Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

Structural and functional model of methane hydroxylase of membrane-bound methane monooxygenase from *Methylococcus capsulatus* (Bath)

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Computer analysis of a wide range of primary sequences showed that α-, β-, and γ-peptides of membrane-bound methane hydroxylase contained 2, 7, and 6 transmembrane helices respectively. Conservative amino acid residues participating in complex formation were revealed. The α- and γ-peptides are suggested to contain mononuclear copper ions with the ligand environment mainly consisting of His residues. The Cu sites are located in the hydrophilic region and are responsible for ESR signals. The active site of β-peptide in which the activation of O_2 and oxidation of CH_4 occur is localized in the hydrophobic region close to the membrane surface. This site is formed by the amino acid residues of four transmembrane helices and one loop between them and is suggested to be a binuclear Cu—Fe or Fe—Fe center. The Cu site of α-peptide transfers electrons to the active site of β-peptide, and the Cu site of γ-peptide is either involved in this process or only stabilizes the protein structure.

Key words: membrane-bound methane monooxygenase, pMMO, membrane-bound methane hydroxylase. copper-containing enzymes, prediction of protein structure.

More than 250 types of bacteria oxidizing methane are known. These bacteria play an important role in the carbon and nitrogen cycles in nature and contain the unique enzyme, methane monooxygenase (MMO). Two types of MMO are known: soluble (sMMO) and membrane-bound (pMMO).[1] All methane-oxidizing organisms contain pMMO. Some of them contain in their genome an information about sMMO synthesis. In these organisms sMMO is synthesized with copper deficit, and in the presence of copper only pMMO is synthesized.[2] The structure and functions of sMMO have been studied in detail.[3] An information on pMMO is more scarce due to difficulties in the preparation of active highly purified samples. pMMO possesses a high activity and a narrower substrate specificity[4—6] and, hence, is of great interest. In essence, pMMO an enzyme system

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containing NADH-oxidoreductase (NAD is nicotinamide adenine dinucleotide, and NADH is the reduced form of NAD), several electron carriers, activators, and methane hydroxylase (pMH).

According to the data of electrophoresis in the polyacrylamide gel, pMH consists of three peptides, α (45 kDa), β (27 kDa), and γ (25 kDa), associated by noncovalent bonds to form a single complex.[7,8] The primary structures of peptides of soluble methane hydroxylase (sMH) and pMH differ strongly. Numerous unsuccessful attempts were made to isolate and purify pMMO and pMH.[7—10] The preparations obtained either had a very low enzyme activity, or the enzyme activity was absent at all due to either distortion of the enzyme structure during purification, or loss of the reducing agent and (or) activator of pMH (the direct natural reducing agent of pMH is unknown so far), or combinations of both these factors. From 1 to 15 copper atoms and from 0 to 2 nonheme iron atoms were found in various preparations of pMMO and partially purified pMH. The presence of Cu^{2+} and Fe^{2+} ions in pMH was confirmed by atomic absorption spectroscopy,[7—12] ESR,[8,9,11—22] and Mössbauer spectroscopy.[23] The low-molecular peptide containing several copper atoms was found. It is assumed[8,24] to be the cofactor of pMH. Acetylene, which irreversibly inactivates pMH, added covalently to β-peptide of pMH.[7,8,25] In similar experiments with sMH, [^{14}C]acetylene was included only to the high-molecular α-peptide[25] containing the binuclear active site. By analogy to sMH, we can assume that the β-peptide of pMH is also a carrier of the active site, which catalyzes O_2 activation and CH_4 oxidation.

The group of bacteria in nature uses the oxidation of ammonium as an energy source for their living activity. In these bacteria, the enzyme system responsible for ammonia oxidation is membrane-bound ammonium monooxygenase (AMO). The soluble form of AMO is unknown. Along with ammonia, AMO catalyzes the oxidation of methane, ethylene, and other compounds, which are oxidized by pMMO. The hydroxylase component of AMO consists of three peptides resembling three peptides of pMH and contains copper and iron.[26] As pMMO, AMO is inactivated by acetylene and some its analogs. The purification of AMO and ammonium hydroxylase meets the same difficulties as those for the purification of pMMO and pMH. These facts suggest that the structure of active sites of ammonium hydroxylase is close, if not identical, to that of pMH. It remains unknown what metal ions are present in particular peptides.

Since it is difficult to study the pMH structure by direct methods (due to the absence of the well characterized highly active and highly purified enzyme), the task of this work was computer analysis of the amino acid sequences of α-, β-, and γ-peptides of pMH from *M. capsulatus* (Bath) with the purpose for elucidating their secondary structure and elements of the tertiary structure, revealing the amino acid residues that participate in the formation of the active site of the enzyme, and establishing the structure of this site.

Experimental

Amino acid sequences of α-, β-, and γ-peptides of pMH from *M. capsulatus* (Bath) were obtained from the TrEMBL* electronic data base. The PHDhtm[27,28] program incorporated in The PredictProtein[29] server** was used to predict transmembrane helices of these peptides. The program predicts transmembrane helices and their topology on the basis of an evolution information, which can be obtained from multiple alignment*** of homological sequences and physicochemical properties of particular amino acids. The accuracy of prediction of transmembrane helices was 89±6% and that of their topology was 86±6%. The program searches for homologs with the specified homology level (not lower than 30% identity) in the SWISS-PROT* and TrEMBL* data banks using the specified sequences, multiply aligns the homologs found, and predicts the length, position in the chain, and topology of transmembrane helices on the basis of the obtained multiple alignment.

The sequences obtained along with results on prediction of transmembrane helices were also used to reveal conservative and semiconservative residues using the Clustal (1.8)**** program.[30] This program allows the multiple alignment on the basis of the physicochemical properties of particular amino acid residues. The final analysis of the obtained multiple alignment with the purpose for revealing conservative residues was performed by the GeneDoc (*v* 2.4.014) program.*****

Revealing the structure of the peptides studies and the structure of the active site of the enzyme, we used the following postulates following from the known data on pMH and principles of molecular biology on the synthesis and construction of metal enzymes:

(1) pMH is formed by three peptides (α, β, and γ);

(2) all peptides are synthesized independently of each other;

(3) as a particular peptide chain leaves from the ribosome, it is spontaneously folded and does not impede the formation of the tertiary structure;

(4) spatial structures of α-, β-, and γ-peptides are unified in the membrane to form native pMH;

(5) any active site of the enzyme is formed by conservative amino acid residues of only one peptide;

(6) predominantly transmembrane helices are arranged in the lipid bilayer;

(7) potential ligands of metal ions of the active site of α-, β-, and γ-peptides of pMH are only conservative residues

* http://www.expasy.ch/sprot/sprot-top.html

** http://www.embl-heidelberg.de/predictprotein/

*** Multiple alignment is performed in three stages: (1) all sequences are compared to each other; (2) the rough phylogenetic tree, which describes groups of similar sequences, is plotted; and (3) multiple alignment of sequences with respect to the phylogenetic tree is performed. Each step in the final multiple alignment consists of the alignment of two sequences or two alignments. This is performed gradually following the order of insertion in the phylogenetic tree.

**** *Clustal* (*v 1.8*), http://www.csc.fi/molbio/progs/clustalw/

***** K. B. Nicholas and H. B. Nicholas, GeneDoc (*v* 2.4.014), http://www.psc.edu/biomed/genedoc/

H, M, C, Y, E, and D,* which can be contained both in the helixed and nonhelixed regions of the polypeptide chain.

When choosing the structure of the active sites, we took into account published data on the experimental data of pMMO and pMH obtained by ESR, ENDOR, Mössbauer, and atomic absorption spectroscopy. The data on the length, position, and topology of transmembrane helices and data on the conservative character of the amino acid residues were used to predict the spatial structure. Then the transmembrane helices were put on the scheme of chain arrangement in the membrane. Similar schemes was created for each peptide of pMH. Analyzing the schemes, we paid attention to the arrangement of the conservative amino acid residues in the secondary structures. Then we spatially approached the helices that could participate in the formation of the assumed active site due to the presence of the necessary amount of favorably arranged conservative amino acid residues.

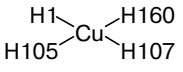
Results and Discussion

The degree of homology of α-peptide was established for three peptides of pMH and 12 peptides of ammonium hydroxylase. For β-peptide (unlike earlier Ref. 32), a similar dependence was established for 112 peptides of pMH and 349 peptides of ammonium hydroxylase. For γ-peptide, it was found for 5 peptides of pMH and 5 peptides of ammonium hydroxylase. The high homology was established only for pMH and ammonium hydroxylase. Therefore, it can be assumed that the amino acid sequences of α-, β-, and γ-peptides of pMH and ammonium hydroxylase are unique. The results of searching for conservative, semiconservative, and nonconservative amino acid residues in the primary sequence of α-, β-, and γ-peptides of pMH and elements of their secondary structure are presented in Figs. 1, 2, and 3. In the description of the structure, the correction to the sequencing results of the N end of peptides was introduced into the amino acid sequence of the studied peptides of pMH.[8] Therefore, the amino acid sequences of α-, β-, and γ-peptides were newly numbered (Figs. 1*b*, 2*b*, and 3*b*).

The α-peptide contains two transmembrane helices ***A*** and ***B*** (Fig. 1) and two large hydrophilic regions of the polypeptide chain localized in cytosole. These regions can form one common domain or two independent domains (***D1*** and ***D2***). Transmembrane helices ***A*** and ***B*** cannot participate in the formation of a complex with the metal ion because they contain only two conservative amino acid residues (H160 and D200) localized at opposite sides of the membrane. The ***A***—***B*** loop cannot either participate in the formation of a complex with the metal ion because their region contains only two residues D193 and D200, which are evidently insufficient for complex formation with the metal ion.

The ***D1*** domain of α-peptide contains 9 conservative amino acid residues (H1, E3, M12, Y18, D19, Y90, H105, H107, and E149), and domain ***D2*** contains 4 conservative amino acid residues (Y259, E284, D336, and D350), which can potentially be ligands of the metal ions. It is known that the ESR spectrum of the native enzyme exhibits the signal characteristic of nonblue copper-containing proteins with the active site of the second type with $Cu^{2+}(d^9)$[8,9,11—22] and belonging to the axial type ($g_z = g_\parallel \cong 2.3$, $g_x = g_y = g_\perp \cong 2.05$) because an unpaired electron is localized on the $d_{x^2-y^2}$ orbital (the nuclear spin of ^{63}Cu and ^{65}Cu is I = 3/2). This signal was first described in Ref. 13. The parameters of the signal for pMMO (g_z = 2.24, $g_{x,y}$ = 2.06, a^{Cu}_z = 19.0 mT, $a^{Cu}_{x,y}$ = 1.0 mT) have later been confirmed in several studies.[8,9,11,12,14—22] Experiments with pMMO, ^{15}N-labeled and ^{63}Cu-substituted, revealed two close signals from different centers of the mononuclear copper.[19—20] The copper ions that manifest the ESR signal are in the hydrophilic region, they are accessible for hydrophilic complex-forming agents (EDTA[17,33] and diethyl dithiocarbamate[34]) and, according to ENDOR data,* contact with the solvent. pMH does not contain other Cu^{2+} sites exhibiting the ESR signal because, after the treatment of pMMO with diethyl dithiocarbamate followed by the extraction of the copper complex, no paramagnetic signal from copper ions was observed by the ESR method.[34] The individually isolated α-peptide contains 1 copper atom, which exhibits the ESR signal[21] close to that from the native enzyme. It was assumed that the copper complex with the polypeptide chain of α-peptide was formed by one copper atom and 3—4 nitrogen atoms of the histidine residues.[21] This assumption does not correspond to the conclusions that the ESR signal from pMH is due to the trinuclear copper center[13—15] but agrees with subsequent conclusions about the mononuclear nature of the Cu centers manifesting the ESR signal.[18—21]

The amino acid residues of domain ***D1*** (H1, H105, H107, M12, Y18, Y90) and, perhaps, H160 of the transmembrane helix ***A*** can participate in the formation of a complex of α-peptide with the copper ion because the latter amino acid residue is localized in the region of the surface layer of helix ***A*** and accessible for complex formation. Domain ***D2*** does not participate in complex formation with the copper ion because it does not contain amino acid ligands, which could be ligands of the copper ion. Thus, the structure of the center exhibiting the ESR signal can most probably be the following:



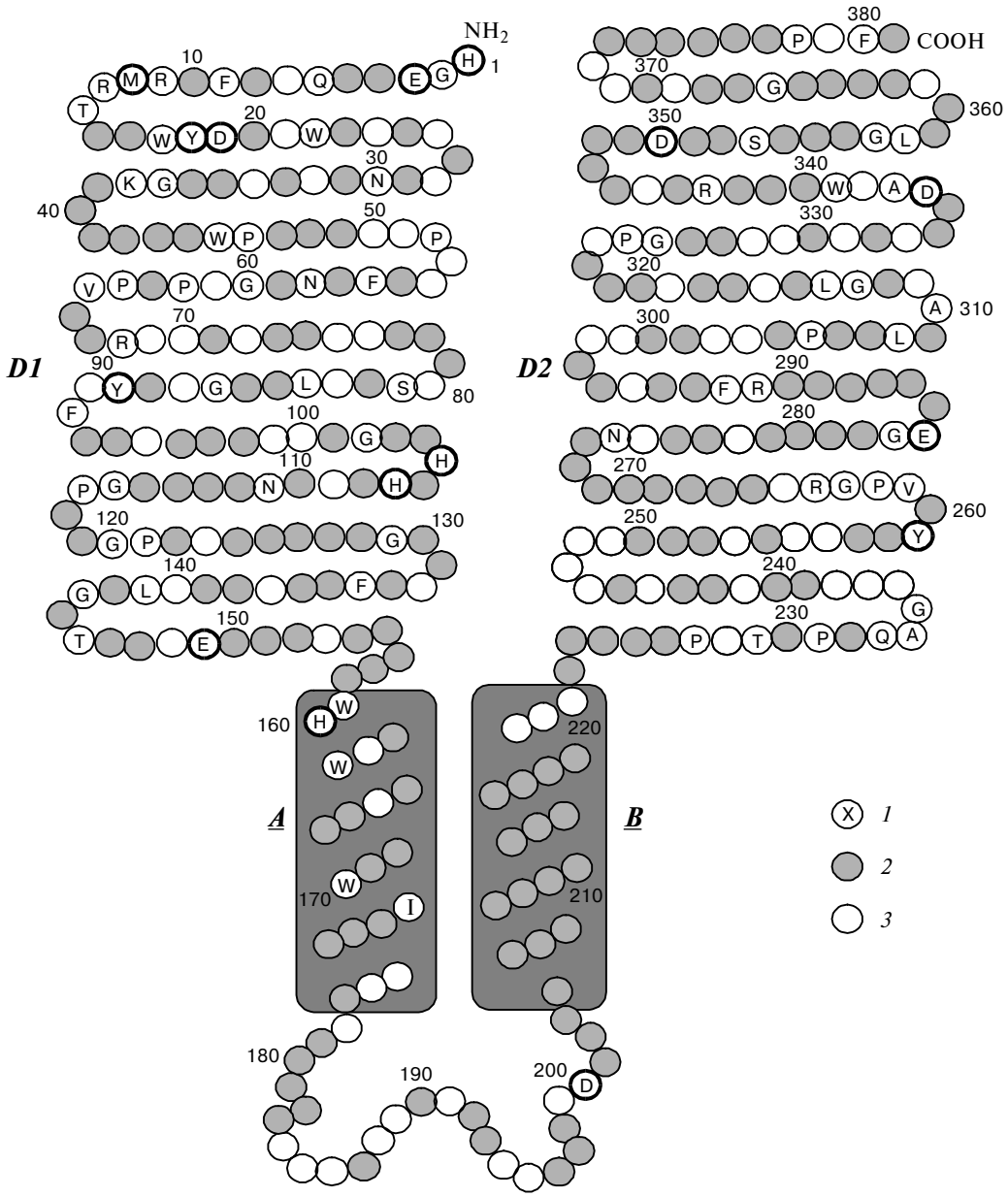
(M12 or Y18 or Y90)

The β-peptide and γ-peptide are not obtained in the individual state and, therefore, the exact composition of metal ions of these peptides is unknown so far. The β-peptide contains 7 transmembrane helices: ***C***, ***D***, ***E***,

* One-letter designations of amino acid residues are used in this work.[31]

* B. Katterle, R. I. Gvozdev, N. Abudu, T. Ljones, and K. K. Andersson, *Biochemical J.*, 2001, in press.

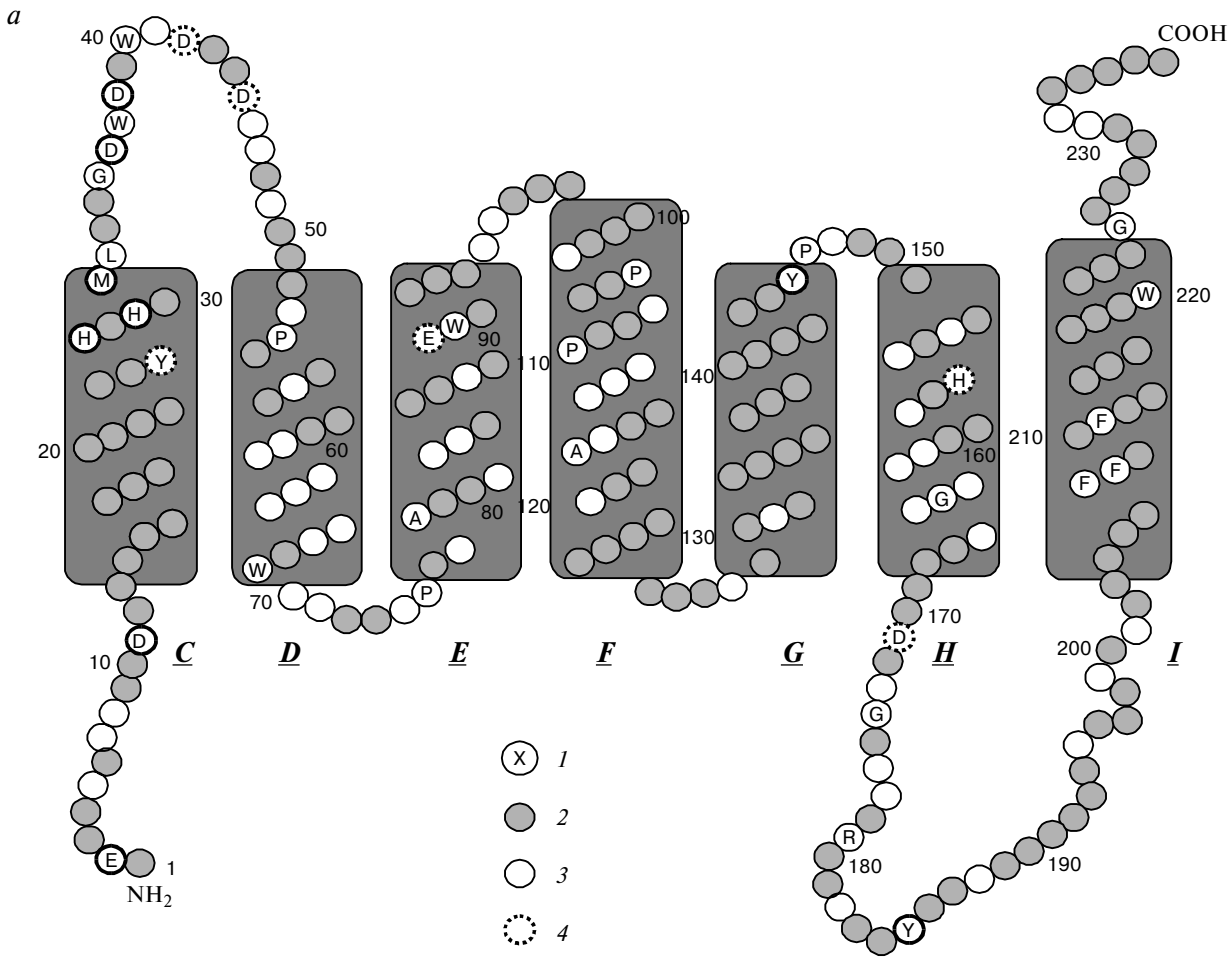
a



b

10	20	30	40	50	60
HGEKSQAAFM	RMRTIHWYDL	SWSKEKVKIN	ETVEIKGKFH	VFEGWPETVD	EPDVAFLNVG
70	80	90	100	110	120
MPGPVFIRKE	SYIGGQLVPR	SVRLEIGKTY	DFRVVLKARR	PGDWHVHTMM	NVQGGGPIIG
130	140	150	160	170	180
PGKWITVEGS	MSEFRNPVTT	LTGQTVDLEN	YNEGNTYF**WH**	**AFWFAIGVAW**	**IGYWSRR**PIF
190	200	210	220	230	240
IPRLLMVDAG	RADELVSATD	RKV**AMGFLAA**	**TILIVVMAMS**	**S**ANSKYPITI	PLQAGTMRGM
250	260	270	280	290	300
KPLELPAPTV	SVKVEDATYR	VPGRAMRMKL	TITNHGNSPI	RLGEFYTASV	RFLDSDVYKD
310	320	330	340	350	360
TTGYPEDLLA	EDGLSVSDNS	PLAPGETRTV	DVTASDAAWE	VYRLSDIIYD	PDSRFAGLLF
370	380				
FFDATGNRQV	VQIDAPLIPS	FM			

Fig. 1. Secondary (*a*) and primary (*b*) structures of α-peptide. *a*. Conservative (*1*), semiconservative (*2*), and nonconservative amino acid residues (*3*). Each circle represents one amino acid residue (one-letter disignations are used). Amino acid residues arranged against the dark background are amino acid residues of transmembrane helices ***A*** and ***B***. The N and C ends of peptide are designated by the corresponding groups (NH_2 and COOH). *b*. The amino acid residues were numbered taking into account results of sequencing.[8] The highlighted and underlined regions of the amino acid residue are transmembrane helices.



b

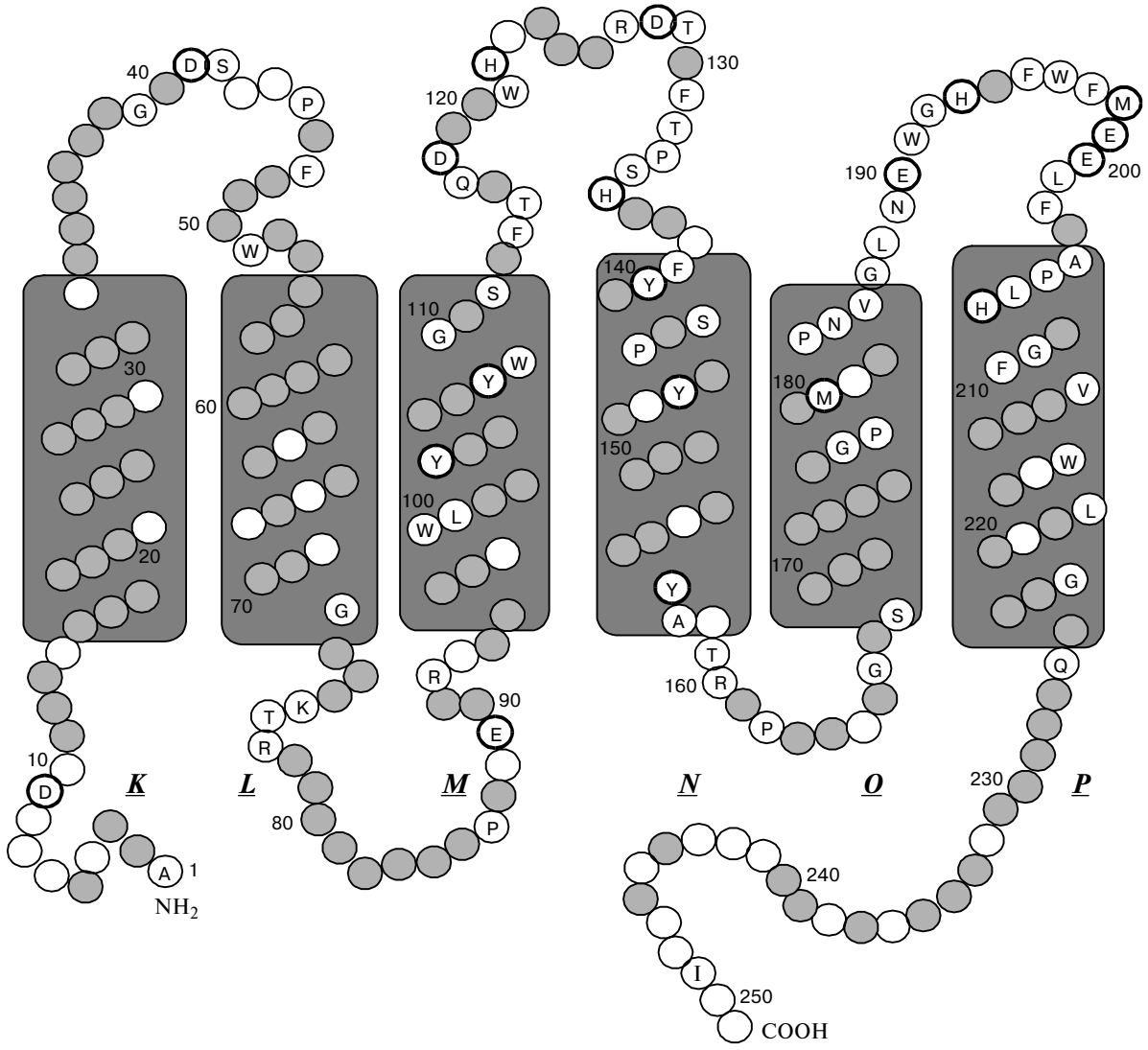
10	20	30	40	50	60
AEAVQVSRTI	DWMA**LFVVFF**	**VIVGSYHIHA**	**M**LTMGDWDFW	SDWKDRRLWV	T**VTPIVLVTF**
70	80	90	100	110	120
PAAVQSYLWE	RYRLP**WGATV**	**CVLGLLLGEW**	**INRY**FNFWG**W**	**TYFPINFVFP**	**ASLVPGAIIL**
130	140	150	160	170	180
DTVLMLSG**SY**	**LFTAIVGAMG**	**WGLIFY**PGNW	**PIIAPLHVPV**	**ENNGMLMS**IA	DIQGYNYVRT
190	200	210	220	230	
GTPEYIRMVE	KGTLRTFGKD	**VAPVSAFFSA**	**FMSILIYFMW**	**HFI**GRWFSNE	RFLQST

Fig. 2. Secondary (*a*) and primary (*b*) structures of β-peptide. *a*. Conservative (*1*), semiconservative (*2*), and nonconservative amino acid residues (*3*); amino acids in the amino acid sequence for which replacements were found (*4*) (Y26, two replacements by H of 36 sequences; D42, one replacement by R of 156 sequences; E89, one replacement by A of 175 sequences; and H157, six replacements by Y and Q and two replacements by R of 452 sequences).*

F, ***G***, ***H***, and ***I*** (Fig. 2 *a*, *b*). Potential ligands of metal ions of the β-peptide arranged in the transmembrane helices can be 7 amino acid residues Y26, H27, H29, and M31 (helix ***C***), E89 (helix ***E***), Y146 (helix ***G***), and H157 (helix ***H***) (Fig. 2). All these residues are group at one side of the membrane. Four residues E2, D11 (N end) and D171 and Y185 of loop ***H***—***I*** at another side of the membrane are insufficient for the formation of a complex with the copper ion because three of them (E2, D11, and D171) are not characteristic complex-forming amino acid residues of the copper ion. These residues can potentially form a complex with the iron, zinc, or magnesium ion, which can participate in the stabilization of the spatial structure of β-peptide. This assumption does not contradict the general structure of

* Taking into account the percentage of a possible experimental error during sequencing nucleotide sequences, these amino acid residues should be considered conservative. (The differences in the number of compared amino acid sequences in analysis of the conservation degree of amino acids are due to the fact that all accessible to the moment both complete and partial amino acid sequences were used in analysis.)

a



b

10	20	30	40	50	60
AAAEAPLLDK	KWLT**FALAIY**	**TVFYLWVRWY**	**EG**VYGWSAGL	DSFAPEFETY	WMN**FLYTEIV**
70	80	90	100	110	120
LEIVTASILW	**G**YLWKTRDRN	LAALTPREEL	RRNF**THLVWL**	**VAYAWAIYWG**	**AS**YFTEQDGT
130	140	150	160	170	180
WHQTIVRDTD	FTPSHIIE**FY**	**LSYPIYIITG**	**FAAFIYAK**TR	LPFFAKGI**SL**	**PYLVLVVGPF**
190	200	210	220	230	240
MILPNVGLNE	WGHTFWFMEE	LFV**APLHYGF**	**VIFGWLALAV**	**MGTLT**QTFYR	FAQGGLGQSL
250					
CEAVDEGLIA	K				

Fig. 3. Secondary (*a*) and primary (*b*) structures of γ-peptide. For designations, see captures for Fig. 1.

β-peptide. Seven conservative amino acid residues (Y26, H27, H29, M31, E89, Y146, H157) at another side of the membrane are localized in four helices. Of four amino acid residues of helix ***C***, only the Y26—H29 or H27—M31 pair can participate in complex formation with the metal ion because the distance between the amino acid residues in these pairs is favorable for the formation of this complex. At the same time, these pairs are localized at opposite sides of helix ***C*** and cannot form (for steric reasons) the same complex with the metal ion. Thus, 5 amino acid residues can potentially participate in complex formation with the metal ion: Y26—H29 (or H27—M31) of helix ***C***, E89, Y146, and H157 of helices ***E***, ***G***, and ***H***, respectively.

Of loops of β-peptide, the ***C***—***D*** loop (D36, D38, D42, D45) can also participate in the formation of a

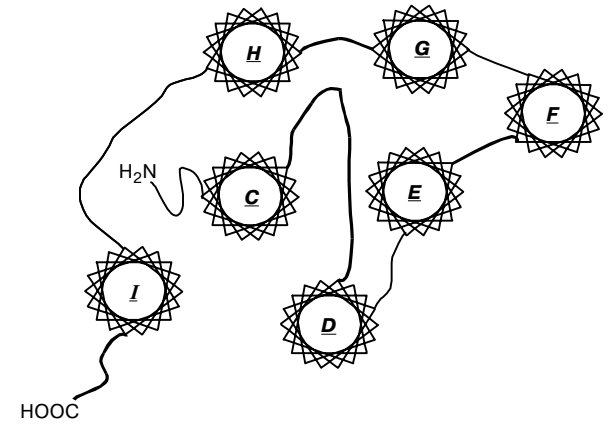


Fig. 4. The most probable run of the polypeptide chain of β-peptide. ***C***, ***D***, ***E***, ***F***, ***G***, ***H***, and ***I*** are transmembrane helices. ***HG***, ***FE***, ***DC***, and ***I***(COOH) are peptide chains above the membrane; (NH_2)***C***, ***DE***, ***FG***, and ***HI*** are peptide chains under the membrane.

complex with the metal ion. When assuming that 4 transmembrane helixes ***C***, ***E***, ***G***, and ***H*** are arranged in the membrane relatively to each other at vertices of the square, the amino acid residues H29, Y26, H157, Y146, and E89, according to the distances and arrangement in the space, are capable of coordinating by metal ions. The following run of the polypeptide chain of β-peptide, which does not interferes the formation of the spatial protein structure, is the most probable (Fig. 4). If the ***C***—***D*** loop is arranged in the space between transmembrane helices ***C***, ***E***, ***G***, and ***H***, amino acid residues D36, D38, D42, and D45 occupy the sites convenient for coordination with the metal. The ***C***—***D*** loop should be arranged in the well-defined site. It is most likely that the ***C***—***D*** loop contains the great number of conservative and semiconservative amino acid residues precisely for this reason. Thus, three parallel helices (***C***, ***E***, ***G***), one antiparallel helix (***H***), and the ***C***—***D*** loop can potentially participate in the formation of the active site of β-peptide. This arrangement of the regions of the polypeptide chain is the only possible for active site formation. The β-peptide does not contain other amino acid ligands, which could be ligands of metal ions and simultaneously would be favorably arranged in the space to form a complex with metal ions.

Amino acid residues that are localized in the space restricted with the ***C***, ***E***, ***G***, and ***H*** helices and the ***C***—***D*** loop are sufficient for the formation of both the mononuclear and binuclear centers. The formation of the trinuclear copper-containing center is excluded almost completely. Examples of possible sites are presented in Fig. 5. The assumed ligand environment of the active site of the β-peptide of pMH is characteristic of both copper and iron ions. Presently, it is virtually impossible to predict, using analysis of the primary protein stucture only, which of these sites is present in the enzyme. Additional experimental data are needed.

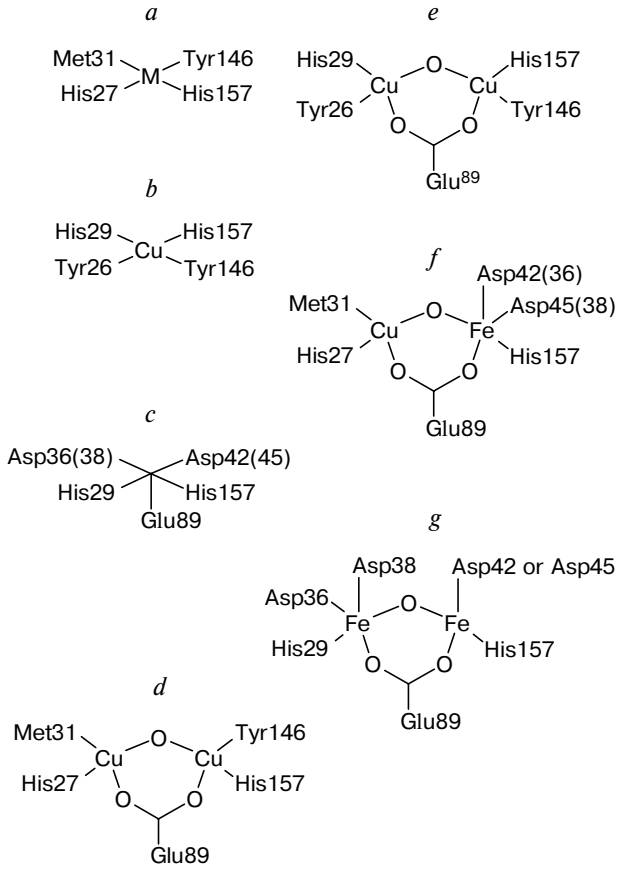


Fig. 5. Tentative structure of active sites in β-peptides: mononuclear (*a*—*c*) and binuclear (*d*—*g*) sites.

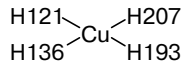
Of two types of the sites (Fig. 5), mononuclear sites *a*—*c* do not use the whole set of potential ligands. The binuclear sites *d*—*g* are more preferential because they possess greater possibilities for two-substrate (O_2 and CH_4) reactions and use almost the whole set of available conservative amino acid residues capable of being ligands of metal ions. Of them sites *f* and *g* can be most probable. Their structure does not contradict the values of isomeric shifts and quadrupole splitting obtained previously by NGR spectroscopy.[23] It is evident from these data that nitrogen and oxygen ligands are present in the nearest environment of the Fe ion. Sites *d*, *e*, and *g* should not manifest the ESR signal due to the strong exchange interaction of the closely arranged metal ions. Site *f* can manifest no ESR signal due to the presence of the Y26 residue in the second coordination sphere. This residue is capable of participating in electron transfer to form the radical and thus affecting the paramagnetic properties of binuclear site *f*. The presence of iron in the active site of β-peptide is favored by the following indirect data: (1) presently none enzyme is known to oxidize the aliphatic C—H bonds involving copper ions; all similar enzymes contain only iron (cytP450, ω-hydroxylase, sMMO); (2) in model experiments with various metal complexes incorporated into zeolites, CH_4

was oxidized to MeOH only on the iron complexes[35] (the replacement of iron by copper complexes gave negative results).

Methane hydroxylases sMH[36] and pMH[37] have a high deuterium-isotope effect in methane oxidation (25—70) and a low deuterium-isotope effect in ethane oxidation (~2—4). These data favor the fact that the mechanisms of methane oxidation and, perhaps, the structures of active sites of these two enzymes are close despite their basically different protein structures.

The active site of pMH is localized in the well between four transmembrane helices. The approach of substrates to the active site in this well is closed from one side by the ***C***—***D*** loop. This restricts the accessibility of large molecules to the active site of the enzyme. The approach of substrates to the active site from another side of the well (it cannot be excluded that this entrance is also closed by the peptide chain) has a long way (the thickness of the biological membrane). In addition, pMH has another set of ligands compared to that of sMH. That is why, most likely, the oxidation rate of large substrates in pMH is lower by tens times than that of small substrates. The active site of sMH is localized in the middle of the well formed by four long helices of α-peptide.[3] However, the entrance to the active site of the enzyme is not closed by the peptide chain from both sides of the well, and this site is accessible for higher-molecular substrates through relatively large holes at the both sides of the well. The mobility of four helices in the region of the active site of pMH, which would facilitate the access of substrate into the active site, is impossible because this region of helices is bound by the binuclear active site of the enzyme. That is why, most likely, pMH possesses a narrower substrate specificity compared to that of sMH. Experiments on selective labeling of β-peptide by [^{14}C]acetylene are also easily explained by the proposed model. The highly reactive oxidized acetylene species (perhaps, ketene[24]) gets into the restricted space of β-peptide, has no time to escape from the volume of the active site of β-peptide, and hence, labels predominantly one of the amino acid residues of four helices and side peptide chain surrounding the active sites. An approximately similar situation is observed for sMH. Due to the long way, the time of escaping the product from the well is much longer than the time of its interaction with the amino acid residues of four helices of the α-peptide of sMH. It is most likely that this explains in part the higher rate of methane oxidation in pMH compared to that in sMH.

The results of searching for conservative, semiconservative, and nonconservative amino acid residues in the amino acid sequence of the γ-peptide and establishing the elements of its secondary structure are presented in Fig. 3. The γ-peptide contains 6 transmembrane helices: ***K***, ***L***, ***M***, ***N***, ***O***, and ***P*** (Fig. 3). Potential ligands of metal ions of the transmembrane helices of the γ-peptide can be 7 amino acid residues: Y103 and Y108 (helix ***M***), Y140, Y146, and Y156 (helix ***N***), M181 (helix ***O***), and H207 (helix ***P***) (Fig. 5). The fragments of the γ-peptide from the N and C ends and loops ***K***—***L***, ***L***—***M***, ***M***—***N***, ***N***—***O***, and ***O***—***P*** are the regions of the γ-peptide arranged in the hydrophilic region. Of them the ***N***—***O*** loop and the peptide region from the C end have no amino acid residue that could participate in complex formation with the metal ion. The loops ***K***—***L*** (D41), ***L***—***M*** (E89), highly conservative loop ***M***—***N*** (D118, H122, D128, and H135), highly conservative loop ***O***—***P*** (E190, H193, M198, E199, and E200), and peptide region from the N end (D9) can potentially participate in complex formation of the metal ion. Amino acid residues of the N end (D9) and ***L***—***M*** loop (E89) are evidently insufficient for complex formation with the metal ion and, hence, the formation of a complex with the metal ion by the amino acid residues from this side of the membrane is impossible. Thus, the formation of the complex of γ-peptide with the metal ion is potentially possible only with the participation of the amino acid residues of helices ***M***, ***N***, ***O***, and ***P*** and loops ***K***—***L***, ***M***—***N***, and ***O***—***P*** at their spatial approach favorable for complex formation. The role of the γ-peptide in the structure and function of pMH and possible presence of some metal ions in it are unknown. Based on the composition of the cultural medium, which is most favorable for the growth of methane-oxidizing bacteria, we can suggest that these metals can be Cu, Fe, Mg, or Zn ions. Of them the Cu^{2+} ion seems preferential because the ESR method using copper isotopes detected two somewhat differed ESR signals,[20] which are characteristic of mononuclear copper with histidine residues as a ligand environment. The mononuclear copper complex can be formed by the amino acid residues (H121, H136, H193, and M198 of the ***M***—***N*** and ***O***—***P*** loops) and Y140 and H207 localized in the surface layer of the transmembrane helices ***N*** and ***P***. Of three peptides only the α- and γ-peptides contain amino acid residues H, whose number satisfies the conclusions of the studies on ESR spectroscopy.[19,20] Therefore, the most probable structure of the Cu site of the γ-peptide can be presented as follows:



(M198 or Y140)

This Cu site either participates in the intramolecular electron transport from the external reducing agent to the active site of the enzyme localized in the β-peptide or participate in the stabilization of the protein spatial structure, or perform both these functions.

Unambiguous principles for molecular recognition, according to which particular helices are arranged in the space, are presently unknown. Therefore, the arrangement of three peptides of pMH in the space remains unclear. Nevertheless, it is evident that three peptides tightly contact with each other because they are isolated

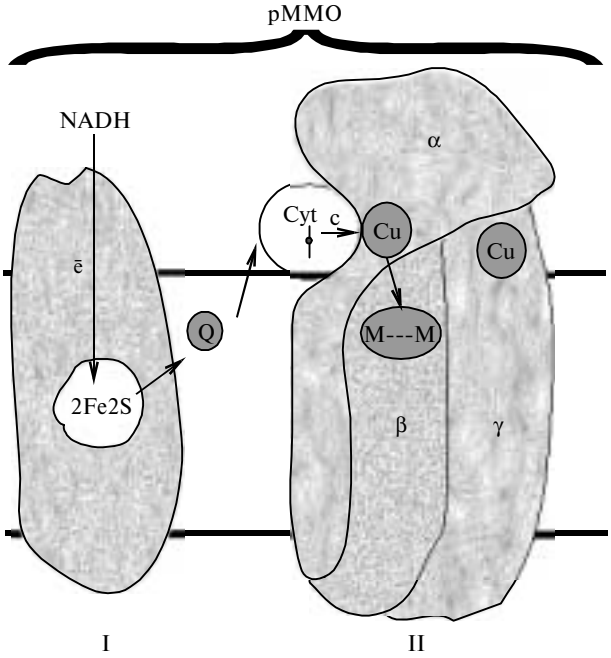


Fig. 6. Tentative models of the structure and electron transport of pMMO: I, NADH-oxidoreductase, and II, pMH.

together due to the specific interaction of their helices during the preparation of the purified protein by different methods. The most short chains between the helices of the β- and γ-peptides arranged in the region of the active sites of pMH most likely participate in the interaction with the hydrophilic domains of the α-peptide thus stabilizing the general spatial structure of pMH.

Based on available data, we can present the general scheme of pMMO functioning as follows (Fig. 6). Electrons from NADH through the system of intramolecular electron transfer of NADH-oxydoreductase are transmitted to hydrophobic hydroquinone Q, which further transmits electrons to a one-electron carrier with the yet unknown nature. It can be one of cytochromes of the c group (Cyt c), localized in the surface region of the membrane. This carrier interacts with the surface area of the hydrophilic part of the α-peptide and transmits an electron to the active site of this peptide containing the Cu^{2+} ion. Then the reduced center of the α-peptide (Cu^{1+}) releases an electron into the hydroxylating site of the β-peptide (it cannot be ruled out that the electron can be transmitted to this site through the γ-peptide) in which molecular oxygen is reduced and one oxygen atom is inserted at the C—H bond of methane to form alcohol and water. The alcohol is transferred to the active site of adjacent methanol dehydrogenase. The enzymatic pMMO complex can use the energy of electron transfer from NADH to the hydroxylating pMH site and that of oxygen reduction to water in the reaction without methane for the transfer of hydrogen ions through the membrane. Members potential thus formed is utilized further in the process of oxidative phosphorilation to form ATP. Perhaps, for this reason all methane-oxidizing bacteria contain genes of pMMO in their genomes and only some of then additionally possess genes of sMMO.

* * *

Thus, based on computer analysis of a wide set of complete and partial amino acid sequences of α-, β-, and γ-peptides of pMH and ammonium hydroxylase, we established that these peptides contain 2, 7, and 6 transmembrane helices, respectively. The most probable amino acid residues participating in complex formation were revealed. It is assumed that pMH is the Cu—Fe-containing enzyme in which copper ions are contained in the α- and γ-peptides and participate in either stabilization of the protein structure or intramolecular electron transfer, or perform both these functions. These ions are localized in the hydrophilic region and responsible for the ESR signals. The activation of O_2 and the oxidation of CH_4 occur in the binuclear (Cu—Fe or Fe—Fe) center of the β-peptide formed by four α-helices and a loop between two of them. This center is localized in a small space of the hydrophobic region near the membrane surface. It cannot be excluded that amino acid residues E2, D11, D171, and Y185 arranged in the hydrophilic region at the opposite side of the β-peptide membrane participate in the stabilization of its spatial structure. It is also assumed that pMMO is a multienzyme complex, which includes NADH-oxyreductase and the system of electron transport from NADH to the active site of pMH. The proposed model of the active site of pMH and the model of intermolecular and intramolecular electron transfer provide good challenges for their purposeful verification and refinement by various physicochemical methods including X-ray structural analysis.

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