An Investigation Towards MIL-101 (Fe-Cu) Structures to Achieve Optimal Activity Across a pH Range

Aida Davari[1], Arastoo Badoei-Dalfard[2*], Zahra Karami[3], Shahriar Dabiri[4]

1. MSc student of Biochemistry, Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran, AidaDavari@sci.uk.ac.ir
2. Professor of Biochemistry, Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran, badoei@uk.ac.ir
3. Associated Professor of Biophysics, Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran, karami@uk.ac.ir
4. Professor of Pathology, Afzalipour Hospital, Pathology and Stem Cell Research Center, Kerman University of Medical Sciences, Kerman, Iran, sh_dabiri@kmu.ac.ir

Abstract

Metal-organic frameworks (MOFs) are compounds consisting of metal and organic linkers with properties such as high porosity, chemical and thermal stability, and structure tunability that have diverse applications including catalysis, biosensors, and drug delivery in the fields of industry, therapy, and medicine. Due to combination of nanozyme and enzyme in biosensing fields to measure metabolites, pH is one of the most effective factors related to functional performance of both nanozyme and enzyme, so it should be optimized. In this study, the bimetallic structure MIL-101 (Fe/Cu) was prepared with different Iron to Copper ratios (5:1, 2:1, 1:1, 1:2, and 1:5) and its Peroxidase-like activity was investigated in the pH range of 4 to 6.5. The results showed that the optimal pH is about 4.5, but the ratio of metals plays a key role in increasing the catalytic activity at other pHs. The compounds (Fe:Cu 1:2) and (Fe:Cu 1:5) compounds had a relatively rapid decrease in activity with increasing pH, like compound (Fe:Cu 5:1), although their absorption level at pH 4 was more than 2 times the value reported for (Fe:Cu 5:1). These findings indicate that designing and tuning the metal ratio in bimetallic MOFs can improve their catalytic efficiency in biosensing applications.

Key words: MOF, Nanozyme, Peroxidase, Fe-Cu, Activity

1. Introduction

Metal-organic frameworks (MOFs) are inorganic-organic crystalline hybrids with one-, two-, or three-dimensional structures composed of metal nodes and organic ligands linked by coordination bonds. The metal ions used in the structure of MOFs are usually transition, alkaline earth, p-block or actinide metals, while carboxylates, amines, nitrates, phosphates, and sulfonates are commonly used as organic linkers. MOFs exhibit properties such as large surface area and pore volume, intrinsic crystalline nature, extremely controllable porosity, tunable and easy modification of topological structure, multiple interaction sites such as hydrogen bonding, van der Waals forces, covalence, etc. with a selective reactant such as an enzyme, and relatively high chemical/thermal stability. All these features have led to be utilized in various fields such as chemical analysis, gas adsorption and separation, biosensing and diagnostics, as well as drug and enzyme loading and delivery (1-3).

Encapsulation of enzymes including lipase (Lip), glucose oxidase (GOx) and laccase (Lac) in MOFs makes them more stable than their free counterparts under harsh conditions like high temperatures, the presence of organic solvents and extreme pH. On the other hand, immobilization of the enzyme allows it to be used multiple times without losing activity (4).

In order to improve the structural stability, catalytic properties and generally better performance of MOF, two dissimilar metal ions are incorporated into a single structure, forming bimetallic MOFs. In bimetallic MOFs, the ratio of metals can be adjusted, which allows the physicochemical properties of bimetallic MOFs to be tuned. Some bimetallic MOFs are MIL-53 (Cr–Fe), CoZn-ZIF-8, MIL-100 (Fe, Ni) and UiO-66 (Zr/Ti) (5, 6).

The characteristics of MOFs have led some to be used as nanozymes. Nanozymes mimic the function of natural enzymes using chemical catalysis and have shown promising applications in cancer therapy, biosensing and wound healing. Currently, the mimicry of natural enzymes by mineral nanomaterials, especially Fe, Cu, Au, Mn, mainly focuses on oxidoreductases such as peroxidase (POD), oxidase (OXD), catalase (CAT) and superoxide dismutase (SOD). It should be noted that such catalytic behaviors can get more stable or unstable under the influence of endogenous or exogenous environmental factors such as pH, oxidation or redox conditions, radiation and electrochemical neurostimulants (7). It has been reported that some iron or copper-containing MOFs have peroxidase-mimicking properties that can produce colored products in the presence of H2O2 with TMB catalysis (8).

In bioassay systems where a combination of nanozyme and enzyme is used to measure metabolites, finding the right pH for optimal performance of both is a challenging issue. For this purpose, in this study, the effect of different buffers and pH ranges from 4 to 6.5 on the peroxidase-like activity of MIL-101 (Fe/Cu) with different structural ratios of copper and iron was investigated.

2. Experimental

2.1 MIL-101 preparation

In order to make MOFs with Iron to Copper ratios of 5:1, 2:1, 1:1, 1:2 and 1:5, first 1.5, 1.2, 0.9, 0.6 and 0.3 g of $FeCl_3.6\ H_2O$ (Iron Chloride Hexahydrate) and conversely $Cu(NO_3)_2.3\ H_2O$ (Copper Nitrate Trihydrate) were weighed and finally dissolved with 800 g of Terephthalic acid in 30 ml of DMF, then the solution was kept in an oven at 120 °C for one day to form MOF structures. Then, the solution was centrifuged at 8000 rpm for 8 minutes, after discarding the supernatant, the precipitate was washed with DMF. The final precipitate was placed in an oven to dry.

2.2 Buffers preparation

For this experiment, Sodium acetate (NaOAc, MW: 82.03 g/mol) buffer was made in the pH range of 4 to 6.5, with concentrations of 40 mM.

2.3 Peroxidase-like activity assay

In order to measure Peroxidase-like activity of MOF, briefly, first, 10 mg of each MOF structure was dissolved in 1 ml of deionized water. Then, in a final volume of 1100 µL, 100 µL of MOF, 40 µL of (3,3',5,5'-Tetramethylbenzidine, 4 M) TMB, 30 µL of H_2O_2 (1.2 mM) and 930 µL buffer were added. Each sample has a control without H_2O_2 at the same pH. The resulting mixture was kept at 37°C for 10 minutes until the desired reaction occurred. Then, the absorbance of each sample is read by a spectrophotometer at the wavelength of 652 nm.

3. Results

During 10 min, the MOF can catalyze H_2O_2 to oxidize TMB and produce a blue color which can be detected at the wavelength of 652 nm, the maximum absorption wavelength of the oxidized TMB product. The absorption peak shouldn't be observed in the UV-Vis spectra without addition of H_2O_2 or MOF, but sometimes interference of MOF components with buffer even in the absence of H_2O_2 can cause a blue color and a uv-visible peak at 652 nm. Hence at each pH besides every sample, a control solution contains buffer with the same pH, MOF and TMB without H_2O_2 is considered. It is noticeable that in MOF structure Copper causes a green color, while Iron causes an orange hue. Thereby, the resulting compounds have the color spectrum of green, orange and intermediates of these two. The higher the percentage of copper used, the closer the combination is to green.

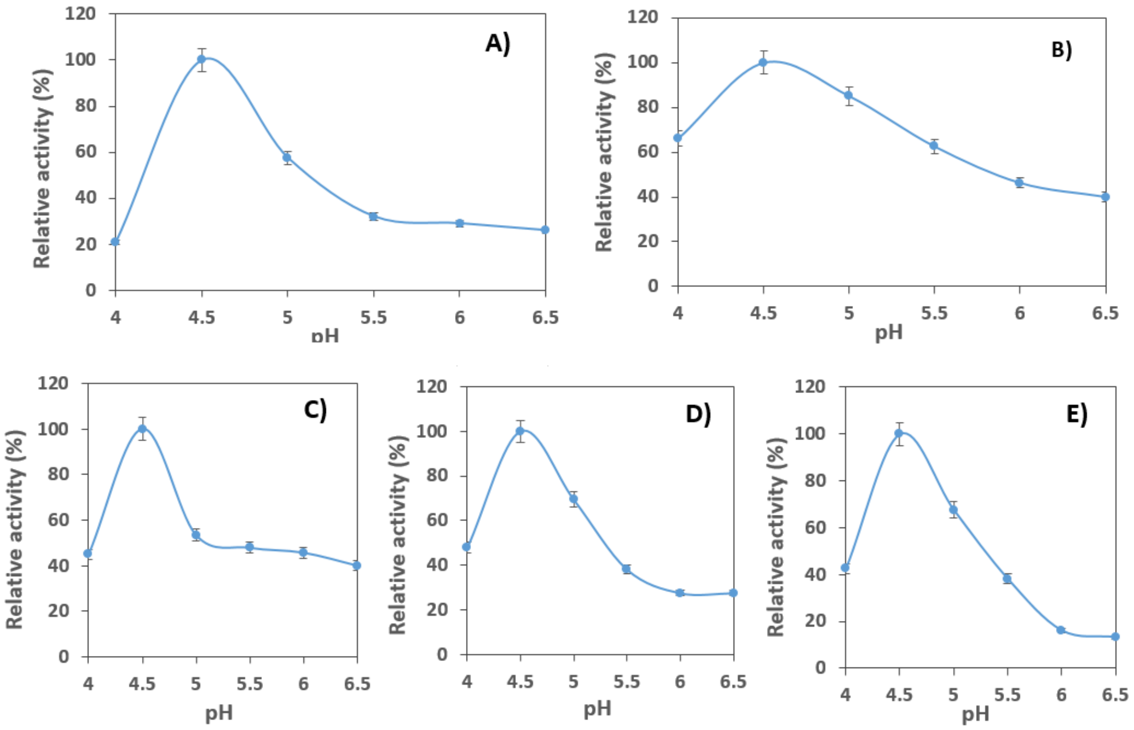

Figure 1: The effect of pH for oxidation of TMB (A) in Fe:Cu (5:1) (B) in Fe:Cu (2:1) (C) in Fe:Cu (1:1) (D) in Fe:Cu (1:2) (E) in Fe:Cu (1:5)

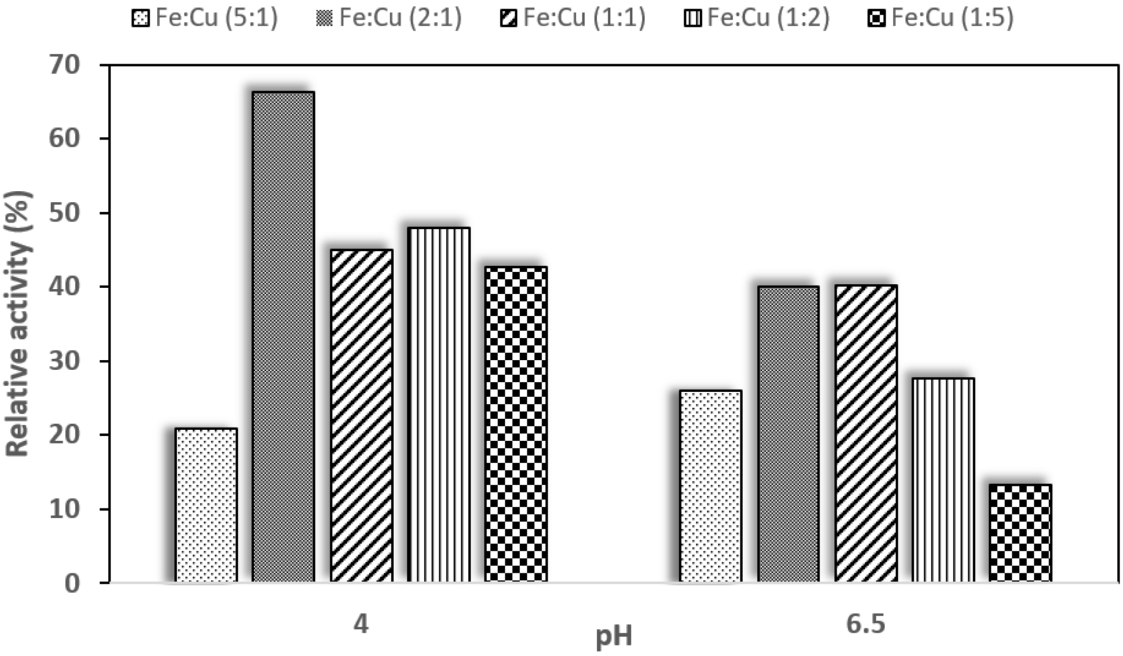

Figure 2: The effect of pH 4 and 6.5 on TMB oxidation

Figure 1 shows that the optimum pH for all 5 compounds is 4.5. Maximum activity (100%) was observed at this pH. For the compound (Fe:Cu 5:1), activity decreased rapidly after pH 4.5, the activity level at pH 4 was about 20% and at pH 6.5 it reached about 30%. While for the (Fe:Cu 2:1), the rate of activity decrease was much lower than all the existing compounds, the peroxidase activity was initially 65% and at pH 6.5 it was about 40%. The (Fe:Cu 1:1) showed the least changes in absorption at different pHs compared to the other compounds, it is clearly visible in fig.1 (C) that the activity level remained constant in the range of 40 to 50% across the range of 4 to 6.5 except pH 4.5. The (Fe:Cu 1:2) and (Fe:Cu 1:5) had a relatively rapid decrease in activity by pH increasing, like (Fe:Cu 5:1). Fig.2 indicates the absorption level for both (Fe:Cu 1:2) and (Fe:Cu 1:5) was more than 2 times the reported value of (Fe:Cu 5:1) at pH 4. The (Fe:Cu 1:5) compound, which has the highest Copper content, showed the lowest activity at pH 6.5, about 10%, almost half the absorption of (Fe:Cu 5:1), although at pH 4, on the contrary, it has twice the activity of (Fe:Cu 5:1).

4. Conclusion

The results of TMB oxidation in the presence of H_2O_2 by MIL-101 (Fe-Cu) showed that the Fe:Cu ratio (1:1) displayed good activity and moderate stability over a wider pH range. While the Fe:Cu ratio (2:1) was the most stable compound, retaining more than 40% of its activity even at pH 6.5. Finally, it can be said that in MIL-101 (Fe-Cu), the proportional presence of Iron and Copper together maintains the stability and significant nanozyme activity of the MOF over a wider pH range from acidic to relatively neutral.

References

[1] Chen T-T, Yi J-T, Zhao Y-Y, Chu X. Biomineralized metal–organic framework nanoparticles enable intracellular delivery and endo-lysosomal release of native active proteins. Journal of the American Chemical Society.140(31):9912-20, 2018.

[2] Xia H, Li N, Zhong X, Jiang Y. Metal-organic frameworks: a potential platform for enzyme immobilization and related applications. Frontiers in Bioengineering and Biotechnology, 8:695, 2020.

[3] Liang S, Wu X-L, Xiong J, Zong M-H, Lou W-Y. Metal-organic frameworks as novel matrices for efficient enzyme immobilization: an update review. Coordination Chemistry Reviews, 406:213149, 2020.

[4] Shortall K, Otero F, Bendl S, Soulimane T, Magner E. Enzyme immobilization on metal organic frameworks: The effect of buffer on the stability of the support. Langmuir, 38(44):13382-9, 2022.

[5] Chen L, Wang H-F, Li C, Xu Q. Bimetallic metal–organic frameworks and their derivatives. Chemical science,11(21):5369-403, 2020.

[6] Kumari A, Kaushal S, Singh PP. Bimetallic metal organic frameworks heterogeneous catalysts: Design, construction, and applications. Materials Today Energy, 20:100667, 2021.

[7] Tang Q, Cao S, Ma T, Xiang X, Luo H, Borovskikh P, et al. Engineering biofunctional enzyme-mimics for catalytic therapeutics and diagnostics. Advanced Functional Materials, 31(7):2007475, 2021.

[8] Yu H, Wu H, Tian X, Zhou Y, Ren C, Wang Z. A nano-sized Cu-MOF with high peroxidase-like activity and its potential application in colorimetric detection of H 2 O 2 and glucose. RSC advances, 11(43):26963-73, 2021.