ISSN 1063-7745, Crystallography Reports, 2006, Vol. 51, No. 5, pp. 817–823. © Pleiades Publishing, Inc., 2006.
Original Russian Text © A.V. Lyashenko, Yu.N. Zhukova, N.E. Zhukhlistova, V.N. Zaitsev, E.V. Stepanova, G.S. Kachalova, O.V. Koroleva, W. Voelter, Ch. Betzel, V.I. Tishkov, I. Bento, A.G. Gabdulkhakov, E.Yu. Morgunova, P.F. Lindley, A.M. Mikhailov, 2006, published in Kristallografiya, 2006, Vol. 51, No. 5, pp. 870–877.

STRUCTURE
OF MACROMOLECULAR COMPOUNDS

Dedicated to the 60th Birthday of M.V. Kovalchuk

Three-Dimensional Structure of Laccase from *Coriolus zonatus* at 2.6 Å Resolution

A. V. Lyashenko[a], Yu. N. Zhukova[a], N. E. Zhukhlistova[a], V. N. Zaitsev[b], E. V. Stepanova[c], G. S. Kachalova[d], O. V. Koroleva[b], W. Voelter[e], Ch. Betzel[f], V. I. Tishkov[g], I. Bento[h], A. G. Gabdulkhakov[a, i], E. Yu. Morgunova[a], P. F. Lindley[h], and A. M. Mikhailov[a]

[a] *Shubnikov Institute of Crystallography, Russian Academy of Sciences, Leninskiĭ pr. 59, Moscow, 119333 Russia*
e-mail: amm@ns.crys.ras.ru
[b] *Centre for Biomolecular Sciences, University of St. Andrews, North Haugh, St. Andrews, Fife KY16 9ST, UK*
[c] *Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskiĭ pr. 33, Moscow, 119991 Russia*
[d] *Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow oblast, 142290 Russia*
[e] *Institute for Biochemistry, University of Tübingen, Germany*
[f] *Department of Biochemistry and Molecular Biology, University of Hamburg, c/o DESY, Bld. 22a, Notkestrasse 85, D-22603 Hamburg, Germany*
[g] *Department of Chemical Enzymology, Moscow State University, Vorob'evy gory, Moscow, 119992 Russia*
[h] *Institute for Technology in Chemistry and Biology, New University of Lisbon, 127 Av. da Republica, 2781-901 Oeiras, Portugal*
[i] *Institute of Protein Research, Russian Academy of Sciences, Institutskaya ul. 4, Pushchino, Moscow oblast, 142290 Russia*

Received May 5, 2006

Abstract—Laccase (oxygen oxidoreductase, EC 1.14.18.1) belongs to the copper-containing oxidases. This enzyme catalyzes reduction of molecular oxygen by different organic and inorganic compounds to water without the formation of hydrogen peroxide. The three-dimensional structure of native laccase from *Coriolus zonatus* was solved and refined at 2.6 Å resolution (R_{factor} = 21.23%, R_{free} = 23.82%, rms deviations for the bond lengths and bond angles are 0.008 Å and 1.19°, respectively). The primary structure of the polypeptide chain and the architecture of the active site were refined. The carbohydrate component of the enzyme was identified. The access and exit water channels providing the access of molecular oxygen to the active site and release of water, which is the reduction product of molecular oxygen, from the protein molecule were found in the structure.

PACS numbers: 87.15.By

DOI: 10.1134/S1063774506050117

INTRODUCTION

Laccases belong to the blue oxidases (oxygen oxidoreductases, EC 1.10.3.2) with broad substrate specificity, which can be enhanced with the use of redox mediators. Various phenols, such as mono-, di-, and polyphenols, serve as substrates oxidized by laccase [1–3]. These enzymes catalyze oxidation of substrate molecules accompanied by reduction of molecular oxygen to water. The *T*1 copper ion of the mononuclear copper site serves as the primary electron acceptor. An electron is transferred from the *T*1 copper ion to the trinuclear site containing one *T*2 copper ion and two *T*3 copper ions (the *T*2/*T*3 site). The oxygen molecule binds at the trinuclear site, where two water molecules are generated after the four-electron transfer from four substrate molecules. The classification of copper ions (three types) in multinuclear copper-containing oxidases (ascorbate oxidase, laccase, and ceruloplasmin) is based on the differences in their spectral and magnetic properties [4–6]. The *T*1 copper ions (the mononuclear site) are characterized by an absorption band with a maximum at 610 nm and are responsible for the blue color of concentrated solutions of these proteins. The *T*2 copper ion ("non-blue copper"/EPR-active copper) shows adsorption at 330 nm. The *T*3 copper ions (EPR-inactive copper) exist as a binuclear cluster composed of two antiferromagnetically coupled copper atoms. The amino-acid residues binding the *T*1, *T*2, and *T*3 copper ions are strictly conserved in all representatives of mononuclear copper-containing oxidases. The first step of the proposed schemes of the evolution of laccases involves the duplication of the common gene precursor encoding the sequence of the blue pra protein

of the cupredoxin series containing mononuclear sites. In the second step of evolution, the third domain is incorporated and the trinuclear site is formed at the interface between the first and third domains.

Laccase has a rather wide field of application in scientific research (genetic engineering, studies of biopolymer structures, etc.) to industrial processes (biosensor technologies, organic synthesis, design of new pharmaceuticals, bleaching of paper slurries and cloths, detoxification of xenobiotics including pesticides and nerve-paralytic agents, and stabilization of beverages). In addition, owing to the ability of laccase to catalyze electroreduction of molecular oxygen to water molecules according to the mediatorless mechanism, the kinetic and electrocatalytic properties of the enzyme as a promising catalyst for electrode processes have attracted considerable attention.

Laccases have been extensively studied by biochemical and spectral methods and X-ray diffraction [7–12], thereby providing insight into many structural and functional features of these enzymes. Knowledge of the three-dimensional structures of laccases from different sources and the elucidation of the mechanism of catalysis are necessary for the more successful practical application of the enzyme. The present study is a continuation of X-ray diffraction investigations of fungi laccase from *Coriolus zonatus*. The results of the preliminary investigation have been reported in [13]. The aim of the present study was to refine the three-dimensional organization of laccase from *Coriolus zonatus*, its active site, water channels, the carbohydrate component, and the primary sequence of this enzyme by X-ray diffraction methods.

EXPERIMENTAL

Isolation and purification of laccase from *C. zonatus*. *Coriolus zonatus* from the collection of the Bach Institute of Biochemistry of the Russian Academy of Sciences (*GenBank Accession number Trametes ochracea*—AB158314) was used as the producer strain of laccase. The strain was preserved on agarized media, which were prepared through dilution of a wart with water in a 1 : 4 ratio (v/v) in the presence of 2% agar at +4°C with a colorless reverse. The sowing material was grown by the surface method in a nutrient medium at initial pH 6.0 containing peptone as the nitrogen source, glucose as the carbon source, and mineral salts at 25–27°C. The following medium was used in all experiments (g/l): glucose, 10.0; peptone, 3.0; KH_2PO_4, 0.6; $ZnSO_4 \cdot 7H_2O$, 0.001; K_2HPO_4, 0.4; $FeSO_4 \cdot 7H_2O$, 0.0005; $MnSO_4$, 0.05; and $MgSO_4 \cdot 7H_2O$, 0.5. The deep cultivation of the producer of the fungus *Coriolus zonatus* was carried out as follows: $CuSO_4$ (0.15 g/l) and $CaCl_2$ (0.5 g/l) were added to the nutrient medium of the above-mentioned composition, and pH was adjusted to 6.0 with a NaOH solution or acetic acid. The medium was sterilized in an autoclave at 1 atm for 30 min. After completion of cultivation, the culture filtrate was separated from micelles by filtration and deep freezing was performed at –40°C.

Extracellular laccase from *Coriolus zonatus* was isolated from the culture liquid (pH 5.0) at room temperature by precipitation with 90% ammonium sulfate for 2 h with constant stirring. The precipitate was collected by centrifugation at 2500*g* for 30 min and again dissolved in a minimum volume of distilled water. Then the sample was applied to a Sephadex G-25 column (100 × 2 cm) equilibrated with a 5 mM potassium phosphate buffer (PPB), pH 6.0. The elution was carried out with the use of the same buffer. The peak containing the enzymatic activity was applied to a DEAE-Toyopearl 650 M column (2 × 20 cm) equilibrated with 5 mM PPB, pH 6.0. The protein was eluted with a linear ionic-strength gradient of PPB (2 × 150 ml), pH 7.2, and the molarity was varied from 5 to 200 mM. The active fractions were combined, dialyzed against 5 mM PPB, pH 6.0, and rechromatographed on a DEAE-Toyopearl 650 M column under the same conditions but with a more gently sloping ionic-strength gradient (2 × 250 ml). A highly homogeneous sample of the enzyme required for crystallization was prepared by high performance liquid chromatography (HPLC) on an FPLC chromatograph using a TSK 3000 gel-filtration column. The elution was performed with 50 mM PPB, pH 6.5, at a rate of 0.5 ml/h. The homogeneity of the sample was checked by electrophoresis (Fig. 1).

Crystallization. Crystals of laccase from *C. zonatus* (Fig. 2) were grown by the hanging-drop vapor-diffusion method. The reservoir solution (0.5 ml) contained 0.2 M ammonium sulfate, 0.1 M sodium acetate (pH 4.6), and 25% (w/v) polyethylene glycol 4000. The drops (6 μl) were composed of the protein (8 mg/ml) in a 50 mM citrate buffer (pH 5.5), 0.1 M ammonium sulfate, 0.05 M sodium acetate (pH 4.6), and 12.5% (*w*/*v*) polyethylene glycol 4000.

X-ray diffraction data collection. X-ray intensities were measured from crystals of laccase from *C. zonatus* at 100 K on a BW6 beamline (DESY, Hamburg, Germany, a CCD detector) at a wavelength of 1.05 Å. The reservoir solution, to which 15% glycerol (v/v) was added, was used as a cryosolution. The experimental structure amplitudes were obtained with the use of the XDS program package [14]. The crystallographic characteristics of the X-ray data set for laccase from *C. zonatus* and details of X-ray data collection are summarized in Table 1.

Determination and refinement of the three-dimensional structure of laccase. The crystal structure was solved by the molecular replacement method using the MOLREP program [15] incorporated in the CCP4 suite of programs [16]. The three-dimensional structure of laccase from *C. zonatus*, which we have established earlier at 3.2 Å resolution [13], was used as the starting model for the structure solution and refinement. The water molecules, copper atoms of the active

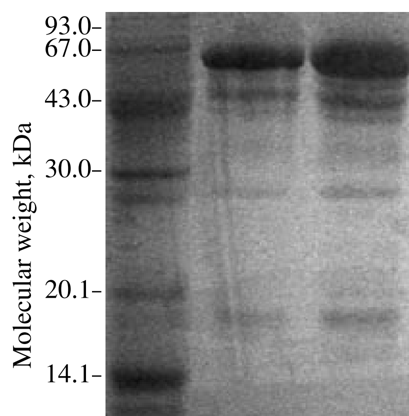

Fig. 1. SDS-electophoretic pattern for laccase from *C. zonatus* (to the right of the figure) and marker proteins with the molecular weights in kDa (to the left).

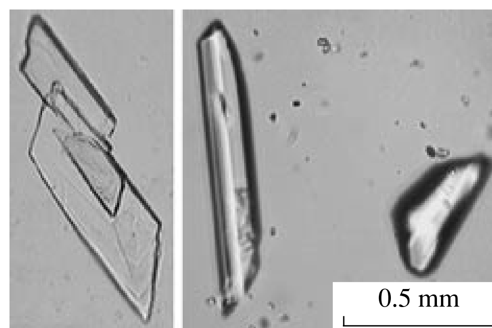

Fig. 2. Crystals of laccases from *C. zonatus.*

site, and carbohydrates of laccase were excluded from the starting model. The true solution corresponded to rotation- and translation-function maxima, for which the R_{fact} and R_{corr} were 38.7 and 63.2, respectively (Fig. 3).

The structure was refined with the use of the CNS [17] and REFMAC [18] programs. The refinement was monitored with the use of the free R factor (R_{free}) calculated for 5% of reflections, which were arbitrarily chosen from the experimental X-ray data. The manual rebuilding was performed with the use of the O [19] and COOT [20] programs based on ($3F_o - 2F_c$), ($2F_o - F_c$), and ($F_o - F_c$) difference Fourier maps, where F_o and F_c are the observed and calculated structure amplitudes, respectively. The average B factors for the structure were calculated with the Baverage program incorporated in the CCP4 suite of programs.

The correctness of the results in the course of refinement was monitored with the Procheck [21], WhatCheck [22], and COOT [20] programs. The refinement statistics for the structure of laccase from *C. zonatus* are given in Table 2.

RESULTS AND DISCUSSION

Laccase from *C. zonatus* is a single-chain molecule consisting of 499 amino-acid residues. The primary structure of the enzyme, which was determined on the basis of results of the present X-ray diffraction study, is shown in Fig. 4. A comparison with the primary structure of laccase from *T. versicolor* (ID PDB 1GYC), which we have used as the starting model in the early steps of X-ray diffraction study of laccase from *C. zonatus* [13], revealed 123 different amino-acid residues.

The laccase under consideration exists as a monomer (Fig. 5) consisting of three cupredoxin domains. The first domain includes residues from 1 to 131 and contains one helix and seven β-strands, the second domain includes residues from 132 to 309 and consists of eleven β-strands, and the third domain includes residues from 310 to 499 and contains three helices and six β-strands. The first domain is linked to the second and third domains by two disulfide bridges (Cys117–Cys205, 2.03 Å; and Cys85–Cys488, 2.04 Å).

Structure of the active site. The copper atoms of the active site were observed as the following four highest-density peaks in ($F_o - F_c$) difference electron density maps: Cu(1), 7.0σ; Cu(2) and Cu(3), 10.5σ; and Cu(4), 7.3σ, where σ is the rms error of the electron density map. The *T*2 and *T*3 copper atoms form a trinuclear cluster located between domains *I* and *III* (Fig. 6).

Table 1. Crystallographic data and details of X-ray data collection for laccase from *C. zonatus*

Sp. gr.	*P*321 (No. 150)
Unit-cell parameters, Å, deg	$a = b = 168.93$, $c = 69.35$ $\alpha = \beta = 90.0$, $\gamma = 120.0$
Molecular weight, kDa	60
Number of molecules per asymmetric unit	1
Wavelength, Å	1.05
Resolution, Å	145.86–2.60
Number of measured reflections	175868
Crystal-to-detector distance, mm	180
Oscillation range, deg	0.5
Rotation range, deg	80
Number of independent reflections	35011
Redundancy	5.02 (4.75)*
Completeness of the set, %	95.07
Mosaicity, deg	0.2
Average $I/\sigma(I)$	15.17 (4.27)*
R_{merge}	7.1 (24.4)*
Matthews coefficient (V_M), Å^3 Da^{-1}	4.8
Solvent content, %	74

* High-resolution data are given in parentheses.

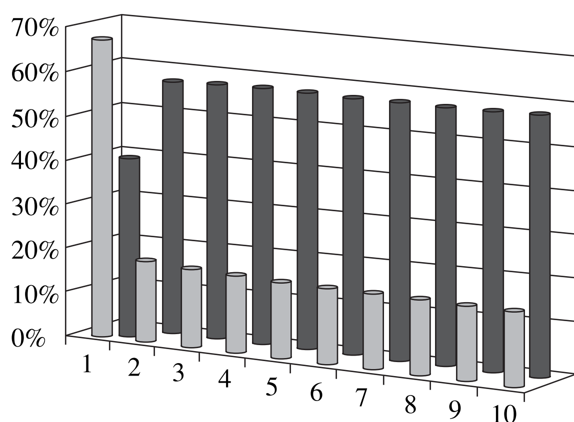

Fig. 3. Graphical representation of the molecular-replacement solution in searching for the starting model for laccase from *C. zonatus* with the use of the MOLREP program [14], where the gray and black columns are R_{corr} and R_{fact}, respectively.

The $T1$ copper atom is located in domain *III* in the vicinity of the substrate-binding site analogously to that found in other multinuclear copper-containing oxidases. The Cu(1) atom is coordinated by two histidine residues (His395 and His458) and a cysteine residue (Cys453) lying in a plane with the copper ion (Table 3, Fig. 6). The axial position on the side of the substrate pocket is occupied by Ile455, which forms the shortest contact (3.74 Å) with the $T1$ copper atom. The amino-acid residue Phe463 at a distance of 3.62 Å from Cu(1) is located on the opposite side. Other fungi laccases also contain aliphatic residues in the axial positions. For example, the axial position in laccase from *C. cinereus* is occupied by the residue Leu462 at a distance of 3.51 Å from the copper ion. In laccase from *T. versicolor*, the corresponding position is occupied by phenylalanine at a distance of 3.6 Å [7, 8]. This three-dimensional configuration of the $T1$ site differs from that found in ascorbate oxidase, laccase *cotA* from *Bacillus subtilis*, and laccase *CueO* from *Escherichia coli*, in which the sulfur atom of methionine occupies the axial position at a distance of approximately 3.0 Å from copper [23–25].

Eight histidines (four amino-acid residues from domain *I* and four residues from domain *III*) are involved in coordination of the copper atoms of the trinuclear (the $T2/T3$ cluster) site (Fig. 6). The Cu(2) and Cu(3) atoms are coordinated by six histidines (each copper atom is coordinated by three amino-acid residues). The N$E2$ atoms of five histidines are involved in coordination, whereas the sixth histidine is involved in coordination through the N$D1$ atom (Table 3). The distance between the copper atoms, Cu(2)–Cu(3), is 4.80 Å. An electron-density peak asymmetrically related to the Cu(2) and Cu(3) atoms is observed in $(F_o - F_c)$ difference maps between these copper atoms. The careful refinement made it possible to identify this peak as an oxygen atom of hydroxyl or water (Table 3). The Cu(2)–O–Cu(3) angle is 156.25°. As a result, the

Fig. 4. Primary sequence of laccase from *C. zonatus* and secondary-structure elements, where *S* is a β-ribbon and *h* is a helix.

coordination sphere of each copper atom can be described as a distorted tetrahedron. The Cu(2) and Cu(3) atoms are located from the Cu(4) atom of the *T*2 type at distances of 4.29 and 3.91 Å, respectively. The Cu(4) atom is coordinated by two histidines (Table 3). A peak found at a distance of 2.79 Å from the latter copper atom was refined as a water molecule. This water molecule and two histidine residues form a trigonal planar configuration of the *T*2 site.

The *T*1 copper atom is linked to the trinuclear site by the tripeptide His452–Cys453–His454, which is conserved in all multinuclear copper oxidases (Table 3, Fig. 6). The distances from the Cu(1) atom (*T*1 type) to the Cu(2), Cu(3) (*T*3 type), and Cu(4) (*T*2 type) atoms are 12.02, 13.12, and 14.68 Å, respectively. The possible electron-transfer path between the mononuclear site and the trinuclear sites has been proposed for the first time for ascorbate oxidase (AO) [23]. The structural similarity of the active sites of AO and laccase implies the similarity of the electron-transfer mechanisms in these proteins. By analogy with AO, the sulfur atom of Cys453 in the coordination sphere of the *T*1 copper atom in laccase from *C. zonatus* accepts an electron from the Cu(+1) ion, which is, correspondingly, oxidized to Cu(+2). Then, the electron is successively transferred to the carbonyl oxygen atom of Cys453 and, through a hydrogen bond, to the N*D*1 atom of His452, the latter serving as a ligand of Cu(3) in the trinuclear site (Fig. 6). The length of the O_Cys453····N*D*1_His452 hydrogen bond involved in the electron transport from the mononuclear site (the *T*1 copper ion) to the trinuclear (the *T*2/*T*3 cluster) site is 2.76 Å.

Water channels. In the molecule of laccase from *C. zonatus*, two water channels leading to the trinuclear site were revealed (Figs. 5 and 7). The water molecules in these channels are involved in numerous hydrogen bonds with each other and with the amino-acid residues lining the walls of the channels. In one channel (Fig. 7), which provides the access of oxygen molecules to the *T*3 copper ions, the O_2, O_61, O_62, O_103, O_41, and O_58 water molecules were located in difference electron density maps. These water molecules are linked to each other by hydrogen bonds (the distances vary from 2.53 to 3.19 Å). The O_2 water molecule is linked to the O_61 water molecule in the channel, is located asymmetrically between the *T*3 copper ions, and serves as a bridge (Fig. 7). Most amino-acid residues lining the walls of this channel, such as *Ala*80, *Ser*110, *His*111, *Ser*113, and *Tyr*116 from domain *I* and *His*454 and *Asp*456 from domain *III* are conserved in the laccase family. These residues are involved in the following hydrogen bonds with the water molecules occupying the first channel: O_*His*111···O_41, 3.38 Å; N*E*2_*His*111···O_61, 3.01 Å; O_*Ser*113···O_103, 2.77 Å; N–*Ser*113···O_103, 3.13 Å; N_*Ser*113···O_58, 3.13 Å; N*E*2_*His*454···O_61, 3.00 Å; and O*D*1_*Asp*456···O62, 2.55 Å.

Table 2. Refinement statistics for the structure of laccase

Crystal	laccase from *C. zonatus*
Resolution range, Å	29.0–2.6 (2.62–2.60)*
Number of reflections in the working set	31805 (2289)*
Number of reflections in the test set (5%)	1691 (116)*
Cutoff of the set, $F >$	σ
Number of non-hydrogen atoms of the protein molecule	3899
Number of water molecules	117
Number of carbohydrate molecules:	
NAG	4
MAN	1
R_{fact}, %	21.23 (31.8)*
R_{free}, %	23.82 (36.8)*
rms deviation for bond lengths, Å	0.008
rms deviation for bond angles, deg	1.19
Average *B* factor, Å^2:	30.405
for main-chain atoms	30.369
for side-chain atoms and water molecules	39.102
Ramachandran statistics, %:	
most favored region	84.7
allowed region	14.8
additionally allowed region	0.2
disallowed region	0.2

Another water channel (Figs. 5 and 7) serves for the transport of water molecules from the *T*2 site to the surface of the protein molecule. The O_7, O_113, O_69, O_21, and O_28 molecules form a chain and are located at distances of 2.55–3.39 Å from each other. Like the amino-acid residues of the first channel, the residues of the second channel, such as *Gly*67 and *Gln*102 of domain *I*, *Asp* 224 of domain *II*, and *Leu*399, *His*402, and *Asp*424 of domain *III*, are conserved in the laccase family and are linked to the water molecules in the channels by a hydrogen bond network. The main-chain oxygen atoms of *Leu*399 and *His*402 are involved in hydrogen bonds with the O_113 water molecule (O_*His*402···O_113, 3.08 Å; O_*Leu*399···O_113, 3.01 Å), which is, in turn, located at a hydrogen-bond distance (2.78 Å) from the water molecule involved in the coordination sphere of the *T*2 copper ion (2.79 Å). The O_69 water molecule forms short contacts with O_Gly67 (3.01 Å) and O*D*1_*Asp*424 (2.6 Å). In addition, two water molecules, O_21 and O_28, from the second channel, which are linked to each other by a hydrogen bond (2.74 Å), are involved in hydrogen bonds with O_*Asp*224 (2.95 Å) and N*E*2_*Gln*102 (2.96 Å), respectively. Analogous access and exit water channels were found in the structures of other laccases as well [9, 24, 26].

Fig. 5. Tertiary structure of laccase from *C. zonatus*.

Carbohydrate component of laccase. Laccase, like all blue oxidases, is a glycoprotein. The carbohydrate component of most laccases comprises 10–13% of the weight of the protein molecule and includes such carbohydrates as hexosamine, glucose, mannose, galactose, and arabinose. High-electron-density regions, which were found in difference-electron-density maps at the periphery of the molecule in the vicinity of the amino-acid residues *Asn*54, *Asn*333, and *Asn*436, were interpreted as carbohydrates (Fig. 5). One of the electron-density regions was interpreted as two *N*-acetylgalactosamines (*NAG*5 and *NAG*1), which are covalently bonded to each other (the bond length is 1.35 Å) and to N*D*2_*Asn*54 (*NAG*5; the bond length is 1.35 Å). In addition, the *NAG*5 molecule is involved in the S*D*_*Met*57···O7_*NAG*5 hydrogen bond (2.65 Å) with the amino-acid residue *Met*57. The O4 atom of *N*-acetylgalactosamine *NAG*2 is located at a hydrogen-bond distance (2.31 Å) from *ND*2_Asn333. The amino-acid residue Asn436 is involved in a covalent bond with the mannose molecule *MAN*3, which is, in turn, covalently bonded to the *NAG*4 molecule.

Heterogeneity of the carbohydrate component and its disorder in protein molecules always present great problems in growing crystals of laccases suitable for X-ray diffraction. Owing to the lability of carbohydrate chains, glycan residues generally cannot be located in electron density maps. Evidently, the covalent bonds between of the carbohydrate chains found in the structure of laccase from *C. zonatus* facilitate ordering of the carbohydrate component in the structure, and the formation of these bonds plays, apparently, an important role in the growth of stable and ordered crystals of this protein.

Mechanism of action of the enzyme. The mechanism of action of multimedia oxidases has been proposed in [26]. The hypothesis of the authors was based on X-ray diffraction studies of the crystal structures of laccase complexes, in which molecular oxygen, peroxide, or azide occupy the bridging positions between the *T*3 copper ions. The structure of laccase from *C. zonatus* is yet another "instant photograph," which fixes one of the possible events of the enzymatic reaction. After the transformation of molecular oxygen into two hydroxide ions, one of the ions is protonated and then migrates as a water molecule into the exit channel, the second hydroxide ion remaining bound to one of the *T*3 copper atoms. After protonation of this hydroxide ion, the second water molecule migrates to the exit channel. The formation of two water molecules occurs after the four-electron transfer from four substrate molecules. Apparently, the amino-acid residues lining the walls of the access channel serve as the proton source. This mechanism assumes a considerable mobility of the

Fig. 6. Structure of the active site of laccase from *C. zonatus*.

Fig. 7. Three-dimensional structure of the water channels including water molecules (filled circles).

Table 3. Distances between the copper atoms and ligands

Type of the copper atom	Atom	Atom_amino-acid residue	Distance, Å
*T*1	Cu(1)	N*D*1_*His*395	2.47
	Cu(1)	S_*Cys*453	2.11
	Cu(1)	N*D*1_*His*458	2.39
*T*3	Cu(2)	N*E*2_*His*111	2.03
	Cu(2)	N*E*2_*His*400	2.03
	Cu(2)	N*E*2_*His*452	1.96
	Cu(2)	O_2	1.93
*T*3	Cu(3)	ND1_*His*66	2.01
	Cu(3)	N*E*2_*His*109	2.19
	Cu(3)	N*E*2_*His*454	2.18
	Cu(3)	O_2	2.96
*T*2	Cu(4)	N*E*2_*His*64	1.93
	Cu(4)	N*E*2_*His*398	1.91

Cu(4) ion of the *T*2 type because it should provide the access of the hydroxide ions to the exit channel. The less rigid configuration of the *T*2 copper atoms relative to the *T*3 copper atom allows higher mobility of the Cu(4) ion. This finding is consistent with the experimental observations. Thus, purified samples of laccases from different sources often lose *T*2 copper atoms either completely or partially.

ACKNOWLEDGMENTS

We thank the Komarov Botanical Institute of the Russian Academy of Sciences for providing a producer strain of extracellular laccase from *Coriolus zonatus*.

This study was supported by the BMFW (Bundesministerium für Forschung und Wissenschaft, contract No. RUS/214) and in part by the Russian Foundation for Basic Research (project no. 05-04-49520).

REFERENCES

1. A. I. Yaropolov, O. V. Skorobogat'ko, S. S. Vartanov, and S. D. Varfolomeyev, Appl. Biochem. Biotechnol. **49** (3), 257 (1994).
2. D. R. McMillin and M. K. Eggleston, in *Multi-Copper Oxidases*, Ed. by A. Messerschmidt (World Scientific Publishing Co. Pte. Ltd, 1997), Chap. 5, p. 129.
3. B. Reinhammar, in *Copper Proteins and Copper Enzymes*, Ed. by R. Lontie (CRC Press, Boca Raton, 1984), Vol. 3, p. 1.
4. B. G. Malmstrom, Annu. Rev. Biochem. **51**, 21 (1982).
5. B. G. Malmstrom, in *Multi-Copper Oxidases*, Ed. by A. Messerschmidt (World Scientific Publishing Co. Pte. Ltd, 1997), p. 1.
6. K. Nakamura and N. Go, *Cellular and Molecular Life Sciences* (Birkhauser, Bassel, 2005), p. 1.
7. V. Ducros, A. M. Brzozowski, K. S. Wilson, et al., Nat. Struct. Biol. **5**, 310 (1998).
8. V. Ducros, A. M. Brzozowski, K. S. Wilson, et al., Acta Crystallogr., Sect. D: Biol. Crystallogr. **57**, 333 (2001).
9. K. Piontek, M. Antorini, and T. Choinowski, J. Biol. Chem. **277** (40), 37663 (2002).
10. T. Bertrand, C. Jolivalt, P. Briozzo, et al., Biochemistry **41**, 7325 (2002).
11. N. Hakulinen, L. L. Kiiskinen, K. Kruus, et al., Nat. Struct. Biol. **9**, 601 (2002).
12. F. J. Enguita, D. Marcal, L. O. Martins, et al., J. Biol. Chem. **279**, 23 472 (2004).
13. A. V. Lyashenko, N. E. Zhukhlistova, E. V. Stepanova, et al., Kristallografiya **51** (2), 305 (2006) [Crystallogr. Rep. **51**, 278 (2006)].
14. W. Kabsch, *International Tables for Crystallography*, Ed. by M. G. Rossmann and E. Arnold (Kluwer, Dordrecht, 2001).
15. A. A. Vaguine, J. Richelle, and S. J. Wodak, Acta Crystallogr., Sect. D: Biol. Crystallogr. **55**, 191 (1999).
16. Collaborative Computational Project, Number 4, Acta Crystallogr., Sect. D: Biol. Crystallogr. **50**, 760 (1994).
17. A. T. Brunger, P. D. Adams, and G. M. Clore, Acta Crystallogr., Sect. D: Biol. Crystallogr. **54**, 905 (1998).
18. G. N. Murshudov, A. A. Vagin, and A. Lebedev, Acta Crystallogr., Sect. D: Biol. Crystallogr. **55**, 247 (1999).
19. T. A. Jones, J.-Y. Zou, S. W. Cowan, and M. Kjeldgaard, Acta Cristallogr., Sect. A: Found Crystallogr. **47**, 110 (1991).
20. P. Emsley and K. Cowtan, Acta Crystallogr., Sect. D: Biol. Crystallogr. **60**, 2126 (2004).
21. R. A. Laskowski, M. W. MacArthur, D. S. Moss, and J. M. Thornton J. Appl. Crystallogr. **26**, 283 (1993).
22. R. Rodriguez, G. Chinea, N. Lopez, et al., CABIOS, Comput. Appl. Biosci., No. 14, 523 (1984).
23. A. Messerchmidt, R. Ladenstein, and R. Huber, J. Mol. Biol. **224**, 179 (1992).
24. F. J. Enguita, L. O. Martins, A. O. Henriques, and M. A. Carrondo, J. Biol. Chem. **278**, 19 416 (2003).
25. S. A. Roberts, A. Weichsel, and G. Grass, Proc. Natl. Acad. Sci. U.S.A. **99**, 2766 (2002).
26. I. Bento, L. O. Martins, and G. G. Lopes, Dalton Trans., 3507 (2005).

Translated by T. Safonova