Polarized Light Induced Molecular Orientation in Laccase for Chiral azo-salen Mn(II), Co(II), Ni(II), Cu(II), Zn(II) Mediators toward Application for Biofuel Cell

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Abstract

We report on syntheses, characterization, and docking of new organic/inorganic hybrid materials composed of chiral salen-type Schiff base complexes having azobenzene groups and laccase, an enzyme catalyzing four-electron reduction of oxygen to water. By using polarized UV-vis and IR spectroscopy, it is confirmed that these hybrid materials indicate Weigert effect (polarized UV light induced anisotropic molecular orientation) because of azobenzene groups. To our knowledge, we find mediator performance of Mn(II) complex-laccase hybrid materials is improved by polarized UV light irradiation for the first time.

Keywords

Weigert effect; Azobenene; Chiral Schiff base; Manganese(II); Laccase

Introduction

Laccase [1-3] has been used as the cathode enzyme of the biofuel cell [4-7] to reduce molecular oxygen to water by four-electron reaction. In order to enhance efficiency of electric power generation, it is important and necessary to improve electron transfer from electrode to enzyme (in the case of laccase, T1 site [1] accepting electrons especially). To overcome this problem, mediators are commonly introduced between cathode-enzyme systems. Since nano-particle mediators can be involved in protein molecules, they have spatial advantage, while they also have disadvantage in view of low current density. On the other hand, metal complex mediators [6] have advantage in current density, though their molecular size and shape make impossible to be include into protein molecules, so long-distance electron transfer may occur generally. Therefore, metal complex mediators having spatially advantage or appropriate methods for spatial tuning have been required so far [8-10].

By the way, besides conventional application with photoisomerization [11], we have widely investigated organic/inorganic hybrid materials composed of functional metal complexes and azobenzene [12, 13, 16-21, 23] in polymer films to induce optical anisotropy (namely anisotropic molecular orientation) induced by linearly polarized UV light due to Weigert effect [14, 15]. The systems have been developed to bidendate chelate chiral Schiff base complexes [12, 13], salen-type chiral Schiff base complexes [16, 17], achiral complexes [18, 19], azo-group containing complexes [20, 21], and without azo-moiety materials (whose metal complexes have large dipole moment) [21, 22]. As a matrix, not only synthetic polymer films but also protein films [22, 23] were also employed and docking of metal complexes into proteins was also discussed [22, 24]. Moreover, circularly polarized UV light induced supramolecular chirality or photo-induced helical supramolecular arrangement
were elucidated recently [18, 25]. Hence, polarized light induced molecular orientation may be promising method to control spatial tuning of metal complexes included in protein molecules or docking on protein surface [26].

Herein, we prepared organic/inorganic hybrid materials composed of chiral salen-type Schiff base Mn(II) (1), Co(II) (2), Ni(II) (3), Cu(II) (4), and Zn(II) (5) complexes (Fig. 1) and laccase. Structural characterization and docking of them will be discussed. We will elucidate to compare degree of optical anisotropy for them caused by Weigert effect after irradiation of polarized UV light up to 10 min, which was observed by using polarized UV-vis and IR spectroscopy. Furthermore, we will attempt to observe changes of electrochemical properties of 1-5 with laccase not only potential performance as mediator but also “spatially photo-tuneable mediator” by polarized UV light irradiation to an electrode of the hybrid materials for the first time to our knowledge. (Fig. 1)

Fig. 1. Structures of complexes 1-5 (M = Mn, Co, Ni, Cu, and Zn, respectively)

2. Experimental

2.1. General procedures

Chemicals of the highest commercial grade available (solvents from Kanto Chemical, organic compounds from Tokyo Chemical Industry and metal sources from Wako) were used as received without further purification.

2.2. Preparation

2.2.1. Preparations of complexes

Treatment of aniline (1.929 g 20.0 mmol) in 15 mL of 6 M HCl and NaNO₂ (1.395 g 20.0 mmol) in 30 mL of H₂O for 30 min at 278 K gave rise to yellow precursor. Treatment of the precursor and o-vanillin (2.9878 g 20.0 mmol) in 30 mL of 10 % NaOH aqueous solution for 1h at 278 K, and the resulting orange precipitates of 2-hydroxy-3-methoxy-5-phenyldiazobenzaldehyde were filtrated and washed with water and ethanol and dried for several days. To a solution of 2-hydroxy-3-methoxy-5-phenyldiazobenzaldehyde (0.5126 g, 2.00 mmol) dissolved in ethanol (60 mL), (1R,2R)-(+) -1,2-diphenylethylendiamine (0.2122 g, 1.00 mmol) was added and stirred at 323 K for 3 h to give brown solution of the ligand. Manganese(II) acetate tetrahydrate (0.2475 g, 1.00 mmol) was added to the resulting solution containing orange deposition for 3 h to give dark brown solution of the complex. This orange compound was filtered and recrystallized from chloroform/diethyl ether to give single crystals for X-ray analysis (only 1, 3, and 4). Basically the other complexes were also prepared in a similar way using the corresponding metal sources.

1: Yield 0.555 g (74.47 %). Anal. Found: C, 60.73; H, 4.56; N, 9.14 %. Calc. for C₄₂H₃₂N₆MnO₄: C, 62.51; H, 4.76; N, 10.17 %. IR (KBr): 1656 cm⁻¹ (C=N).

2: Yield 0.6686 g (88.12 %). Anal. Found: C, 62.29; H, 4.65; N, 10.27 %. Calc. for C₄₂H₃₂N₆CoO₄: C, 62.21; H, 4.74; N, 10.12 %. IR (KBr): 1658 cm⁻¹ (C=N).
3. **Preparations of hybrid materials of complex (I-5)+PMMA**

Acetone solution (0.5 mL) of a complex (1-5) (0.0019 g in 5 mL acetone) and acetone solution (2 mL) of polymethylmethacrylate (PMMA) (10%) were mixed and dropped onto a PMMA film and dried for 2 days at 298 K to give rise to hybrid materials 1+PMMA through 5+PMMA.

2.2.3. **Preparations of hybrid materials of complex (I-5)+laccase**

Acetone solution (0.5 mL) of a complex (1-5) (0.0019 g in 5 mL acetone) and laccase (0.0152 g in 2 mL tris buffer) were mixed and dropped onto a PMMA film and dried for 2 days at 298 K to give rise to hybrid materials 1+laccase through 5+laccase.

2.3. **Physical measurements**

Elemental analyses (C, H, N) were carried out with a Perkin-Elmer 2400II CHNS/O analyzer at Tokyo University of Science. Infrared spectra were recorded as KBr pellets on a JASCO FT-IR 4200 plus spectrophotometer in the range of 4000-400 cm⁻¹ at 298 K. Electronic spectra were measured on a JASCO FT-IR 4200 plus spectrophotometer in the range of 800-200 nm at 298 K. Circular dichroism (CD) spectra were measured on a JASCO V-570 UV/VIS/NIR spectrophotometer in the range of 800-200 nm at 298 K. Electrochemical (cyclic voltammetry, CV) measurements were carried out on a BAS SEC2000-UV/ VIS and ALS2323 system with Ag/AgCl electrodes range of -0.50–0.80 V vs. Ag/Ag⁺. ²H-NMR spectra were recorded on a JEOL JMN-300 spectrometer (300 MHz) in CDCℓ₃. Photo-illumination were carried out using a lamp (1.0 mW/cm²) with optical filters (UV λ = 200-400 nm) leading to a sample by using optical fibers and polarizer through optical filters.

2.4. **X-ray crystallography**

Prismatic single crystals of 1 (brown), 3 (orange), and 4 (brown) were glued on top of a glass fiber and coated with a thin layer of epoxy resin to measure the diffraction data. Intensity data were collected on a Bruker APEX2 CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). Data analysis was carried out with a SAINT program package. The structures were solved by direct methods with a SHELXS-97 [27] and expanded by Fourier techniques and refined by full-matrix least-squares methods based on F² using the program SHELXL-97 [27]. An empirical absorption correction was applied by a program SADABS [28]. All non-hydrogen atoms were readily located and refined by anisotropic thermal parameters. All hydrogen atoms were located at geometrically calculated positions and refined using riding models. Analysis of 1 indicated partial racemization of chiral ligands during preparation. There are two crystallographically independent molecules in the asymmetric units.

Crystallographic data for 4 (CCDC 1457179).

C₄₂H₃₄N₆MnO₄, crystal size 0.96 mm × 0.93 mm × 0.96 mm, Mₑ = 834.75, monoclinic, space group P2₁(#4), a = 12.1008(15) Å, b = 16.718(2) Å, c = 23.170(3) Å, β = 94.1111(16)°, V = 4673.1(10) Å³, Z = 4, D calc = 1.186 mg/m³, F(000) = 1540, R = 0.0835, wR = 0.2427 (16762 reflections), S = 1.490, Flack parameter = 0.892(17). (where \( R_1 = \sum w(|F_o| - |F_c|) / \sum w|F_o| \), \( R_2 = \sum w(|F_o|^2 - |F_c|^2) / \sum w|F_o|^2 \), w = 1/σ²(Fo)).

Crystallographic data for 4 (CCDC 1457180).

C₄₂H₃₄N₆NiO₄, crystal size 0.07 mm × 0.06 mm × 0.05 mm, Mₑ = 745.46, monoclinic, space group group P2₁(#4), a = 12.3257(18) Å, b = 12.2272(18) Å, c = 23.428(3) Å, β = 99.2308(2)°, V = 3483.9(9) Å³, Z = 4, D calc = 1.421 mg/m³, F(000) = 1552, R = 0.0384, wR = 0.1032 (10358 reflections), S = 0.765, Flack parameter = 0.054 (17). (where \( R_1 = \sum w(F_o) - \sum F_c) / \sum w F_o |, R_2 = \sum (w(F_o)^2 - w(F_c)^2) / \sum (w(F_o)^2)^{1/2}, w = 1/(σ²(F_o) + (0.1P)^2/0.3989P) \), P = \( F_o + 2F_c^2/3 \).

Crystallographic data for 5 (CCDC 1457182).

C₄₂H₃₆CuN₆O₇, crystal size 0.16 mm × 0.10 mm × 0.08 mm, Mₑ = 823.32, triclinic, space group P1 (#1), a = 11.4817(15) Å, b = 12.1012(17) Å, c = 15.358(2) Å, α = 93.619(2)°, β = 92.131(2)°, γ = 107.221(2)°, V = 2103.65(5) Å³, Z = 2, D calc = 1.347 mg/m³, F(000) = 852, R = 0.0580, wR = 0.1568 (7457 reflections), S = 1.067, Flack parameter = 0.054 (17). (where \( R_1 = \sum w(F_o) - \sum F_c) / \sum w F_o |, R_2 = \sum (w(F_o)^2 - w(F_c)^2) / \sum (w(F_o)^2)^{1/2}, w = 1/(σ²(F_o) + (0.1P)^2/0.3989P) \), P = \( F_o + 2F_c^2/3 \).

2.5 **Computational methods**

All calculations were performed using the Gaussian 09W software Revision D.01 (Gaussian, Inc.). The gas phase geometry optimizations were carried out using TD-DFT with
B3LYP functional. The vertical excitation energy was calculated with the Lanl2dz for Mn, Co, Ni, Cu, Zn and with the 6-31+G(d) basis set for H, C, N and O method based on the singlet ground state geometry [29].

3. Results and discussion

3.1. Crystal structures of 1, 4, and 5

The molecular structures of 1, 4, and 5 are depicted in Figs. 2-4, respectively. Complexes 1, 4, and 5 afford a six-coordinated octahedral [MnN₂O₂], four-coordinated square planar cis-[NiN₂O₂], and five-coordinated square pyramidal [CuN₂O₃] coordination geometries having 2, 0, and 1 water molecules as axial ligands. Selected bonds are written in each figure caption. In all crystals, azo-groups afford a trans configuration, and λ-configuration in diamine ligands. Most of them are within normal values for the analogous Schiff base metal complexes [30-32]. The molecular packing in solid state is essentially formed by weak van der Waals forces predominantly.

3.2. Docking of 1-5 into laccase

Firstly, in order to estimate solution structures of complexes, experimental (acetone solutions) and simulated (based on TD-DFT optimized structures) UV-vis and CD spectra of 1-5 were compared as shown in Fig. 5. Qualitatively, experimental spectra were reasonably reproduced and assigned by simulated ones generated according to the literature procedures [16]. For example, intense π−π* peaks of 1-5 appeared at 430, 431, 413, 434, and 407 nm, respectively, in actual solutions, while the corresponding simulated peaks of 1-5 appeared at 430, 431, 413, 434, and 407 nm, respectively. The simulated CD spectra of the complexes by TD-DFT support that a certain supramolecular arrangement was induced in chiral laccase matrix by irradiation of linearly polarized UV light. Predominant absorption band of electronic transition (419 nm, HOMO-1(189) to LUMO+3(191)) will be also associated increase in current density of 1 observed experimentally (see later section).

Secondly, difference of CD spectra of solutions indicated keeping secondary structure (exhibiting random coil and α-helix band at 195-220 nm generally [33]) of laccase after docking a complex 5 (without α-helix band) as to be 5+laccase regardless of CD intensity emerged by chiral 5. Moreover, gradual change of UV-vis spectra with increasing amount of 5 added was also exhibited characteristic band shift around 400 nm (Fig. 6). Both spectral features suggested that a hybrid material (5+laccase) is quantitatively formed by intermolecular interaction between laccase and 5 at a certain surface region of laccase molecules without structural changes of peptide chains.

Thirdly, computational docking simulation using GOLD suite [34] with PDB 1GYC structure of this laccase [3] was performed (Fig. 7). Judging from recent standard [35], the best fitting score was obtained by assuming including a complex (5) in a pocket region around T1 site binding by hydrogen...
Fig. 3. Crystal structures of 4 showing selected atom labeling scheme. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) are Ni1-N3=1.837(4), Ni1-O4=1.837(3), Ni1-N6=1.857(3), Ni1-O2=1.860(3), Ni2-O6=1.842(3), Ni2-O8=1.843(3), Ni2-N9=1.850(3), Ni2-N12=1.862(4).

Fig. 4. Crystal structures of 5 showing selected atom labeling scheme. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) are Cu1-O2=1.909(6), Cu1-O4=1.920(6), Cu1-N3=1.923(7), Cu1-N6=1.939(7), Cu1-O9=2.387(6), Cu2-N9=1.931(7), Cu2-O8 1.934(6), Cu2-O6=1.940(6), Cu2-N12=1.985(7), Cu2-O10=2.443(6).
bonds or aromatic π-π interaction. Some nearest intermolecular distances are C1(Phe285 of laccase) – O1(5) = 0.2818 nm, C2(Phe285) – H1(5) = 0.2381 nm, C3(Phe285) – C4(5) = 0.2796 nm, H2(Phe285) – C5(5) = 0.2286 nm, H3(Gly392) – H4(5) = 0.1948 nm, H3(Gly382) – C8(5) = 0.2932 nm, H5(Gly392) – H6(5) = 0.1718 nm.

**Fig. 5.** Experimental (acetone solutions; black) and simulated (TD-DFT; red) UV-vis and CD spectra based on TD-DFT optimized structures of 1-5 with electronic distribution
3.3. Polarized UV-vis and IR spectra after linearly polarized UV light irradiation

Fig. 8 depicts polarized UV-vis absorption spectra and the corresponding circular diagrams of angular dependence of absorbance of $\pi-\pi^*$, $n-\pi^*$, and d-d bands at 270, 335, and 685 nm, respectively, for $1+l$acase (as a cast film) after linearly polarized UV light ($<350$ nm) irradiation up to 10 min. Besides drastic change of $\pi-\pi^*$ and $n-\pi^*$ bands due to photoisomerization of azobenzene moiety from trans to cis, the optical anisotropy of (cis)-azobenzene compound increased because of Weigert effect (molecular reorientation with dipole moment of (cis)-azobenzene to be perpendicular to the electric vector of linearly polarized UV light) [14], which was also observed in other bis-bidentate Schiff base metal complexes having azobenzene moiety even in laccase matrix (forming hybrid material by regularly docking) [23]. Although UV-vis spectra indicated overlapped information of a complex and laccase especially in UV region, polarized IR spectra (reading C=N and N=N bands) can observe separated information. For all systems, parameters derived from UV-vis and IR spectra reasonably indicated increasing such anisotropy (Tables S1-S4).
Table S1. The R values from polarized UV-vis spectra of for 1 through 5+laccase after polarized UV light irradiation.

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<th>UV irradiation (min)</th>
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Table S2. The S values from polarized UV-vis spectra of for 1 through 5+laccase after polarized UV light irradiation.

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Table S3. The R values from polarized IR spectra of for 1 through 5+laccase after polarized UV light irradiation.

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3.4. Electrochemical behavior and photo-induced molecular orientation of a complex

In order to investigate novel performance of 1-5 as mediator to laccase on RRDE [10] as the final goal (Fig. 9), firstly redox behavior of 1-5 before and after UV light irradiation was confirmed (Fig. 10). As known well, cis-trans photoisomerization of azobenzene moiety of ligand results in slight changes of both redox potentials and current densities for all 1-5. Therefore, the cis form and its redox potential should be assumed for species after UV light irradiation. Secondly, comparison between laccase and hybrid materials of a complex and laccase was investigated (Fig. 11). Even keeping trans form without UV light condition, current density predominantly increased for 1-4, while complicated behavior could be observed due to redox reaction by ligand for 5. Therefore, complexes (1-4, especially 1) could act as a good mediator for laccase by formation of hybrid materials. Thirdly, differences of CV before and after polarized UV light irradiation, which was expected to
lead to not only cis-trans photoisomerization but also anisotropic molecular orientation, were compared for hybrid materials 1 through 5 + laccase (Fig. 9). Based on examination above, shift of redox potential mainly results from cis-trans photoisomerization [36], while increasing current density mainly can be regarded as improving performance as a mediator. Although 2+laccase, 3+laccase, 5+laccase indicated only slight difference, 1+laccase and 4+laccase indicated drastic changes of CV features. As for 4+laccase, a new peak emerged at about -0.5 V, which can be attributed to additional redox by ligand moiety except for original metal ion moiety. On the other hand, as for 1+laccase, increasing current density without shift of redox potentials could be observed, which can be ascribed to improve performance as a mediator to laccase. Its reason is molecular orientation of cis form 1 in the pocket of laccase molecular surface. As elucidated by docking simulation, it is fine tuning of molecular orientation of 1 to form suitable hydrogen bonding pathway between 1 and laccase to conduct electron [37, 38].

Fig. 9. CV (scan rate 0.05 Vs-1) measured using RRDE electrodes for Nafion coated hybrid materials 1 through 5+laccase after polarized UV light for 0 (black) and 10 (red) min.

Fig. 10. CV (scan rate 0.05 Vs-1) measured using RRDE electrodes for Nafion coated a complex 1-5 after UV light irradiation for 0 (black) and 10 (red) min to confirm effects from trans to cis photoisomerization of a complex.
4. Conclusions

In summary, we prepared five chiral Schiff base metal complexes whose ligand incorporate azobenzene moiety, which can exhibit Weigert effect (anisotropic molecular orientations) by polarized UV light irradiation. These complexes can bind to laccase in the pocket on the surface near T1 site. By polarized UV light irradiation to 1+ laccase, it indicated novel increasing current density, which is attributed to molecular orientation tuning in laccase. In order for application cathode material for biofuel cell, this photo-tuneable mediator will be a new promising molecular design strategy. Further study on this method and the analogous materials are in progress now.

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References


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