he would probably say something about training for a lifetime to learn to listen to Nature in preference to Nature's interpreters [30].

Acknowledgments

This work was supported by National Institutes of Health Grant GM40466. We thank Dr. LeeAnn Higgins and Thomas P. Krick for assistance in collecting and interpreting the mass spectrometry data and Dr. David D. Thomas for the use of the ISS K2 Fluorometer.

References

[1] M. Katagiri, B.N. Ganguli, I.C. Gunsalus, A soluble cytochrome P-450 functional in methylene hydroxylation, J. Biol. Chem. 243 (1968) 3543–3546.

[2] A.Y. Lu, K.W. Junk, M.J. Coon, Resolution of the cytochrome P-450-containing omega-hydroxylation system of liver microsomes into three components, J. Biol. Chem. 244 (1969) 3714–3721.

[3] D.Y. Cooper, S. Narasimhulu, O. Rosenthal, R.W. Estabrook, Role of cytochrome P-450 in mixed-function oxidations, Adv. Chem. 77 (1968) 220–234.
[4] A.G. Hildebrandt, R.W. Estabrook, Evidence for the participation of cytochrome b_5 in hepatic microsomal mixed-function oxidation reactions, Arch. Biochem. Biophys. 143 (1971) 66–79.
[5] C.A. Tyson, J.D. Lipscomb, I.C. Gunsalus, The roles of putidaredoxin and P450 complexes in methylene hydroxylation, J. Biol. Chem. 247 (1972) 5777–5784.
[6] I.C. Gunsalus, C.A. Tyson, J.D. Lipscomb, Cytochrome P-450 reduction and oxygenation systems, in: T.E. King, H.S. Mason, M. Morrison (Eds.), Oxidases and Related Redox Systems, Proceedings of the 2nd International Symposium, University Park Press, Baltimore, MD, 1973, pp. 583–603.
[7] J.D. Lipscomb, S.G. Sligar, M.J. Namtvedt, I.C. Gunsalus, Autooxidation and hydroxylation reactions of oxygenated cytochrome $P450_{cam}$, J. Biol. Chem. 251 (1976) 1116–1124.
[8] I.C. Gunsalus, J.R. Meeks, J.D. Lipscomb, V.P. Marshall, E. Münck, P.G. Debrunner, Bacterial monooxygenases-P450 cytochrome system, in: O. Hayaishi (Ed.), Molecular Mechanisms of Oxygen Activation, Academic Press, New York, 1974, pp. 561–613.
[9] S.G. Sligar, P.G. Debrunner, J.D. Lipscomb, I.C. Gunsalus, A role of the putidaredoxin COOH-terminus in $P\text{-}450_{cam}$ (cytochrome *m*) hydroxylations, Proc. Natl. Acad. Sci. USA 71 (1974) 3906–3910.
[10] B.G. Fox, W.A. Froland, J.E. Dege, J.D. Lipscomb, Methane monooxygenase from *Methylosinus trichosporium* OB3b. Purification and properties of a three-component system with high specific activity from a type II methanotroph, J. Biol. Chem. 264 (1989) 10023–10033.
[11] B.J. Wallar, J.D. Lipscomb, Dioxygen activation by enzymes containing binuclear non-heme iron clusters, Chem. Rev. 96 (1996) 2625–2657.
[12] B.G. Fox, Y. Liu, J.E. Dege, J.D. Lipscomb, Complex formation between the protein components of methane monooxygenase from *Methylosinus trichosporium* OB3b: identification of sites of component interaction, J. Biol. Chem. 266 (1991) 540–550.
[13] B.G. Fox, W.A. Froland, D.R. Jollie, J.D. Lipscomb, Methane monooxygenase from *Methylosinus trichosporium* OB3b, Methods Enzymol. 188 (1990) 191–202.
[14] W.A. Froland, K.K. Andersson, S.-K. Lee, Y. Liu, J.D. Lipscomb, Methane monooxygenase component B and reductase alter the regioselectivity of the hydroxylase component-catalyzed reactions: a novel role for protein–protein interactions in an oxygenase mechanism, J. Biol. Chem. 267 (1992) 17588–17597.
[15] A. Shevchenko, M. Wilm, O. Vorm, M. Mann, Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels, Anal. Chem. 68 (1996) 850–858.
[16] J. Colby, H. Dalton, Resolution of the methane monooxygenase of *Methylococcus capsulatus* (Bath) into three components, Biochem. J. 171 (1978) 461–468.
[17] A.C. Rosenzweig, C.A. Frederick, S.J. Lippard, P. Nordlund, Crystal structure of a bacterial non-haem iron hydroxylase that catalyses the biological oxidation of methane, Nature 366 (1993) 537–543.
[18] N. Elango, R. Radhakrishnan, W.A. Froland, B.J. Wallar, C.A. Earhart, J.D. Lipscomb, D.H. Ohlendorf, Crystal structure of the hydroxylase component of methane monooxygenase from *Methylosinus trichosporium* OB3b, Protein Sci. 6 (1997) 556–568.
[19] S.L. Chang, B.J. Wallar, J.D. Lipscomb, K.H. Mayo, Solution structure of component B from methane monooxygenase derived through heteronuclear NMR and molecular modeling, Biochemistry 38 (1999) 5799–5812.
[20] S.G. Sligar, I.C. Gunsalus, A thermodynamic model of regulation: modulation of redox equilibria in camphor monooxygenase, Proc. Natl. Acad. Sci. USA 73 (1976) 1078–1082.
[21] K.E. Paulsen, Y. Liu, B.G. Fox, J.D. Lipscomb, E. Münck, M.T. Stankovich, Oxidation–reduction potentials of the methane monooxygenase hydroxylase component from *Methylosinus trichosporium* OB3b, Biochemistry 33 (1994) 713–722.
[22] S.C. Pulver, W.A. Froland, J.D. Lipscomb, E.I. Solomon, Ligand field circular dichroism and magnetic circular dichroism studies of component B and substrate binding to the hydroxylase component of methane monooxygenase, J. Am. Chem. Soc. 119 (1997) 387–395.
[23] M.P. Hendrich, E. Münck, B.G. Fox, J.D. Lipscomb, Integer-spin EPR studies of the fully reduced methane monooxygenase hydroxylase component, J. Am. Chem. Soc. 112 (1990) 5861–5865.
[24] S.L. Chang, B.J. Wallar, J.D. Lipscomb, K.H. Mayo, Residues in *Methylosinus trichosporium* OB3b methane monooxygenase component B involved in molecular interactions with reduced- and oxidized-hydroxylase component: a role for the N-terminus, Biochemistry 40 (2001) 9539–9551.
[25] B.J. Wallar, J.D. Lipscomb, Methane monooxygenase component B mutants alter the kinetics of steps throughout the catalytic cycle, Biochemistry 40 (2001) 2220–2233.
[26] B.J. Brazeau, J.D. Lipscomb, Kinetics and activation thermodynamics of methane monooxygenase compound Q formation and reaction with substrates, Biochemistry 39 (2000) 13503–13515.
[27] J.C. Nesheim, J.D. Lipscomb, Large isotope effects in methane oxidation catalyzed by methane monooxygenase: evidence for C-H bond cleavage in a reaction cycle intermediate, Biochemistry 35 (1996) 10240–10247.
[28] B.J. Brazeau, B.J. Wallar, J.D. Lipscomb, Unmasking of deuterium kinetic isotope effects on the methane monooxygenase compound Q reaction by site-directed mutagenesis of component B, J. Am. Chem. Soc. 123 (2001) 10421–10422.
[29] B.J. Brazeau, J.D. Lipscomb, Key amino acid residues in the regulation of soluble methane monooxygenase catalysis by component B, Biochemistry 42 (2003) 5618–5631.
[30] I.C. Gunsalus, Learning, Annu. Rev. Microbiol. 38 (1984) 13–44.