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Bovine serum albumin-cobalt(II) Schiff base complex hybrid: an efficient artificial metalloenzyme for enantioselective sulfoxidation using hydrogen peroxide

Jie Tang,[ac] Fuping Huang,[a] Yi Wei,[a] Hedong Bian,*[ab] Wei Zhang[a] and Hong Liang*[a]

An artificial metalloenzyme (BSA-CoL) based on the incorporation of cobalt(II) Schiff base complex {CoL, H_2L = 2,2'-[(1,2-ethanediyl)bis(nitrilopropylidyne)]bisphenol} with bovine serum albumin (BSA) has been synthesized and characterized. Attention is focused on the catalytic activity of this artificial metalloenzyme for enantioselective oxidation of a variety of sulfides with H_2O_2. The influences of the parameters such as pH, temperature, concentration of catalyst and oxidant on the thioanisole as model are investigated. Under optimum conditions, BSA-CoL as hybrid biocatalyst is efficient for the enantioselective oxidation of a series of sulfides, producing corresponding sulfoxides in excellent conversion (up to 100%), chemoselectivity (up to 100%) and good enantiomeric purity (up to 87% *ee*) in certain cases.

Introduction

Optically active sulfoxides are useful building blocks as intermediates,[1] auxiliaries[2-3]

*[a]State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (School of Chemistry and Pharmacy, Guangxi Normal University), Guilin, 541004, P. R. China. Email: huangfp2010@163.com

*[b]School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, Key Laboratory of Chemistry and Engineering of Forest Products, Nanning, 530008, P. R. China. E-mail: gxunchem@163.com

[c]Guilin Normal College, Guilin 541001, P. R. China.

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and ligands[4] in modern organic synthesis.[5] They are also constituted important biologically active compounds[6] in the pharmaceutical industry.[7-9] During the past few decades, intense effort has been devoted to the development of various synthetic methods toward enantioenriched sulfoxides.[10] Among the available approaches developed, it is widely appreciated that the asymmetric oxidation of corresponding prochiral sulfide is the most powerful and reliable route.[3,10]

Many methods including different catalysts (natural enzymes,[11-13] organocatalysts[14] and metal complexes[15]) have found most applications in the asymmetric oxidation of prochiral sulfides;[16] high level enantioselectivity, satisfactory conversion and yield have already been achieved.[17-18] However, each system has obvious limitations that include tedious separation process, relatively toxic reaction conditions or expensive chiral metal complexes.[10,19] Artificial metalloenzyme which is a promising new kind of eco-compatible hybrid biocatalysts, represents an attractive alternative for enantioselective catalysis.[20-21] They are not only able, like enzymes, to work under mild conditions with high activity,[22-24] but can also be stably used in a wide range of substrates like homogeneous catalysts.[25] For example, Viktor Mojr and co-workers[26] reported that smaller α-cyclodextrin conjugate was a suitable catalyst for the oxidation of *n*-alkyl methyl sulfides while the larger β-cyclodextrin conjugates were optimal catalysts for the oxidation of sulfides carrying bulkier substituents.

Artificial metalloenzymes are resulting from the introduction of a metal-complex with catalytic activity into macromolecular hosts[27-30] (such as a protein, DNA or an antibody) with covalent[31-33] or coordination bonds,[7,20] as well as using supramolecular anchoring.[34-36] Therefore, various proteins (*e.g.*, myoglobin, serum protein, avidin or streptavidin) providing a chiral cavity[21] as effective chiral hosts widely used for synthesis of artificial metalloenzymes.[34] Yoshihito Watanabe *et al.*[37-40] employed apo-Mb and metal (Schiff base) complexes in preparing hybrid metalloproteins and those were able to catalyze enantioselective sulfoxidation. Kyohei Watanabe and co-workers[41] reported that the recombinant human serum albumin (HSA) mutant-heme complex showed high peroxidase activity. In Valentina Oliveri's study,[42] a novel artificial superoxide dismutase enzyme based on the non-covalent

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conjugation of bovine serum albumin (BSA) and the manganese(III) complex was investigated.

In the context of supramolecular anchoring of metal cofactors within a host protein, the serum albumin play a key role.[43] Among them, BSA is attractive because of its abundance, stability, low cost, ease of purification, excellent water solubility and different substrate accessibility.[38,44] However, the metalloenzymes using BSA as protein scaffold generally display low activity in asymmetric sulfide oxidation. Atif Mahammed *et al*.[35] have reported that the BSA-conjugated manganese complex as the catalyst for the enantioselective oxidation of prochiral sulfides obtaining good enantioselectivity (up to 74% *ee*) but very low yield (16%). The incorporation of a $VOSO_4$ complex into BSA by Anca Pordea and co-workers[21] afforded a racemic mixture in the asymmetric oxidation of thioanisole.

Schiff base complexes as cofactor for artificial metalloenzyme play an important role in asymmetric catalysis due to their catalytic active properties[10] and easy methods of syntheses.[45] In contrast to the remarkable success obtained of manganese[46-48], iron[49-50] vanadium[22,51] metal complexes, few studies have demonstrated the catalytic role of cobalt complexes in enantioselective sulfoxidation.

Previously, various strong oxidants were used for the oxidation of sulfides.[52-55] However, these oxidants not only corroded the equipment but also generated large amounts of wastes during the reaction process.[56] As an alternative, hydrogen peroxide (H_2O_2) is one of the most noteworthy "green oxidants" offering the advantages of being abundant,[8] cheap,[21] relatively safe,[50] atom-economical[11] and environmentally benign with the formation of water as the only by-product.[57]

Considering these remaining challenges and endeavoring to guide principles of green chemistry, in this paper, we designed a BSA-cobalt(II) Schiff base complex hybrid as biocatalyst for asymmetric sulfoxidation. The influence of parameters through optimizing reaction conditions was studied using thioanisole as a model substrate in the presence of "green oxidant" H_2O_2. Comparing with related biomimetic systems,[22-24,26] this artificial metalloenzyme provided efficient catalytic activity for a broad panel of prochiral sulfides. In addition, it avoided a common

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problem which was over-oxidation of the sulfoxides to sulfones.[58] Especially for thioanisole, over-oxidation to sulfone was either entirely absent or very minimal in most cases.

Experimental section

Materials

All starting chemicals were commercially available reagents of analytical grade and were used without further purification. BSA was purchased from Sigma-Aldrich. All the sulfides were obtained from Energy Chemical. The preparation of 2,2'-[(1,2-ethanediyl)bis(nitrilopropylidyne)]bisphenol (H_2L) was described in previous publication.[59] Cobalt(II) Schiff base complex (CoL) and BSA were dissolved in dimethyl sulfoxide (DMSO) and 0.05 M Tris (trihydroxymethyl aminomethane) buffer solution, pH 8.0, respectively, to prepare as standard stock solution (concentration: 1 mM).

Physical measurements methods

UV-Vis spectra were recorded on a Cary 100 UV-visible spectrophotometer. The FT-IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrophotometer with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter ratio. ESI-MS (electrospray ionization mass spectrum) spectra were recorded on a Bruker HCT Electrospray Ionization Mass Spectrometer. HPLC experiments were carried out using UV2302II/P2302II High performance liquid chromatograph. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker NMR. Optical rotations were measured with a WXG-4 polarimeter.

Synthesis of CoL

A mixture of $CoCl_2 \cdot 6H_2O$ (0.5 mmol), H_2L (0.5 mmol) and ethanol (15 ml) were placed in a 15 ml Teflon-lined stainless steel vessel, heated at 80 °C for 72 h and then cooled to room temperature at a rate of 5 °C per hour. The resulting crystalline product was washed with ethanol and dried in air. Color: reddish brown. Yield: 60% (based on Co(II)). *Anal*. Calc. for $C_{40}H_{44}Cl_2Co_3N_4O_4$: C, 53.83; H, 4.97; N, 6.28.

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Found: C, 53.63; H, 4.92; N, 6.15%. EIS-MS *(m/z)*: $[C_{20}H_{22}CoN_2O_2]^+$, 381.10. FT-IR (KBr phase, cm^{-1}): 3438s, 2977w, 1636s, 1540m, 1492w, 1441m, 1384m, 1329m, 1257w, 1218w, 1140w, 1101w, 1044w, 848m, 746m, 689w, 623w, 585w, 538m.

Crystal structure determination of CoL

X-ray structure analysis was performed at 293 K using Agilent Supernova diffractometer (Mo, λ = 0.71073 Å). The structure was collected at 293(2) K and solved by direct methods using SHELxs-97[60] and refined by full-matrix least-squares techniques against F^2 with SHELxs-97.[61] Anisotropic thermal parameters were assigned to all non-hydrogen atoms. The organic hydrogen atoms were generated geometrically, the hydrogen atoms of the water molecules were located from difference maps and refined with isotropic temperature factors. Analytical expressions of neutral-atom scattering factors were employed, and anomalous dispersion corrections were incorporated. The crystallographic data and structural refinement details were summarized in Table 1.

Table 1 Crystal data and structure refinement for CoL

Formula	$C_{20}H_{22}ClCo_{1.5}N_2O_2$	**γ (°)**	68.160 (4)
Formula weight	446.24	**V (Å^3)**	953.2 (4)
Crystal system	Triclinic	**Z**	2
Space group	*P-1*	**D_c (g m^{-3})**	1.555
a (Å)	8.327 (2)	**Goodness-of-fit on F^2**	1.087
b (Å)	9.764 (3)	**θ range for data Collection (°)**	2.3 to 26.4
c (Å)	13.148 (4)	**Reflections collected/unique**	10974/ 3881 [R(int) = 0.0246]
α (°)	75.049 (4)	**Final R indices [$I > 2\sigma(I)$]**	R_1 = 0.0277 ωR_2 = 0.0821
β (°)	78.964 (4)	**R indices (all data)**	R_1 = 0.0360 ωR_2 = 0.863

Synthesis of BSA-CoL

The design of artificial enzyme was performed by mixing BSA standard stock solution (1 mM) with the CoL standard stock solution (1 mM) at equal concentrations

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and incubated overnight at 0 °C. The hybrid solution was dialyzed (3 cycles) in an ice bath, leading to the separation of the protein-complex hybrid from the non-hybridised complex solution. Then, the hybrid were purified by sephadex G75 gel chromatography. The protein concentration was estimated by Coomassie Brilliant Blue method[62] after concentration.

Titration of CoL by a solution of BSA

Increasing amounts (from 0 to 1.5 molar equivalents, final concentration in the 0-6.75 μM range) of a 1 mM solution of BSA (BSA was first solubilized in 0.05 M phosphate buffered saline solution (PBS), pH 7.45) were added to a 4.5 μM solution of CoL in a 0.05 M PBS solution, pH 7.45. The absorbance of the solution A_s was recorded between 250 and 500 nm, 5 min after each addition. The same experiment was performed to measure the absorbance A_0 of the complex CoL alone. After correction of the dilution induced by the volume of cofactor added, the difference $\Delta A = A_0 - A_s$ at 362 nm was plotted as a function of the number of equivalents of BSA added. This allows to determine the stoichiometry of the complex CoL with BSA.[50]

Molecular docking of BSA-CoL

To understand further the right binding site and binding interaction forces of CoL on BSA, the molecular docking of CoL with BSA was carried out using docking software Autodock 4.2 (http://autodock.scripps.edu/, free software). The crystal structure of BSA (PDB ID: 3V03) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Firstly, all the water molecules were removed, and then hydrogen atoms were added to the protein structure.[63] Secondly, the partial atomic charges of BSA and CoL were calculated using Gasteiger-marsili[64] and Kollman methods,[65] respectively. Thirdly, the different conformers of sorafenib were generated by using Lamarkian genetic algorithm (LGA) with the same parameters of each docking. Finally, according to the Autodock scoring function, the dominating configuration of the BSA-CoL with minimum binding energy can be obtained.[66] After the molecular docking, the lowest Gibbs free energy of binding was

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illustrated by PyMol (http://www.pymol.org/).

Typical experimental procedure for enantioselective sulfoxidation reaction

A mixture of catalyst (2.7 μmol) and sulfide (0.27 mmol) in 2 ml of phosphate buffer (PB) solution (pH 5.1) was stirred in a test tube (5 ml capacity) for 5 hour at room temperature. Then the reaction mixture was cooled to 0 ℃ and 30% H_2O_2 (1.5 equiv.) as the oxidant was added under stirring. After another 20 hours, the solution was quenched with sodium sulfite solution and extracted with dichloromethane (2 ml ×5). Finally, the combined organic solutions were dried over by anhydrous sodium sulfate, filtered, and evaporated under vacuum. The same procedure was followed with the cofactor alone or without the cofactor. A sample of the crude reaction mixture re-dissolved in a minimum amount of isopropanol before being analyzed by HPLC with Chiralcel OB-H or Chiralpak AS-3 (4.6*Φ* mm × 250 mm) column (Daicel Chemical Industries, Tokyo) at room temperature. Further purification was achieved by chromatography on silica gel then was taken for the NMR analysis. The enantiomeric excess (*ee*) and chemoselectivity were calculated using the formulas: *ee* % = [peak area (S-R)/(S+R)] × 100%; chemoselectivity % = [peak area SO/(SO+SO_2)] × 100%, SO = sulfoxide, SO_2 = sulfone. Conversions were based on sulfide substrate; yields were referred to isolated product after column chromatography and were based on substrate; the absolute configurations of the sulfoxides were assigned by comparison of HPLC elution orders and sign of optical rotation reported in the literature.

Methyl phenyl sulfoxide. Yellow oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 3:1) (89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.59 (dd, *J* = 7.90, 1.4 Hz, 2H), 7.49-7.41 (m, 3H), 2.66 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 145.6, 133.7, 131.0, 129.3, 127.3, 123.4, 43.9. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (80:20), 0.8 ml/min, 254 nm, t_r (*S*) = 14.0 min, t_r (*R*) = 24.0 min, $[\alpha]_D^{20}$ = +84.3° (*c* 1.0, acetone), {ref. 67, $[\alpha]^{20}_D$ = +130° (*c* 1.7, acetone) for (*R*) 89% *ee*}.

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2-Chlorophenyl methyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (29%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.99 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.57 (td, *J* = 7.7, 1.2 Hz, 1H), 7.48 (td, *J* = 7.6, 1.6 Hz, 1H), 7.43 (dd, *J* = 7.9, 1.1 Hz, 1H), 2.86 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 143.6, 132.0, 129.8, 129.8, 128.2, 125.4, 41.7. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (90:10), 1 ml/min, 254 nm, t_r (*S*) = 11.3 min, t_r (*R*) = 22.0 min, $[\alpha]_D^{20}$ = +151.2° (*c* 1.0, THF), {ref. 67, $[\alpha]_D^{20}$ = +139.0° (*c* 1.0, THF) for (*R*) 80% *ee*}

3-Chlorophenyl methyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (40%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.69 (t, *J* = 1.1 Hz, 1H), 7.53 (qd, *J* = 3.8, 1.3 Hz, 1H), 7.50 (td, *J* = 4.3, 2.0 Hz, 2H), 2.77 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 147.9, 135.8, 131.2, 130.6, 123.7, 121.6, 44.0. Determination of *ee* by HPLC analysis: *n*-hexane/2-PrOH (90:10), 1 ml/min, 254 nm, t_r (*S*) = 15.0 min, t_r (*R*) = 21.5 min, $[\alpha]_D^{20}$ = +93.5° (*c* 1.0, acetone), {ref. 67, $[\alpha]^{20}_D$ = +128.8° (*c* 1.0, $CHCl_3$) for (*R*) 99% *ee*}.

3-Bromophenyl methyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.84 (t, *J* = 1.7 Hz, 1H), 7.67-7.64 (m, 1H), 7.58 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 2.77 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 148.0, 134.2, 130.9, 126,5, 123.6, 122.0, 44.0. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (80:20), 1 ml/min, 254 nm, t_r (*S*) = 10.7 min, t_r (*R*) = 16.1 min, $[\alpha]_D^{20}$ = +105.4° (*c* 1.0, acetone), {ref. 67, $[\alpha]_D^{20}$ = +116.3° (*c* 1.2, acetone) for (*R*) 97% *ee*}.

4-Chlorophenyl methyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.63-7.60 (m, 2H), 7.55-7.52 (m, 2H), 2.74 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 144.2, 137.3, 131.1, 129.7, 128.9, 125.0, 44.0. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (90:10), 1 ml/min, 254 nm, t_r (*S*) = 14.2 min, t_r (*R*) = 23.7 min, $[\alpha]_D^{20}$ = +76.6° (*c* 1.0, acetone), {ref. 67,

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$[\alpha]^{20}_D$ = +97° (*c* 2.0, acetone) for (*R*) 78% *ee*}.

4-Bromophenyl methyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.72-7.68 (m, 2H), 7.57-7.53 (m, 2H), 2.75 (s, 3H); ^{13}C NMR(126 MHz, $CDCl_3$) δ (ppm): 144.9, 132.7, 132.6, 129.0, 125.5, 125,2, 44.0. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (80:20), 0.8 ml/min, 254 nm, t_r (*S*) = 12.2 min, t_r (*R*) = 17.7 min, $[\alpha]_D^{20}$ = +80.0° (*c* 1.0, acetone), {ref. 67, $[\alpha]^{20}_D$ = +77° (*c* 1.8, acetone) for (*R*) 80% *ee*}.

4-Methylphenyl methyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.53-7.49 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 2.68 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 142.3, 141.5, 130.0, 129.8, 123.5, 123.3, 43.9, 21.4. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (50:50), 0.5 ml/min, 254 nm, t_r (*S*) = 9.7 min, t_r (*R*) = 16.5 min, $[\alpha]_D^{20}$ = +112.3° (*c* 1.0, acetone), {ref. 67, $[\alpha]^{20}_D$ = +150.4° (*c* 1.17, acetone) for (*R*) > 99% *ee*}.

4-Methoxyphenyl methyl sulfoxide. Yellow oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 2:3) (98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.61-7.56 (m, 2H), 7.04-7.00 (m, 2H), 3.84 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 162.0, 136.5, 129.5, 125.5, 114.9, 114.5, 55.5, 43.9. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (80:20), 1 ml/min, 254 nm, t_r (*S*) = 12.2 min, t_r (*R*) = 24.6 min, $[\alpha]^{20}_D$ = +122.2° (*c* 1.0, $CHCl_3$) {ref. 67, $[\alpha]^{20}_D$ = -113.8° (*c* 3.09, $CHCl_3$) for (*S*) 83% *ee*}.

Vinyl phenyl sulfoxide. Yellow oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 3:1) (23%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.57 (dt, *J* = 4.6, 2.7 Hz, 2H), 7.48-7.41 (m, 3H), 6.55 (m, J = 16.5, 9.6, 2.7 Hz, 1H), 6.17-5.81 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 143.3, 143.0, 132.1, 131.2, 129.4, 125.7, 124.6, 120.6. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (90:10), 1 ml/min, 254 nm, t_r (*S*) = 18.8 min, t_r (*R*) = 30.1 min, $[\alpha]^{20}_D$ = -115.1° (*c* 1.0, acetone), {ref. 67, $[\alpha]^{20}_D$ = -262.4° (*c* 1.7, acetone) for (*S*) 85% *ee*}.

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Methyl benzyl sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate/petroleum ether, *v/v* = 1:1) (87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.43-7.36 (m, 3H), 7.33-7.30 (m, 2H), 4.02 (dd, *J* = 69.3, 12.8 Hz, 2H), 2.48 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 130.5, 130.0, 129.7, 129.2, 129.0, 128.5, 60.4, 37.3. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (90:10), 1.5 ml/min, 210 nm, t_r (*S*) = 13.7 min, t_r (*R*) = 18.2 min, $[\alpha]^{20}_D$ = +21.7° (*c* 1.0, acetone) {ref. 67, $[\alpha]^{20}_D$ = -33.6° (*c* 3.0, acetone) for (*S*) 58% *ee*}.

Methyl *tert*-Butyl Sulfoxide. Colorless oil, purified by flash chromatography on silica gel (ethyl acetate) (95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): (400 MHz, $CDCl_3$) 2.39, 2.37, 2.35, 2.34, 2.33, 2.33, 2.33, 2.31, 1.33, 1.25, 1.23, 1.23, 1.21, 1.20, 1.20, 1.19, 1.19, 1.17; ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 52.2, 31.4, 22.2. Determination of *ee* by HPLC analysis: Chiralcel chiralpak AS-3, *n*-hexane/2-PrOH (90:10), 1.0 ml/min, 210 nm, t_r = 32.76 min, t_r = 40.36 min.

Methyl *n*-Octyl Sulfoxide. White solide, purified by flash chromatography on silica gel (ethyl acetate) (98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.49 (1 H, td, *J* 7.5, 1.1), 2.09 (1 H, d, *J* 1.3), 1.66-1.53 (1 H, m), 1.43-1.26 (5 H, m), 0.88 (2 H, dd, *J* 7.2, 6.3); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 53.6, 39.7, 37.4, 30.7, 28.1, 27.9, 27.8, 21.6, 13.1. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (98:2), 0.6 ml/min, 210 nm, t_r (*S*) = 26.4 min, t_r (*R*) = 29.5 min. $[\alpha]^{20}_D$ = -17.8° (*c* 1.0, acetone) {ref. 67, $[\alpha]^{25}_D$ = -71.3° (*c* 1, acetone) for (*R*) 85.1% *ee*}.

Methyl *n*-Dodecyl methyl Sulfoxide. White solid, purified by flash chromatography on silica gel (10% methanol in dichloromethane) (56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.57, 2.43, 2.42, 2.40, 2.23, 2.02, 1.54, 1.52, 1.23, 1.19, 0.90, 0.88, 0.88, 0.87, 0.83, 0.81, 0.79; ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 54.5, 40.7, 34.3, 31.9, 29.6, 29.5, 29.3, 29.2, 28.8, 24.9, 22.7, 15.4, 14.1. Determination of *ee* by HPLC analysis: Chiralcel OB-H, *n*-hexane/2-PrOH (98:2), 0.5 ml/min, 220 nm, t_r (*S*) = 22.2 min, t_r (*R*) = 24.4 min.

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Results and discussion

Description of the crystal structure

Complex CoL crystallized in the non-centrosymmetric space group *P*-1. Selected bond lengths and bond angles were given in Table S1. Single-crystal X-ray crystallographic analysis of CoL reveals a linear trinuclear Co(II) cluster (Fig. S1), in which Co1 atom is located on a crystallographic inversion center and is coordinated octahedrally by four O atoms from two Schiff base ligands (Co1-O1 = 2.1401(15) Å, Co1-O2 = 2.1268(15) Å). Co1 is bridged to Co2 through two μ_2-phenolato-O atoms from a Schiff base ligand and a μ_2-Cl atoms, with the Co1···Co2 distance of 2.8105(6) Å. Co2 is coordinated to two imino-N atoms as well as two μ_2-phenolato-O atoms from a Schiff base ligand and one μ_2-Cl atom. The average Co-N and Co-O bond lengths are 1.8795 and 1.8810 Å, respectively.

Spectroscopic characterization of the hybrid

The artificial metalloenzyme is dark brown, attesting to the presence of the Co complex in the protein.[68] Fig. S3 shows the UV-visible spectra of CoL, BSA and BSA-CoL hybrid at equal concentrations. The UV-visible spectra of the BSA-CoL hybrid is dominated by a single charge transfer (CT) band in the UV region. The spectra show that attachment of the CoL to BSA results a shift in λ_{max} from 279 to 274 nm accompanied by a substantial increase in its intensity. On the other hand, the spectra of the complex in the presence of BSA depict one band in the UV region with maxima at 366 instead of 362 nm, together with a slight increase in its intensity. In addition, subtraction of BSA-CoL and BSA spectra leads to a difference spectrum that is similar to the spectrum of CoL revealing the presence of interaction between the CoL complex and the BSA.[25,69]

Insertion of the cofactor CoL in the BSA

The UV spectrum of a 4.5 μM solution of CoL was recorded in 0.05 M PBS, pH 7.45, alone or in the presence of BSA. The presence of BSA allows only a slight 4 nm shift

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of the band to be observed, from 362 to 366 nm, together with a decrease in its intensity (Fig. S4). The slight shift of the band is in agreement with no change in the coordination sphere of the metal[42,70] Furthermore, it have been proven by several authors that the decrease in absorbance might probably be the sign that the cofactor inserts into a hydrophobic environment.[50]

In order to check the stoichiometry of the BSA-CoL, increasing amounts of BSA were added to a 4.5 μM solution of CoL in 0.05 M PBS, pH 7.45. The difference between the absorbance value at 362 nm of the solution of BSA-CoL (A_s) and that of CoL alone (A_0), $\triangle A_{362nm}$, was plotted against the BSA/CoL ratio (Fig. S4 (insert)). $\triangle A_{362nm}$ clearly increases linearly with the BSA/CoL ratio until 1.0 equivalent of BSA has been added to CoL, and then reaches a plateau. This indicates that the BSA is able to bind one CoL.[70] For a BSA/CoL ratio higher than 1.0, $\triangle A_{362nm}$ slightly decreases, which could be due to non-specific surface interaction of the CoL cofactor with BSA in excess in the solution.[50]

Molecular docking of BSA-CoL

Molecular simulation is beneficial to further understand the interaction of CoL with BSA. The globular protein BSA consists of three structurally similar domains and each domain is also divided into subdomains A and B.[71] The best ranked result, which has the lowest free energy for BSA-CoL is shown in Fig. 1. As shown in Fig. 1(a), CoL molecule is inserted into the hydrophobic cavity of subdomain IB (site I) of BSA. The detailed docking results are presented in Fig. 1(b). The model shows that CoL is surrounded by hydrophobic residues such as Phe-133, Leu-122, Leu-115, Ala-128 and Trp-134, polar residue Tyr-160, as well as charge hydrophobic residues Lys-136 and Glu-125. It is implied that CoL is bond to the site essentially via hydrophobic and Van Der Waals forces.[72] Similarly, P. Bourassa *et al.*[73] also reported anticancer tamoxifen, its metabolites 4-hydroxytamoxifen or endoxifen could be bound within a hydrophobic cavity in subdomain IB of BSA.

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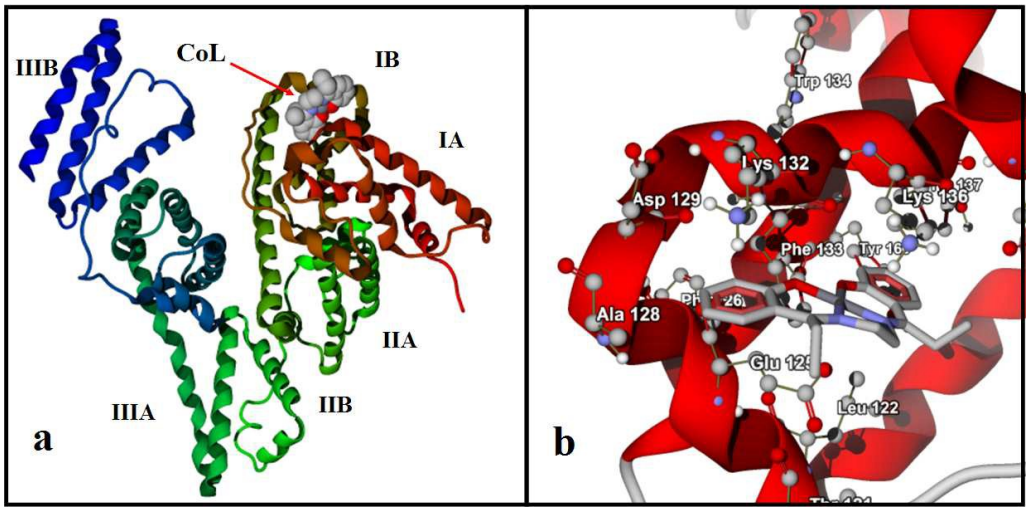

Fig. 1(a) The each subdomain of BSA and the minimum energy docking conformation between CoL with BSA. The BSA was presented by ribbon structure. CoL was presented by sphere model; **(b)** The hydrophobic and hydrophilic amino acid residues surrounding the CoL within 10,000 Å;

Asymmetric sulfoxidation

In an effort to fulfill the demands of "economical and environmental" benefits,[74] the first attempt to use H_2O_2 as oxidant in asymmetric oxidation of sulfides was carried out with methyl phenyl sulfide, one of the most frequently used model substrates for initial sulfoxidation experiments (Scheme 1). As shown in Fig. 1, blank reaction with thioanisole gave only 16% conversion with 52% chemoselectivity and the major product was racemic. When only HSA was present, the reaction afforded 95% conversion with 100% chemoselectivity and 14% *ee* of the sulfoxide, meanwhile 85% conversion with 100% chemoselectivity and 8% *ee* were obtained in the presence of BSA. This clearly demonstrates that the chiral environment provided by the protein has a determinant influence on the stereoselective step.[75] In contrast, the sulfoxide formed with CoL alone was found to be almost a racemic mixture with an enantiomeric excess of only 3% in favour of the *R* enantiomer due to the non-achiral nature of the catalyst.[19] Clearly no other case studied here could match the performance of the SA-CoL (BSA-CoL and HSA-CoL) in terms of enantioselectivity. Interestingly, although BSA is homologous to HSA,[76] BSA can catalyze the sulfoxidation combined with CoL affording higher enantiomeric excess (40% *ee* for the *R*-enantiomer). These suggest that the host protein has an influence on the enantioselection.[35,46-50] This enantiomeric excess can be compared to those obtained

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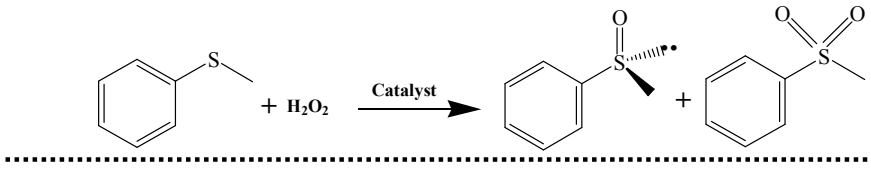
for this type of reaction in the presence of some other artificial metalloenzymes. For example, only 13% *ee* in favour of the *S* isomer was obtained in the oxidation of thioanisole using a rather bulky iron-porphyrin complex associated with a neocarzinostatin variant, NCS-3.24, as catalyst. The lower enantioselectivity may be due to the fact that the metal cofactor lay outside the binding site of the protein[70]. At the opposite, the insertion of Fe-corrole into BSA led to 38% *ee* in favour of the *R* isomer was reported by Atif Mahammed and Zeev Gross.[35] In our case, the molecular modelling experiments (Fig. 1(a) and Fig. 1(b)) show that the CoL molecule is embedded in the hydrophobic environment of BSA which provides the asymmetric environment around the catalyst. This could induce a enantioselectivity in the transfer of the oxygen atom to the sulfur atom. It seems reasonable, therefore, to afford 40% *ee* in favour of the *R* isomer. Additionally, the polar amino (Tyr-160) acid surrounding complex CoL (Fig. 1(b)) also enhances its reactivity and selectivity. [47]

Scheme 1 Enantioselectivity of the thioanisole oxidation using aqueous 30% H_2O_2 as oxidant at room temperature.



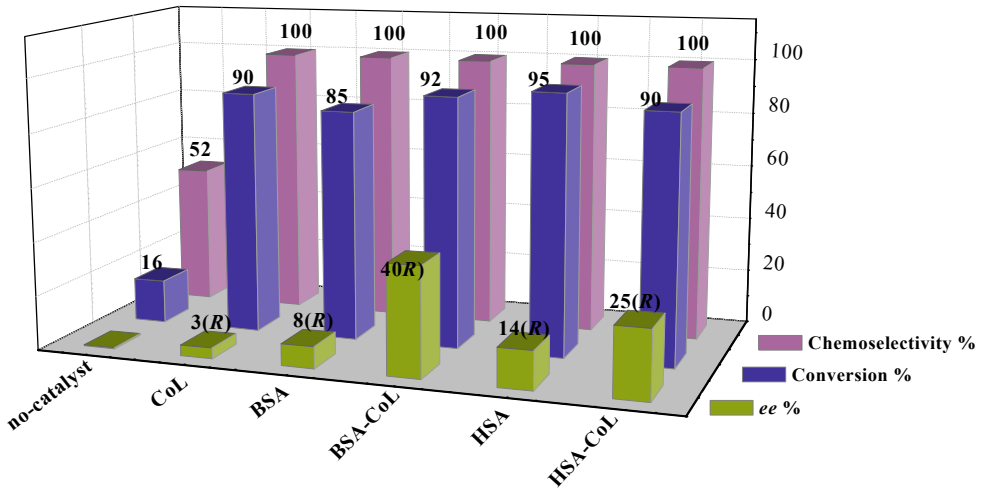


Fig. 2 Screening of catalysts for oxidation of thioanisole at room temperature for 20 h. The mol ratios of H_2O_2: thioanisole (0.27 mmol) were 150:100. The sulfoxide of thioanisole was in the *R* configuration.

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The effect of oxidants such as *tert*-butyl hydrogen peroxide (TBHP), sodium hypochlorite (NaClO) and sodium periodate ($NaIO_4$), were also tested along with 30% aqueous H_2O_2, and respective data were shown in Fig. 3. All these oxidants gave fairly good conversion (> 90%) and excellent product selectivity (100%). Remarkably, H_2O_2 effectively oxidized the sulfide affording the highest *ee* value (up to 40%). This indicates the exclusive activation of BSA-CoL by H_2O_2 in this reaction.

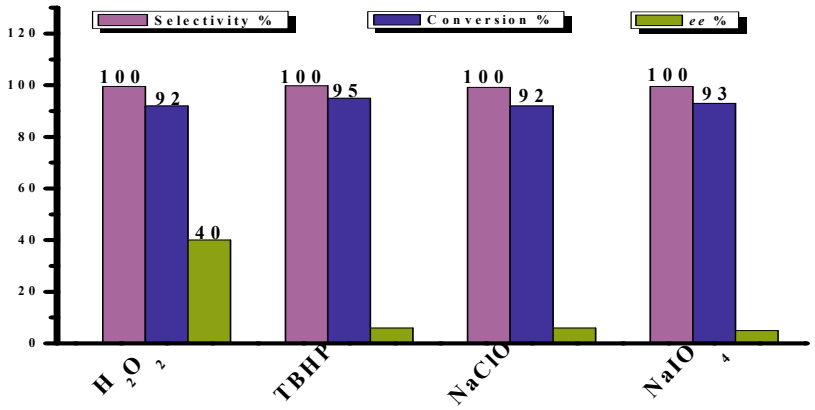

Fig. 3 Screening of oxidants for oxidation of thioanisole catalyzed by BSA-CoL in PB (pH 5.1) at room temperature after 20 h. The mol ratios of oxidant: thioanisole: BSA-CoL (2.7 μmol) were 150:100:1. The sulfoxide of thioanisole was in the *R* configuration.

Influence of different pH

Following the results above, the reaction parameters such as pH value, substrate concentration, oxidants or catalyst concentration, reaction temperature were investigated in an attempt to optimize the enantioselective sulfoxidation and, in particular, to improve the efficiency of the transformation. The experiments for pH-dependent oxidation were carried out in the presence of H_2O_2 at room temperature (Fig. 4). The chemoselectivities were observed quite similar in all cases (> 95 %). We found that a maximum yield of 92% was achieved at pH 5.1 and the yield decreased at pH 4.7 (87%). When the pH was over 5.1, the yield decreased drastically. Although the best enantioselectivity (47% *ee*) was found at pH 6.0, pH of 5.1 was adopted as the favorable pH in light of the optimum yield and chemoselectivity.

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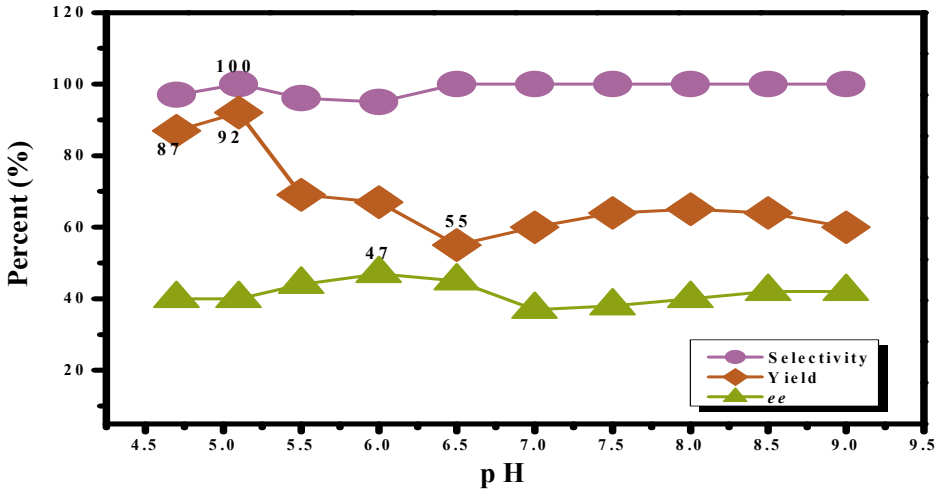

Fig. 4 Effect of different pH on the oxidation of thioanisole in PB at room temperature for 20 h. The mol ratios of H_2O_2: thioanisole: BSA-CoL (2.7 μmol) were 150:100:1. The sulfoxide of thioanisole was in the *R* configuration.

Influence of substrate mixing time

Due to the poor solubility of the substrate, the premixing time was considered in this study. The results, which were summarized in Table 2, showed that both conversion and yield increased steadily up to 5 h and attained a maximum value of 96% (entry 5) while no change in the chemoselectivity (100%) was observed (in the range of 1-7 h). A significant decrease in conversion (63%) was afforded when the mixing time was increased from 5 to 8 h, while the *ee*'s slightly increased to 48% (entry 8). Therefore, subsequent catalyst evaluation were conducted 5 h (taken as optimum) for thioanisole premixing time to optimize other reaction parameters.

Table 2 Effect of substrate mixing time on the oxidation of thioanisole[a]

Entry	Time (h)	Conversion (%)	Yield (%)	Chemoselectivity (%)	*ee* (%)
1	1	79	79	100	44
2	2	88	88	100	44
3	3	92	92	100	40
4	4	92	92	100	44
5	5	96	96	100	44
6	6	85	85	100	44
7	7	85	85	100	45
8	8	63	58	92	48

[a] Reactions were performed in PB (2 ml, pH 5.1) at room temperature for 20 h. The ratios of H_2O_2: thioanisole: BSA-CoL (2.7 μmol) were 150:100:1.

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Influence of substrate concentration

As shown in Table 3, 100% chemoselectivity in 96% yield and 44% *ee* was obtained by oxidation of 0.135 mM thioanisole (entry 3) with 1 mol% BSA-CoL. Moreover, 0.203-0.338 mM substrate concentrations (entries 4-6) were also investigated. Yield and enantioselectivity dropped to 78% and 34%, respectively, while the chemoselectivity (100%) was not affected.

Table 3 Effect of substrate concentration on the oxidation of thioanisole[a]

Entry	Concentration (mM)	Conversion (%)	Yield (%)	Chemoelectivity (%)	*ee* (%)
1	0.034	80	68	85	44
2	0.068	85	75	88	46
3	0.135	96	96	100	44
4	0.203	75	75	100	38
5	0.270	78	78	100	38
6	0.338	75	75	100	34

[a] Reactions were performed in PB (2 ml, pH 5.1) at room temperature for 20 h. The ratios of H_2O_2: thioanisole: BSA-CoL (2.7 μmol) were 150:100:1. The sulfoxide of thioanisole was in the *R* configuration.

Influence of oxidant and catalyst concentration

Using the conditions identified above, we next explored the effect of oxidant concentration on the catalytic sulfoxidation at 0.135 mM substrate concentration. Increasing the concentration of the oxidant from 0.135 to 0.203 mM, the oxidation of thioanisole gave a marginal increase in the conversion from 99 to 100% along with an increasing in the chemoselectivity from 88 to 96% (Fig. 5 (a)). However, the conversion dropped quickly (down to 38%) when H_2O_2 concentration beyond 0.203 mM. It also showed a steady decrease in the sulfoxide selectivity from 100 to 76%, whereas, the enantiomeric excess increased (from 44 to 63% *ee* for the *R*-enantiomer). The reason might be interpreted in terms of the oxidative destruction of the catalyst[77] and the existence of a kinetic resolution process due to excess oxidant.[78-79] Although increasing the amount of oxidant was desirable from a enantioselectivity standpoint, 0.203 mM was adopted as the optimum choice in light of the high conversion and chemoselectivity.

It is well known that the concentration of catalyst is an important factor in

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catalyzed oxidation.[80] Clearly, the maximum of the curve corresponded to the optimal catalyst concentration (Fig. 5(b)). In the absence of the catalyst, the oxidant reaction gave only 16% conversion and racemic product. Phenyl methyl sulfoxide was obtained when the catalyst concentration was 1.35 μM (1 mol% of substrate) with 96% conversion in 44% *ee*. Higher catalyst concentration didn't show positive effect on catalytic activity but with somewhat less conversion (80-85%) and *ee* value (38-41%). However, excellent chemoselectivities were obtained in all the cases except blank reaction.

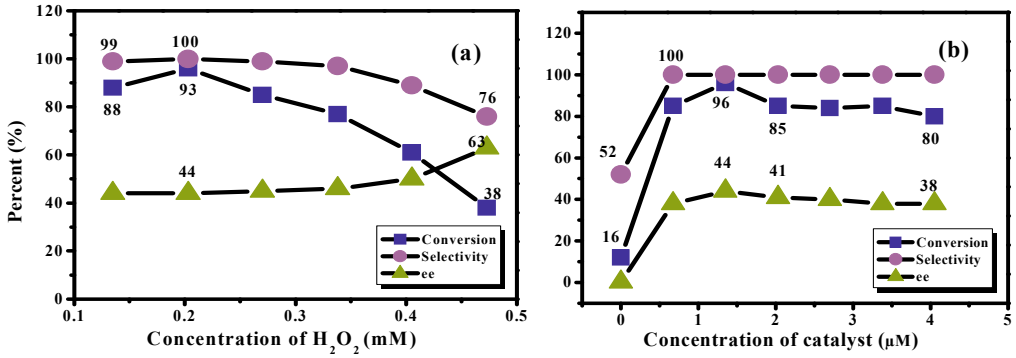

Fig. 5 Effect of the concentration of **(a)** oxidant (H_2O_2) and **(b)** catalyst (BSA-CoL) on the oxidation of thioanisole in PB (2 ml, pH 5.1) at room temperature for 20 h. The sulfoxide of thioanisole was in the *R* configuration.

Influence of other factors

Low solubility of the sulfides in pure water could decrease the rate of the catalyzed oxidation which is the reason for low enantioselectivity.[81] Solubilization of the substrate requires a small amount of organic solvents in some reactions.[82-83] So the effect of different organic co-solvents was further investigated. Disappointingly, from the results shown in Table 4, the use of methanol in concentration of 5% (*v/v*) significantly decreased the conversion (entry 2), analogous to the results previously found by Sergei V. Dzyuba and co-workers.[84] As a possible explanation, organic solvent could results protein destruction although a higher solubility of substrate could be obtained. Similar results also occurred when EtOH, acetone and THF as co-solvents (entries 3-5). Comparing with the polar co-solvents (MeOH, EtOH and acetone), the weak polar co-solvent (THF) afforded the lowest conversion, yield and

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ee value (entry 5). In the absence of co-solvent, the effect of reaction temperature was investigated. The catalyst showed maximum activity at 25 °C (entry 1). A temperature decreased to 0 °C resulted in a lower yield while the *ee* was not significantly affected (entry 6). Meanwhile, a decrease both in yield (92%) and stereoselectivity (34% *ee*) was observed when the temperature was increased to 50 °C (entry 7). It seems likely, the higher temperature make denaturation of the artificial metalloenzyme[85] or temperature effect on activation energies for different enantiomers. Comparing to 20 h at 0 °C, a higher *ee* (up to 50%) together with a higher yield (up to 98%) were achieved by prolonging the reaction time (28 h) (entry 8). Decreasing the H_2O_2 dismutation would provide a very efficient oxygen transfer to sulfide[86] because H_2O_2 dismutation could result catalyst destruction *via* hydroxyl radical generation.[4] Thus we also screened the adding H_2O_2 speed at 0 °C. Nevertheless, similar levels of activity and enantiomeric purity (entries 9-11) were observed confirming no H_2O_2 dismutation in this sulfoxidation system or the catalyst has a good stability.

Table 4 Effect of the co-solvent, temperature and adding oxidant rate on the oxidation of thioanisole[a]

Entry	Temperature (°C)	Co-solvents	Conversion (%)	Yield (%)	Chemoselectivity (%)	*ee* (%)
1	25	None	96	96	100	44
2	25	MeOH	82	82	100	42
3	25	EtOH	81	81	100	41
4	25	Acetone	75	75	100	35
5	25	THF	55	55	100	34
6	0	None	85	85	100	44
7	50	None	92	92	100	34
8[b]	0	None	98	98	100	50
9[b,c]	0	None	87	87	100	50
10[b,d]	0	None	92	92	100	49
11[b,e]	0	None	93	93	100	50

[a] Reactions were performed in phosphate buffer (2 ml, pH 5.1) for 20 h. The ratios of H_2O_2: thioanisole: BSA-CoL (2.7 μmol) were 150:100:1, aqueous 30% H_2O_2 was added all at once. [b] Reaction was carried at 0 °C for 28 h. [c] H_2O_2 was added in two portions (20.5 μl per hour). [d] H_2O_2 was added in three portions (13.7 μl per hour). [e] H_2O_2 was added in four portions (10.25 μl per hour). The sulfoxide of thioanisole was in the *R* configuration.

To monitor the reaction process and investigate the existence of a kinetic resolution

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process during the course of the reaction, the time course curve of oxidation thioanisole under the above optimized reaction condition was observed over a period of hours. The data as given in Fig. 6(a) indicated that the chemoselectivity and conversion increased steadily at 0 °C and attained a maximum value of 88% and 100% at 20 and 28 h, respectively, while enantioselectivity (46-49% *ee*) remained nearly constant throughout the course of the reaction. Similar results were presented in Fig. 6(b). The reaction was finished after 6 h at 25 °C affording a maximum value of conversion (96%), whereas chemoselectivity (99-100%) and enantioselectivity (43-45% *ee*) remained steady. This reaction profile, particularly with no change in chemoselectivity, indicates that the oxidative kinetic resolution of the product is either not occurring or negligible.[87]

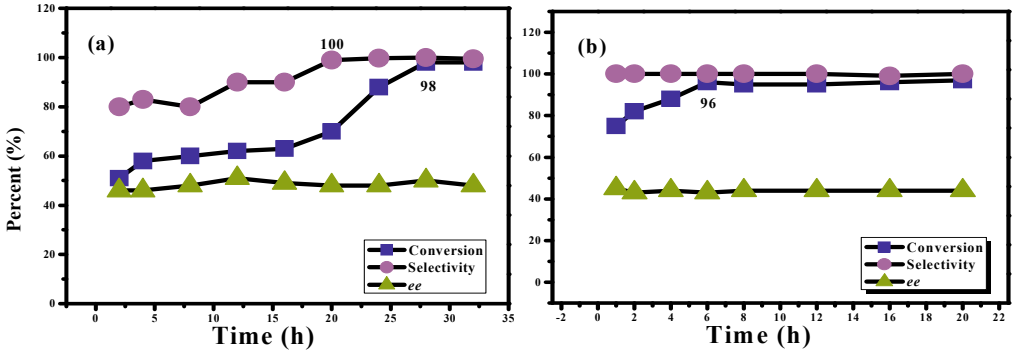

Fig. 6 The time profile diagram of oxidation of thioanisole at 0 °C (a) and 25 °C (b). Reactions were performed in PB (2 ml, pH 5.1). The ratios of H_2O_2: thioanisole: BSA-CoL (2.7 μmol) were 150:100:1. The sulfoxide of thioanisole was in the *R* configuration.

Oxidation of different sulfides

Having identified the optimized conditions, the scope of the asymmetric sulfoxidation was investigated (Scheme 2). As shown in Table 5, all the products of aryl alkyl sulfides obtained were of *R* configuration except for vinyl phenyl sulfoxide (entry 9). The results indicated that the sulfides containing electron-withdrawing groups on the phenyl of the methyl phenyl sulfide (entries 2-6) had a stronger influence on the reactivity and enantioselectivity of the oxidation than those containing electron-donating groups (entries 7-8). For example, the introduction of electron-donating Me (entry 7) and OMe (entry 8) groups at the *para* position resulted

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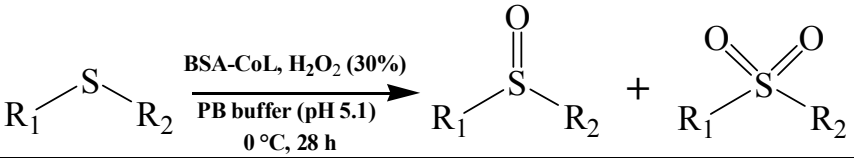
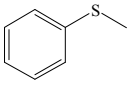
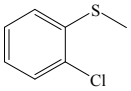
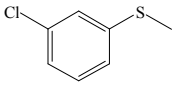
in similar activity and *ee*'s to unsubstituted methyl phenyl sulfide. In contrast, the introduction of electron-withdrawing Cl and Br groups at the *para* position (entries 5-6), *meta* position (entries 3-4) or *ortho* position (entry 2) of aromatic ring of the sulfide gave (*R*)-sulfoxides with higher enantioselectivity (ranging from 66 to 87%) and much lower conversion (ranging from 28-67%) than those observed for thioanisole. It was interesting to note that increasing the steric bulk of the aromatic moiety of the sulfide led to an increase in enantioselectivity: 87% and 85% *ee* for the sulfoxidation of *o*-chlorophenyl methyl sulfoxide and *m*-bromophenyl methyl sulfoxide, respectively (entries 2, 4), in contrast with the results previously found by Maria Annunziata M. Capozzi and co-workers.[88] A steric constraint also was detected in the reaction of sterically bulky vinyl phenyl sulfide and benzyl phenyl sulfide. They both caused a drastic reduction in stereoselectivity (entries 9-10) and afforded the opposite enantiomers with only 35 and 10% *ee*, respectively. It was noteworthy that good yields and excellent chemoselectivitives could be achieved even fort the sulfides with branched or longer alkyl groups instead of aryl (entries 11-13), but disappointing enantiomeric excesses were obtained. Oxidation of methyl *n*-octyl sulfoxide produced the corresponding sulfoxide with only 19% *ee* (*R*) while both methyl *tert*-butyl sulfoxide and methyl *n*-dodecyl methyl sulfoxide afforded racemic mixture.

Table 5 Enantioselective oxidation of various prochiral sulfides using BSA-CoL[a]



Entry	Substrate	Conversion (%)	Yield (%)	Chemoselectivity (%)	*ee* (%) (configuration)[b]
1		98	98	100	50 (R)
2		28	19	68	87 (R)
3		50	40	80	71 (R)

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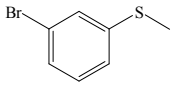
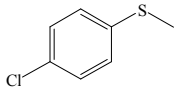
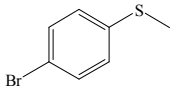
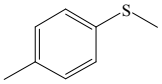
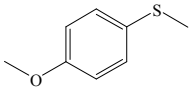
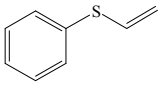
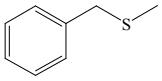
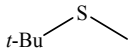
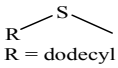
4		**65**	**46**	**71**	**85 (R)**
5		**67**	**63**	**94**	**67 (R)**
6		**56**	**44**	**79**	**66 (R)**
7		**93**	**91**	**98**	**62 (R)**
8		**100**	**98**	**98**	**69 (R)**
9		**44**	**23**	**51**	**35 (S)**
10		**98**	**87**	**89**	**10 (R)**
11		**95**	**95**	**100**	**–**
12		**99**	**98**	**99**	**19 (R)**
13		**57**	**56**	**98**	**–**

[a] Reactions were performed in phosphate buffer (2 ml) at 0 °C for 28 h; The ratios of H_2O_2: substrate: BSA-CoL (2.7 μmol) were 150:100:1. Aqueous 30% H_2O_2 was added all at once. [b] Assigned by HPLC elution order with known literature data (see SI for details).

Conclusions

In summary, we have achieved efficient enantioselective sulfoxidation with wide scope of application in water by using a BSA-CoL metalloenzyme/H_2O_2 system. This procedure offers several major advantages: (1) apply man-made catalyst under biological catalytic mild conditions which carrying out in aqueous solution without any additive; (2) the use of the most accessible and cheapest oxidant (H_2O_2) and protein (BSA); (3) highly efficient for the selective oxidation of structurally diverse sulfides in good to high activity and enantiomeric excess; and (4) the method conform to several of the guiding principles of green chemistry. A further study of the reaction mechanism and scope of this reaction is underway.

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