Lysine-modulated synthesis of enzyme-embedded hydrogen-bonded organic frameworks for efficient carbon dioxide fixation

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Abstract

Carbonic anhydrase (CA) is an important carbon fixation enzyme. Immobilization of CA can expand its application in the realm of adsorption, catalysis, and so on. As a typical metal-free framework, hydrogen-bonded organic frameworks (HOFs) featuring mild synthesis process, exquisite framework structure and good enzyme compatibility have been used for enzyme embedding. However, the catalytic performance of CA-embedded HOFs (CA@HOFs) is limited by the micropore size of HOFs and the slow adsorption of CO₂. Herein, CA@Lys-HOF-1 was synthesized by introducing lysine (Lys), a basic amino acid, during the coprecipitation of CA and HOFs for CO₂ fixation. The addition of Lys enlarged the average pore size of HOF-1 from 1.8 to 3.2 nm, whereas the introduced -NH₂ groups increased the initial adsorption of CO₂ from 0.55 to 1.21 cm³ g⁻¹. Compared to CA@HOF-1, the activity of CA@Lys-HOF-1 was enhanced by 71.25%, and the corresponding production of CaCO₃ was enhanced by 12.7%. After eight reaction cycles, CA@Lys-HOF-1 still maintained an output of 9.97 mg of CaCO₃ every 5 min, 83.7% of the initial production. It is hoped that the CA@Lys-HOF-1 reported offers a platform for efficient and continuous fixation of CO₂.
Keywords: Carbon dioxide fixation, carbonic anhydrase, hydrogen-bonded organic frameworks, enzyme catalysis, enzyme immobilization

INTRODUCTION

Carbon dioxide (CO$_2$) capture and utilization (CCU) is one of the ever-increasing research topics which can contribute to addressing environmental and ecological issues$^{[1-4]}$. Enzymes such as formate dehydrogenase (FDH), ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO), and carbonic anhydrase (CA) can accurately activate CO$_2$ to lower the reaction energy barrier, which have received great attention as a green and feasible solution to CCU$^{[5-7]}$. CA exhibits the highest catalytic rate among all carbon-fixation enzymes, and thus has great potential for the selective transformation of CO$_2$ to HCO$_3^-$.$^{[8]}$ However, enzymes that leave the bodies of organisms are easy to inactivate and difficult to reuse$^{[9-11]}$. One frequently used method to address the above issues is to immobilize enzymes inside carrier materials$^{[12]}$. Porous framework materials, such as metal-organic frameworks (MOFs)$^{[13-17]}$, covalent organic frameworks (COFs)$^{[18-22]}$, and hydrogen-bonded organic frameworks (HOFs)$^{[23-25]}$, bearing high specific surface area, high porosity, exquisite framework structure and excellent designability, are emerging carriers for enzyme immobilization. Particularly, HOFs are framework materials linked by hydrogen bonds$^{[26-29]}$, which can be reversibly repaired by simple recrystallization$^{[30,31]}$ and possess better biocompatibility due to the absence of metal ions$^{[32,33]}$. These advantages make HOFs an excellent candidate as enzyme immobilization carriers$^{[34]}$. For example, HOF-21 synthesized by Bao et al. can recover its original structure after immersion in aqueous or anionic source solution for 48 h$^{[35]}$. Tang et al. designed TA-HOFs capable of in situ embedding enzymes with different surface charges and molecular weights$^{[36]}$. Enzyme in TA-HOFs exhibited remarkably enhanced stability. However, HOFs with micropore size and balanced -NH$_2$/-COOH groups usually showed restricted mass transfer and lower affinity with CO$_2$, therefore exhibiting reduced apparent enzyme activity.

Herein, HOF-1 composed of two units of Tetrakis(4-amidiniumphenyl)methane and tetrakis (4-carboxyphenyl) methane was selected for CA embedding$^{[37]}$. The structure of HOF-1 was modulated by introducing basic amino acids during the synthesis process. Briefly, carbonic anhydrase@HOF-1 (CA@HOF-1) was prepared by a coprecipitation method, in which CA was in situ embedded. The structure of CA@HOF-1 was modulated by altering the species and amount of amino acid. We found that basic amino acids, especially lysine (Lys), can interact with the carboxyl monomer of HOF-1 to occupy some of the hydrogen bond formation sites, thus causing defects of HOF-1 to promote mass transfer. Meanwhile, the introduced -NH$_2$ groups also facilitated the initial adsorption of CO$_2$. Compared to CA@HOF-1, CA@Lys-HOF-1 showed a 12.7% enhancement in CO$_2$ fixation efficiency. When CO$_2$ was introduced at a flow rate of 25 mL min$^{-1}$, CaCO$_3$ precipitation reached 12.26 mg after 5 min of ventilation. After 8 cycles, CA@Lys-HOF-1 maintained an output of 9.97 mg CaCO$_3$ every 5 min. It is believed that Lys-HOF-1 is an ideal framework material for CO$_2$-converting enzyme embedding.

EXPERIMENTAL

Materials and chemicals

Tetrakis(4-amidiniumphenyl)methane tetrahydrochloride (TAM, 95%) was purchased from Jilin Chinese Academy of Sciences-Yanshen Technology Co., Ltd. Tetrakis(4-carboxyphenyl)methane (C$_{29}$H$_{20}$O$_8$, 98%) was obtained from Shanghai Macklin Biochemical Technology Co., Ltd. γ-poly-l-glutamic acid (PLGA, 92%), glycine (C$_2$H$_5$NO$_2$, 98%), serine (C$_3$H$_7$NO$_3$, 98%), arginine (C$_6$H$_{14}$N$_4$O$_2$, 98%), and lysine (C$_6$H$_{14}$N$_2$O$_2$, 98%) were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Carbonic anhydrase (EC 4.2.1.1) was acquired from Sigma-Aldrich Co., Ltd. All other chemicals were used as received without any purification.
Synthesis of HOF-1
Tetrakis(4-amidiniumphenyl)methane tetrahydrochloride (10 mg) was dissolved in H$_2$O (2.5 mL) to form solution A. Tetrakis(4-carboxyphenyl)methane (7.5 mg) was dispersed in H$_2$O (2375 μL), followed by the addition of aqueous ammonium hydroxide solution (1% v/v, 125 μL) to form solution B. Thereafter, solution B was added to solution A under stirring conditions at room temperature. Precipitation occurred immediately upon mixing. The reaction mixture was left to stir gently in the dark for 1 h. The HOF-1 material was then recovered by centrifugation, washed, dispersed, and centrifuged three times in H$_2$O to remove any unreacted precursors.

Synthesis of CA@HOF-1
Tetrakis(4-amidiniumphenyl)methane tetrahydrochloride (10 mg) was dissolved in H$_2$O (1.25 mL) to form solution A. An aqueous solution of CA (1.25 mL of 2 mg mL$^{-1}$ stock solution) was added to solution A and stirred at room temperature for 10 min to form solution B. Tetrakis(4-carboxyphenyl)methane (7.5 mg) was dissolved in 2375 μL of H$_2$O and 125 μL of 1% NH$_4$OH to form solution C. Solution C was then added dropwise to solution B under stirring. The mixture was then left to stir gently for another 1 h to ensure the completion of the synthesis. Thereafter, CA@HOF-1 was collected by centrifugation and then washed, dispersed, and centrifuged three times in H$_2$O to remove the unreacted precursors and loosely adsorbed CA.

Synthesis of CA@amino acid-HOF-1
Tetrakis(4-carboxyphenyl)methane (7.5 mg) was dissolved in 2375 μL of H$_2$O and 125 μL of 1% NH$_4$OH to form solution A. An aqueous solution of CA (0.625 mL of 4 mg mL$^{-1}$ stock solution) and amino acid (0.625 mL of 1/10/20 mg mL$^{-1}$ stock solution) was added to solution A and stirred at room temperature for 10 min to form solution B. Tetrakis(4-amidiniumphenyl)methane tetrahydrochloride (10 mg) was dissolved in H$_2$O (1.25 mL) to form solution C. Solution C was then added dropwise to solution B under stirring. The mixture was then left to gently stir for another 1 h to ensure the completion of the synthesis. Thereafter, CA@amino acid-HOF-1 was collected by centrifugation and then washed, dispersed, and centrifuged three times in H$_2$O to remove any unreacted precursors and loosely adsorbed CA.

Characterization
The sample morphologies were analyzed by scanning electron microscopy (SEM, S-4800, Hitachi) and transmission electron microscopy (TEM, JEM-100CX II, JEOL). The chemical compositions of the samples were identified by Fourier transform infrared (FTIR) spectrometer (Nicolet-6700, Nicolet). The crystal structures of the samples were identified by X-ray diffraction (XRD, X' Pert Pro), with 2-theta ranging from 10° to 90° by a step width of 0.033° with 15.24° min$^{-1}$ speed at 40 mA and 40 kV. The specific surface area and pore size distribution of the samples were examined by Brunauer-Emmett-Teller (BET) method based on N$_2$ adsorption/desorption isotherms on an AUTOSORB-1 surface area and pore size analyzer (Quantachrome Instruments).

Conversion of CO$_2$
CA@HOF-1 and CA@Lys-HOF-1 were used for the conversion of CO$_2$. In a typical experiment, nitrogen was first injected into the aqueous phase of the reaction device for 10 min to remove CO$_2$ gas in the solution. Then, CO$_2$ with a rate of 25 mL min$^{-1}$ was injected into the system. A sample was taken every 5 min for follow-up reaction. Specifically, pH value of the reaction solution was detected by a pH meter and kept at 7.9, which was adjusted by adding 5 mol L$^{-1}$ NaOH. Then, a certain amount of reaction solution was mixed with 670 mmol L$^{-1}$ calcium chloride solution. The mixture was shaken at 200 r min$^{-1}$ to form CaCO$_3$ precipitate. The precipitated CaCO$_3$ was filtered by a filter paper with an average pore diameter of 2.5 μm, which was then dried overnight and weighed to determine the relative yield of CaCO$_3$. 
RESULTS AND DISCUSSION

Preparation and Characterization of CA@HOF-1 and CA@Lys-HOF-1

A reported HOF material (HOF-1) was chosen for the embedding of CA\textsuperscript{[37]}. The preparation and structure regulation of CA@HOF-1 are shown in Figure 1. Briefly, HOF-1 was prepared by mixing tetrakis(4-amidiniumphenyl)methane tetrahydrochloride (monomer 1) solution and tetrakis(4-carboxyphenyl)methane (monomer 2) solution under stirring at room temperature \textsuperscript{[Supplementary Figure 1]}. CA@Lys-HOF-1 was prepared by introducing CA and lysine (Lys) during the HOF-1 synthesis process. For control, CA@HOF-1 was also prepared by only adding CA in the HOF-1 synthesis. In advance of the discussion about CA@HOF-1 and CA@Lys-HOF-1, the chemical composition and physical structure of HOF-1 were characterized by FTIR, XRD and $^{13}$C-NMR, which are shown in Supplementary Figures 2 and 3.

The topological structures of all samples were also examined by SEM and TEM. As shown in Figure 2 and Supplementary Figure 4, both CA@HOF-1 and CA@Lys-HOF-1 maintain the rod-like structure of HOF-1 with similar dimensions (around 300 nm wide). This indicates that the incorporation of CA and Lys did not alter the structure and size of HOF-1 during crystallization. As depicted in Figure 2C, some defects can be observed on the surface of CA@Lys-HOF-1, while CA@HOF-1 and HOF-1 remain intact. Figure 2D further shows that CA@HOF-1 and CA@Lys-HOF-1 well maintain the crystal structure of HOF-1 with the characteristic peaks at 2\textdegree\theta values of 8.6\textdegree, and 17.4\textdegree. The results of FTIR analysis of CA@HOF-1 and CA@Lys-HOF-1 are shown in Figure 2E. The absorption band centered at around 1030 cm\textsuperscript{-1} may be assigned to the N-H bending vibration of amide in Lys, indicating the introduction of Lys into HOF-1. To reveal the variation of surface area and pore size distribution of HOF-1 after modulation and enzyme embedding, the N\textsubscript{2} adsorption-desorption isotherms of HOF-1, CA@HOF-1, and CA@Lys-HOF-1 were examined \textsuperscript{[Supplementary Figure 5]}. As shown in Figure 2F and Table 1, CA@Lys-HOF-1 shows a larger pore size and broader size distribution, which may facilitate the transfer of CO\textsubscript{2} from the particle surface to CA. CA@Lys-HOF-1 also has a larger surface area due to the defects caused by Lys, which provides more sites for CO\textsubscript{2} conversion. Then, the CO\textsubscript{2} adsorption tests were performed and the results are shown in Figure 2G and
Table 1. BET analysis and initial CO₂ adsorption volume of HOF-1, CA@HOF-1, and CA@Lys-HOF-1

<table>
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<th>HOF-1</th>
<th>CA@HOF-1</th>
<th>CA@Lys@HOF-1</th>
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<td>Surface area (m² g⁻¹)</td>
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<td>19.7</td>
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<tr>
<td>Main pore diameter (nm)</td>
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<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Initial CO₂ adsorption volume (cm³ g⁻¹)</td>
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<td>0.546</td>
<td>1.214</td>
</tr>
</tbody>
</table>

Figure 2. TEM images of (A) HOF-1; (B) CA@HOF-1; and (C) CA@Lys-HOF-1; (D) XRD patterns; (E) FTIR spectra; (F) pore size distribution; and (G) CO₂ adsorption capacity of HOF-1, CA@HOF-1, and CA@Lys-HOF-1.

Supplementary Figure 6. The CO₂ adsorption of CA@Lys-HOF-1 is higher when the CO₂ partial pressure is low, a result due to the relatively larger pore size. With an increase in CO₂ partial pressure, CA@Lys-HOF-1 reaches CO₂ adsorption saturation most quickly, mainly owing to the higher amount of -NH₂ with high CO₂ affinity on the particle surface. As the pressure continued to increase, the final amount of CO₂ adsorption of CA@Lys-HOF-1 was close to that of HOF-1 and CA@HOF-1.

Subsequently, EDS mapping was performed to further investigate whether enzymes were embedded in HOF-1. As shown in Figure 3, the distribution of C and N (the main elements in the two monomers of HOF-1) match the morphology of materials, which can also prove the successful synthesis of HOF-1. Particularly, S (the characteristic element of CA) is uniformly dispersed in the two samples of CA@HOF-1 and CA@Lys-HOF-1, indicating the successful embedding of CA.
Catalytic activity of CA@Lys-HOF-1

In this work, five different types of (poly) amino acids [Supplementary Figure 7] were adopted to regulate the structure and catalytic performance of CA@HOF-1. Specifically, the hydrogen bonds in HOF-1 are formed between -NH$_2$ and -COOH of the two monomers, while amino acids are organic compounds containing both -NH$_2$ and -COOH in the molecule. Amino acids may occupy part of the hydrogen bond formation site, and interfere with the synthesis of HOF-1 through competitive ligand interaction, thus altering the pore size of HOF-1 and influencing the mass transfer.

When $\gamma$-poly-l-glutamic acid (PLGA) was introduced, its -COOH groups can rapidly bind to monomer 1, inhibiting the formation of HOF-1. [Supplementary Figure 8]. This was probably due to the preferential combination of -COOH in PLGA and -NH$_2$ in monomer. Among the other four types of amino acid-modulated CA@HOF-1, the CA@Lys-HOF-1 exerted the highest activity [Supplementary Figures 9 and 10].

The catalytic activity of HOF-1, Lys-HOF-1, CA@HOF-1, and CA@Lys-HOF-1 were tested by dispersing them individually in solution for CO$_2$ conversion. The activity was reflected by detecting the pH change in the solution after the ventilation of CO$_2$. The solution without catalyst samples was chosen as control, the $\Delta$ pH was measured in real-time, and the value at the highest point was chosen to reflect the catalyst activity. Figure 4A and B shows that the activity of CA@Lys-HOF-1 was enhanced. The higher activity of CA@Lys-HOF-1 may be attributed to the defects resulting from Lys, which promoted mass transfer. The -NH$_2$ groups in Lys were also introduced into the material, which fortifed the affinity between the materials and CO$_2$ [Supplementary Figure 11]. Furthermore, we investigated the effect of Lys concentrations on the activity. As seen in Figure 4C and D, moderate concentration of Lys is favorable for HOF-1 modulation, thus acquiring the most active CA@Lys-HOF-1. It should be noted that the results of Arg modulation are shown in Supplementary Figure 12, which also shows the same result.
Figure 4. (A and B) Catalytic activity of HOF-1, Lys-HOF-1, CA@HOF-1, CA@Lys-HOF-1, and Free CA; (C and D) Catalytic activity of CA@HOF-1 modulated by different concentrations of Lys.

Stability and CO$_2$ fixation ability of CA@Lys-HOF-1

Subsequently, the stability, reusability, and CO$_2$ fixation ability of CA@Lys-HOF-1 were investigated. As shown in Figure 5, Lys-HOF-1 exhibits better protection of CA at different pH values. Regarding the thermal stability, the protective ability of Lys-HOF-1 for CA began to decrease when the temperature was higher than 60 °C. This may be owing to the weakened strength of hydrogen bonds within the carrier material after Lys modification, which made Lys-HOF-1 more prone to decomposition at higher temperatures. The reusability of CA@Lys-HOF-1 was also evaluated for its importance in industrial applications. As shown in Figure 5C, CA@Lys-HOF-1 shows excellent recyclability. After the 8th cycle of reaction, CA@Lys-HOF-1 still maintained 83.7% of its initial activity, and demonstrated unaltered morphological and crystal structure [Supplementary Figure 13].

Finally, to examine the CO$_2$ fixation ability of CA@Lys-HOF-1, CO$_2$ was introduced at a flow rate of 25 mL min$^{-1}$, and the CaCO$_3$ mineralization reaction was performed after 5 min of ventilation. In detail, the reaction rates of the four samples, including HOF-1, Lys-HOF-1, CA@HOF-1, and CA@Lys-HOF-1, were assessed by measuring the amount of CaCO$_3$ precipitate produced. As shown in Figure 5D, the CaCO$_3$ precipitate amount of HOF-1, Lys-HOF-1, CA@HOF-1, and CA@Lys-HOF-1 is, respectively, 4.81 mg, 6.29 mg, 10.88 mg, 12.26 mg. The production of CaCO$_3$ enabled by CA@Lys-HOF-1 was the highest, again validating the superiority of CA@Lys-HOF-1 in fortifying CO$_2$ fixation processes. Moreover, a hot filtration$^{[38]}$ experiment was performed over CA@Lys-HOF-1 for CO$_2$ mineralization. As shown in Supplementary Figure 14, no more increment in the production of CaCO$_3$ is observed after the filtration process, suggesting the heterogeneous nature of our catalytic system.
CONCLUSIONS

In summary, HOF-1 modulated by amino acids was synthesized through a coprecipitation method for CA immobilization. By regulating the type and concentration of introduced amino acids, CA@Lys-HOF-1 with optimized activity and desirable stability was obtained. Compared with unmodulated CA@HOF-1, the activity of CA@Lys-HOF-1 was enhanced by 71.3%, whereas the CO₂ fixation efficiency reflected by CaCO₃ production was enhanced by 12.7%. This could be ascribed to the large pore size of CA@Lys-HOF-1 that facilitated CO₂ transfer as well as the abundant surface -NH₂ groups that promoted CO₂ adsorption. Moreover, CA@Lys-HOF-1 maintained over 80% of the initial activity after the 8th cycle reaction. Our findings may pave the way for the immobilization of CA and other CO₂-fixation enzymes.

DECLARATIONS

Authors’ contributions
Carried out the catalyst preparation, characterization, and catalytic tests, and prepared the draft manuscript: Zhang B
Performed part of the catalyst characterization: Chu Z, Zhang J, Wu Z
Performed the TEM characterization: Yang D, Wu H
Planned the study, analyzed the data and wrote the manuscript: Shi J, Jiang Z

Availability of data and materials
Not applicable.
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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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REFERENCES