

Size-Selective Biocatalysis of Myoglobin Immobilized into a Mesoporous Metal–Organic Framework with Hierarchical Pore Sizes

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S Supporting Information

ABSTRACT: The protein myoglobin has been successfully immobilized into a mesoporous metal–organic framework with hierarchical pore sizes, which demonstrates interesting size-selective biocatalysis as well as superior catalytic activities toward small substrate oxidation compared to its mesoporous silica material counterpart.

Biocatalysis has long been of great interest in both academia and industry.¹ However, the successful utilization of proteins as biocatalysts in chemical, pharmaceutical, and food industries largely relies on the ability to successfully stabilize them in what is often an unnatural environment while retaining their functions and activities.² Immobilization of the biocatalysts on solid supports presents the advantages of enhanced stability as well as ease of separation and facile recovery for reuse.³ In addition, if a solid support possesses a hierarchy of pore sizes, with large pores for protein ingress and small pores for diffusion of reactants and products, the size- or shape-selective catalysis, which usually cannot be exhibited by native proteins in homogeneous systems,³ could be expected. Over the past 2 decades, extensive efforts have been dedicated to the search for various types of host matrix materials, and current attention has been focused on mesoporous silica materials owing to their high surface areas as well as tunable and uniform pores.^{2,3} Nevertheless, because of the lack of specific interactions with protein molecules, mesoporous silica materials suffer from leaching during the reaction process, which in return results in a loss of activity upon reuse; additionally, no size- and shape-selective catalysis has ever been observed presumably because of the difficulty in achieving hierarchical pores for mesoporous silica materials.^{2,3} These have been recognized as some major hurdles limiting their applications in biocatalysis, thus prompting the search for new types of host matrix materials.²

One of the most promising host matrix candidates is the mesoporous metal–organic framework (MOF) material, which has been advanced in recent years.⁴ Compared with mesoporous silica materials, mesoporous MOFs possess higher surface areas and pore walls composed of functional organic groups, which could afford specific interactions with protein molecules, thus avoiding leaching. In addition, mesoporous MOFs can be tailored to possess hierarchical pores with mesopores to accommodate biomolecules and micropores to selectively allow diffusion of reactants and products, thus possibly resulting in size- or shape-selective catalysis.

Recently, we showed for the first time the successful immobilization of the enzyme MP-11 into a mesoporous MOF, which exhibited enzymatic catalysis superior to that of the mesoporous silica material.⁵ Although MP-11 is a “microenzyme” with relatively small molecular dimensions,⁶ this work paved the first step to developing mesoporous MOFs as a new type of host matrix material for biocatalysis application and also encouraged us to explore the possibility of immobilizing larger protein molecules into mesoporous MOFs.⁷ In this contribution, we report that the protein myoglobin (Mb) can indeed be immobilized into a mesoporous MOF in spite of its apparent larger molecular size than the pore sizes of the mesoporous MOF; the resulting Mb@mesoMOF not only exhibited biocatalysis superior to that of the mesoporous silica counterpart but also demonstrated interesting size-selective biocatalysis due to the hierarchical pore sizes of the mesoporous MOF.

Mb is a small oxygen-binding protein of muscle cells with molecular dimensions of about $2.1 \times 3.5 \times 4.4$ nm.⁸ It contains a single polypeptide chain of 153 amino acid residues and a heme prosthetic group in a hydrophobic pocket (Figure 1a).⁹

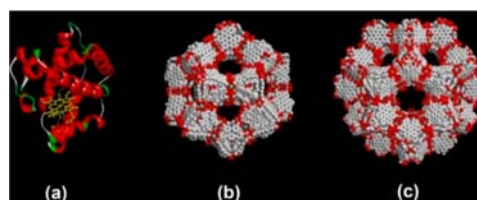


Figure 1. (a) Molecular structure of myoglobin (PDB 3LR7, ferric horse heart myoglobin). (b) 3.9-nm-diameter cage. (c) 4.7-nm-diameter cage in Tb-mesoMOF.

Biocatalysis of Mb is usually evaluated through its peroxidative activity attributed to the heme prosthetic group.¹⁰ Considering its framework stability in buffer solutions, we continued to employ the recently reported mesoporous MOF Tb-mesoMOF as the host matrix for the “proof of concept” studies on immobilizing Mb. Crystallographically, Tb-mesoMOF consists of nanoscopic cages of 3.9 and 4.7 nm diameter (Figure 1b,c)¹¹ and features type IV sorption behavior with hierarchical pore sizes of 0.9, 3.0, and 4.1 nm as revealed by N₂ gas sorption studies at 77 K (Figure S1a,b in the Supporting Information, SI). Because a Mb molecule possesses a highly dynamic and flexible structure, we expect that it could squeeze into Tb-

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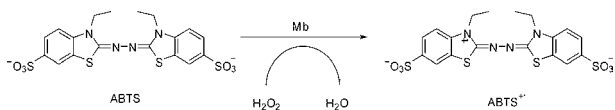
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mesoMOF through the mesopores via a specific orientation,⁷ and the 4.7-nm-diameter cages may serve as the ideal room to accommodate the Mb molecules.

To explore the possibility of immobilizing the Mb molecule, freshly prepared Tb-mesoMOF crystals were immersed in a Mb solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and placed in an incubator at 37 °C. The uptake of Mb by Tb-mesoMOF at different time points was determined by using the BCA method for protein determination,¹² and a saturated loading of 9.1 $\mu\text{mol g}^{-1}$ was reached after ~ 94 h. The Mb-saturated Tb-mesoMOF sample (hereafter denoted as Mb@Tb-mesoMOF) was then washed with a fresh buffer solution several times until the supernatant became colorless to fully remove loosely attached Mb molecules on the surface. The successful immobilization of large Mb molecules into Tb-mesoMOF was confirmed by optical image and UV-vis spectroscopy studies. The color of a Tb-mesoMOF crystal turns dark brown after being saturated with Mb (Figure S2a in the SI), which strongly indicates the ingress of Mb molecules into Tb-mesoMOF. Solid-state UV-vis absorption spectroscopy studies revealed that Mb@Tb-mesoMOF exhibited a Soret band at ~ 412 nm, which represents a slight bathochromic shift compared to the Soret band of ~ 410 nm for Mb in a buffer solution (Figure S2b in the SI), indicating interactions between the trapped Mb molecules and the framework of Tb-mesoMOF.¹³ N₂ sorption isotherms (Figure S1a in the SI) measured at 77 K indicated that the BET surface area of Tb-mesoMOF decreases from 1935 m² g⁻¹ (Langmuir surface 3247 m² g⁻¹) to 462 m² g⁻¹ (Langmuir surface 642 m² g⁻¹) after saturation with Mb. Pore-size distribution analysis revealed that the pore size of Mb@Tb-mesoMOF is predominately around 0.8 nm while the pore sizes of 4.1 and 3.0 nm observed in Tb-mesoMOF disappeared (Figure S1b in the SI). We deduced from these observations that Mb molecules block the two types of mesopores, while the remaining micropores of ~ 0.8 nm can provide a pathway for small substrates to access the active Mb centers housed in the 4.7-nm-diameter cages. The results from gas sorption studies also support the successful immobilization of Mb into Tb-mesoMOF.

Mb is known to perform peroxidation of organic substrates by the use of hydrogen peroxide,¹⁰ and its peroxidase activity is usually assessed with the assay of 2,2'-azinobis(3-ethylbenzthiazoline)-6-sulfonate (ABTS) as a redox indicator by monitoring the rate of increase in absorbance at 660 nm ($\epsilon = 12 \text{ mM}^{-1} \text{ cm}^{-1}$ for ABTS^{•+}) subsequent to the addition of peroxide (Scheme 1).¹⁴ Because mesoporous silica materials

Scheme 1. Reaction Scheme for Oxidation of ABTS by Hydrogen Peroxide Catalyzed by Mb



have been widely investigated for protein immobilization,^{2,3} we selected SBA-15¹⁵ for comparison. SBA-15, with the pore size monodistributed around 8.5 nm, adsorbs Mb (hereafter denoted as Mb@SBA-15) with a lower loading capacity of 7.0 $\mu\text{mol g}^{-1}$ presumably because of its lower surface area (BET surface area $\sim 900 \text{ m}^2 \text{ g}^{-1}$; Figure S3 in the SI) compared to Tb-mesoMOF. Assays of ABTS oxidation were conducted to study the kinetics of biocatalysis for Mb@Tb-mesoMOF, Mb@SBA-15, and free Mb at room temperature in a HEPES buffer.

As shown in Figure 2a, free Mb in solution demonstrates a very fast initial rate of $3.27 \times 10^{-4} \text{ mM s}^{-1}$ for ABTS^{•+}

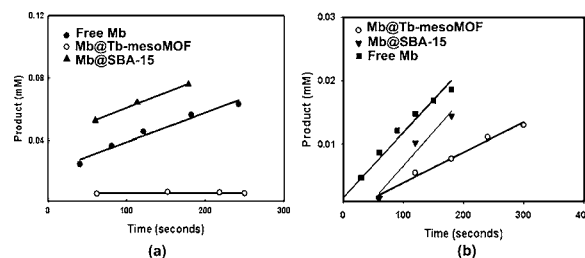


Figure 2. Kinetic traces for the oxidation of ABTS (a) and THB (b) by free Mb, Mb@Tb-mesoMOF, and Mb@SBA-15 with hydrogen peroxide in a HEPES buffer.

formation, as expected, and a high initial rate of $2.00 \times 10^{-4} \text{ mM s}^{-1}$ is observed for Mb@SBA-15 (Table 1), which is

Table 1. Summary of Catalysis Results of Oxidizing ABTS and THB in the Presence of 10 mM Hydrogen Peroxide in a HEPES Buffer

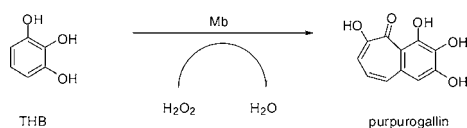
	free Mb ^a	Mb@SBA-15	Mb@Tb-mesoMOF
initial rate for ABTS ^{•+} formation (mM s ⁻¹) ^b	3.27×10^{-4}	2.00×10^{-4}	8.33×10^{-6}
initial rate for THB oxidation ^c (mM s ⁻¹)	1.02×10^{-4}	8.96×10^{-5}	4.80×10^{-5}
average rate for THB oxidation ^d (mM s ⁻¹)	8.20×10^{-6}	6.10×10^{-6}	1.55×10^{-5}

^aDiluted to 0.5 μM in a HEPES buffer. ^bInitial rate calculated in the first 4 min. ^cInitial rate calculated in the first 5 min. ^dAverage rate over 1 h.

consistent with the values reported in the literature.¹⁶ However, an extremely low initial rate of $8.33 \times 10^{-6} \text{ mM s}^{-1}$ is found for Mb@Tb-mesoMOF, which is almost inactive for ABTS^{•+} formation despite a larger amount of Mb trapped in Tb-mesoMOF compared to SBA-15. Because Tb-mesoMOF is inactive for the reaction (Table S1 in the SI), we reasoned that the very low initial rate of Mb@Tb-mesoMOF for ABTS^{•+} formation should be attributed to its small available pore size of ~ 0.8 nm, which does not allow the large ABTS substrate with a molecular dimension of $10.1 \times 17.3 \text{ \AA}$ to access the active Mb centers housed in Tb-mesoMOF. This prompts us to evaluate its possible size-selective biocatalysis performance by assaying a smaller substrate.

Polyphenols are also routinely used to evaluate the peroxidase activity of Mb, although its activity is about one-fourth of the ABTS system.¹⁷ To evaluate the biocatalytic activity as well as to confirm the possible size-selective biocatalysis performance of Mb@Tb-mesoMOF, we selected the small polyphenol 1,2,3-trihydroxybenzene (THB) (or pyrogallol), which has molecular dimensions of $5.7 \times 5.8 \text{ \AA}$, as the substrate by monitoring its oxidation at 320 nm for the formation of the corresponding purpurogallin dimer product (molecular dimensions $5.8 \times 7.5 \text{ \AA}$; Scheme 2). Given that the small substrate molecule can access the active Mb centers through the 0.8 nm pores, which can also facilitate the exit of the product molecule, we expected that Mb@Tb-mesoMOF should exhibit a much higher activity of oxidizing THB compared to ABTS.

Scheme 2. Reaction Scheme for Oxidation of THB by Hydrogen Peroxide Catalyzed by Mb



As expected, Mb@Tb-mesoMOF demonstrates a high initial rate of $4.80 \times 10^{-5} \text{ mM s}^{-1}$ using THB as the substrate (Figure 2b and Table 1), which is about half that of free Mb with a rate of $1.02 \times 10^{-4} \text{ mM s}^{-1}$. The slower initial rate for Mb@Tb-mesoMOF compared to free Mb should originate from the slow diffusion of the substrates from solution into Mb@Tb-mesoMOF through the micropores of 0.8 nm, whereas the observed high rate of $8.96 \times 10^{-5} \text{ mM s}^{-1}$ for Mb@SBA-15 can be attributed to the severe leaching of Mb (Figure S4 in the SI), which actually was also responsible for the high initial rate for the ABTS^{•+} formation in Mb@SBA-15. Nevertheless, after 1 h, Mb@Tb-mesoMOF exhibits a much faster average rate of $1.55 \times 10^{-5} \text{ mM s}^{-1}$ compared to those of Mb@SBA-15 (rate $6.10 \times 10^{-6} \text{ mM s}^{-1}$) and free Mb (rate $8.20 \times 10^{-6} \text{ mM s}^{-1}$). The significant lower average rates for Mb@SBA-15 and free Mb can be attributed to the internal decomposition of the protein active site in solution,¹⁸ which inversely indicates the enhanced stability of Mb enforced by the Tb-mesoMOF framework.

The above results of kinetic studies also indicate that Mb@Tb-mesoMOF indeed demonstrates selective biocatalysis of oxidizing small THB over large ABTS because of the size effect, which, to the best of our knowledge, has never been reported before.

One of the important issues for biocatalysis is the reusability of the catalysts. To evaluate the recyclability of Mb@Tb-mesoMOF, we checked its catalytic activities at different cycles. The reaction rates of THB oxidation for Mb@Tb-mesoMOF fluctuate from 3.84×10^{-5} to $4.84 \times 10^{-5} \text{ mM s}^{-1}$ over 15 cycles (Figure S5 in the SI); it slightly decreases to $3.20 \times 10^{-5} \text{ mM s}^{-1}$ at the 16th cycle, representing a ~33% activity drop compared to that of the first cycle (Table S2 in the SI). In comparison, the activity of Mb@SBA-15 decreases abruptly, with more than 40% activity loss after the first cycle, and less than 18% activity remained at the third cycle (Figure S5 and Table S2 in the SI). The fast decay of Mb@SBA-15 can be ascribed to the severe leaching of Mb, which was detected in the supernatant (Figure S4 in the SI). No Mb leaching was observed for Mb@Tb-mesoMOF over the 16 cycles of reactions, and the Tb-mesoMOF host could still maintain its framework integrity after catalytic cycles, as evidenced by the powder X-ray diffraction (PXRD) studies (Figure S6 in the SI). We reasoned that the capability of Mb@Tb-mesoMOF to retain activity for at least 15 cycles could be attributed to the strong interactions between the Tb-mesoMOF framework and the Mb molecules trapped in the 4.7-nm-diameter cages, which prevent their escape from the MOF host matrix. This also suggests that the Mb molecules can be greatly stabilized via the mesoporous MOF host matrix.

In summary, we have demonstrated the successful immobilization of the Mb into a mesoporous MOF with hierarchical pore sizes, which exhibited unprecedented size-selective biocatalysis as well as superior catalytic activities toward small substrate oxidation compared to the mesoporous silica material SBA-15. The interesting size-selective biocatalysis, together with enhanced stability and excellent recyclability for Mb

encapsulated in the Tb-mesoMOF, not only promises that mesoporous MOFs could serve as a new type of host matrix material to immobilize proteins for biocatalysis applications but also makes mesoporous MOFs stand out from traditional host matrix materials for protein immobilization.

■ ASSOCIATED CONTENT

📄 Supporting Information

Experimental procedures, UV-vis spectra, N₂ sorption isotherms, pore-size distribution plots, PXRD patterns, and table of reaction rates. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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