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MOFzyme: FJU-21 with Intrinsic High Protease-Like Activity for Hydrolysis of Proteins

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Abstract

In this work, metal-organic frameworks (MOFs) FJU-21 was synthesized by solvothermal method. The crystal structure of FJU-21 was characterized by XRD and BET and it was applied to the catalytic hydrolysis of bovine serum albumin. The effects of reaction pH, temperature and reaction time on the catalytic activity of FJU-21 were studied. FJU-21 were found to possess an intrinsic enzyme mimicking activity similar to that found in trypsin. The Michaelis constant (K_m) of the artificial protease (0.18×10^{-3} - 0.20×10^{-3} M^{-1}) was about 15-fold lower than that free trypsin (2.7×10^{-3} M^{-1}) and about 3-fold lower than that of soluble Cu(II) oxacyclen (0.54×10^{-3} M^{-1}). The K_{cat} of FJU-21 is 102 times higher than that of soluble Cu(II) oxacyclen catalysts and, indicating a much higher affinity of BSA for FJU-21 surface. FJU-21 could be reused for eleven times without losing in its activity.

Keywords

Metal-Organic Frameworks (MOFs), FJU-21, Protease Mimetics, Reusability

1. Introduction

The preparation and application of artificial mimic enzymes have become an increasingly important focus for research in recent years because natural enzymes have many problems such as a sensitivity to catalytic activity in the environment condition, lower stability, difficulty in purification and storage [1] [2]. In such

context, many mimic enzymes have been discovered [3] [4], such as fullerene derivatives [5], gold nanoparticles [6] [7], rare earth nanoparticles [8] and ferromagnetic nanoparticles [9] [10] [11] and so on. Proteolysis, hydrolysis of protein into amino acids or small fragments, is widely applied to the utilization of agricultural yields, seafood and meat byproducts as well as to the improvement of nutritional and functional properties of proteins. Therefore, a large number of protein mimetic enzymes have also attracted a lot of research interest due to the harsh conditions of natural protease hydrolysis of proteins and the low hydrolysis efficiency, and difficult to reuse repeatedly. Therefore, many kinds of protease-mimics have been developed during the last decades. Such as Hegg [12] and co-workers designed and synthesized Cu ([9] ane N_3) $C1_2$ hydrolyzed Gly-Gly and bovine serum albumin(BSA) for the first time under physiological pH conditions. The cyclized Cu(II) complex is used as a catalytic center and ruthenium ion as a binding unit to prepare artificial metalloproteinase, *i.e.* $[Cu(II)Cyc]_3(Gua)_3{}^{MeO}PCD$ [13], which catalyzes the hydrolysis of β-globulin at pH 7.0 and 4˚C. Molybdenum peroxide amino complex, $MoO(O_2)_2$(a-leucine)(H_2O) [14] was synthesized for photocatalytic hydrolysis of human pepsin. However, these protein mimic enzymes have lower catalytic activity and poorer selectivity. Therefore, it is highly desirable to design a mimetic enzyme with high selectivity and large degrees of rate acceleration.

Metal-organic frameworks (MOFs) are formed by self-assembly of metal ions or metal cluster units and organic ligand molecules, it is a porous crystalline material having a periodic multidimensional structure and also known as porous codination polymers (PCPs) or organic-inorganic hybrid materials. MOFs also have adjustable pore size, easy functionalization and extremely high specific surface area and porosity, making them suitable for catalysiss [15] [16], separation [17], gas storage [18], sensing, biomedical imaging and drug delivery and has attracted much attention in science and biological systems. These advantages of MOFs suggest that it to be a material suitable for enzyme mimics. Indeed, Cu-MOF [19] was synthesized for catalyzing hydrolysis of bovine serum albumin and casein by solvothermal method. Despite the many achievements in MOF development, we believe that the MOF-based catalytic field is still in the immature stage. Here we report that FJU-21 as protease mimetics to catalyze hydrolysis of bovine serum albumin (BSA).

2. Methods

2.1. Chemicals and Instrumentation

All commercial chemicals were used without further purification. Cuprous iodide (CuI) O-xylene, Dichlorosulfoxide ($SOCl_2$), ethanol (99.5%), NaOH, bovine serum albumin (BSA) were purchased from Sigma-Aldrich. X-ray powder diffraction (XRD) experiments were conducted on a D/max-3B spectrometer with Cu Kα radiation. Scans were made in the 2^{θ} range 3˚ - 40˚ with a scan rate of 10˚ min^{-1} (wide angle diffraction). BET surface areas and pore volumes were

measured through nitrogen adsorption/desorption measurements using a Micromeritics Tristar II surface area and porosity analyzer.

2.2. Synthesis of FJU-21

FJU-21 is a copper-based MOFs material with 5-triazole isophthalic acid as ligand copper as the active center of metal. $SOCl_2$ (6 mL) and DMF (150 mL) were mixed and stirred at 5˚C for 24 h, slowly added with hydrazine hydrated DMF solution (5 mL of hydrazine hydrate, 0.1 mM), stirred at room temperature for 48 h, washed three times with DMF and diethyl ether, and filtered to get a white solid. That is N, N-dimethyl-2-azidoethylamine (DMAZ). And then DMAZ (4 g), 5-aminoisophthalic acid (3.38 g) were dissolved in o-xylene (50 mL) and condensed at 120˚C for 16 h to obtain a white precipitate and washed three times with ethanol gave a white solid, 5-triazole isophthalic acid (H_2L). CuI (0.0191 g), H_2L (0.023 g), DMF (3 mL) and H_2O (2 mL) were added to the crystallization flask and stirred for 10 min, and transferred to an oven at 85˚C for 24 h. It was naturally cooled to room temperature and washed with ethanol three times to obtain a green needle-like solid FJU-21.

2.3. Protease-Like Activity of FJU-21

The effect of pH, temperature, FJU-21 concentration on the protease-like activity of FJU-21 was investigated. Add BSA solution (15 mM, 10 mL) to FJU-21 by gentle vortex mixing, and the peroxidase-like activity of FJU-21 was measured at pH 9.0, the temperature at 37˚C, 50˚C and 70˚C, and the time from 0 to 80 min. The FJU-21 concentration was 1.2 mM. Take out of 1 mL of reaction mixture periodically at a fixed shaking speed. Buffers used in this experiment were acetate buffer (pH 4.0 - 6.0) and borate buffer (pH 8.0 - 10.0). The effect of pH on catalytic efficiency was investigated by varying the buffer complex to produce different pH at 50˚C. Fresh solutions were used in all tests.

The peptide solution was separated from the solid composite by centrifugation at 10,000 rpm for 10 minutes at 4˚C. Bovine serum protein solutions without added materials were taken as the control group. The degree of cleavage of proteins was measured by SDS-PAGE. The rate of protein cleavage was measured by monitoring the decrease in the intensity of the electrophoretic bands. The kinetic data were collected at various C_0 (0 - 1.2 mM) at the optimum pH 9.0 and various temperatures (50˚C, 70˚C).

2.4. Reusability of FJU-21

To evaluate the reusability of FJU-21, the insoluble catalyst has been recycled from the reaction mixture after the catalytic reaction. FJU-21 was reacted with BSA in boric acid buffer for 12 min. After each reaction, it was re-purified by ethanol and centrifuged. The re-purified FJU-21 was used for the next reaction and subjected to SDS-PAGE electrophoresis analysis. The recycling was conducted eleven times. The catalytic activity is not less than 80% compared with

the first time, indicating that FJU-21 still maintains high catalytic activity and stability during the reaction.

3. Results and Discussion

3.1. The Characterization of FJU-21

The X-ray diffraction (XRD) pattern of the as-synthesized FJU-21 is shown in **Figure 1(a)**. The diffraction peaks all corresponded to the products synthesized in the literature [20]. The adsorption – desorption isotherms of FJU-21 (**Figure 1(b)**) are of type Ⅳ, the BET specific surface area of FJU-21 is 341 m^2/g, the pore volume was 0.1 $cm^3 \cdot g^{-1}$ and the average pore size was 3.19 nm. All of the results mentioned above confirmed that FJU-21 was successfully synthesized.

3.2. Protease-Like Activity of FJU-21

The protease-like activity of FJU-21 was measured while varying the pH from 6.5 to 9.0, the temperature from 37˚C to 70˚C using BSA as a substrate. While the buffer solution containing a protein substrate (15 μM) was shaken with FJU-21, the protein disappeared was observed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [21] [22]. When the temperature is 37˚C, hydrolysis was very weak and the intensities of BSA bands reduced slowly within 80 min, which means the catalytic activity of FJU-21was very low at 37˚C. When the temperature was raised from 37˚C to 70˚C, the intensities of BSA bands reduced and a myriad of fragments appeared after 12 min, resulting in a significant loss in intensity of the BSA band and a concomitant smear on the SDS-PAGE gel. The band of BSA disappeared after 12 min, which revealed the enhancement of the digestion activity at 70˚C (**Figure 2**). Nevertheless, FJU-21 acted as the digestion enzyme and cleaved BSA. Temperature and pH values are key factors influencing the digestion results. We found that pH 9.0 was suitable for the reaction (**Figure 3**).

The proteolytic activity of FJU-21 was further examined by kinetic measurements. Within the range of the FJU-21concentrations considered, typical

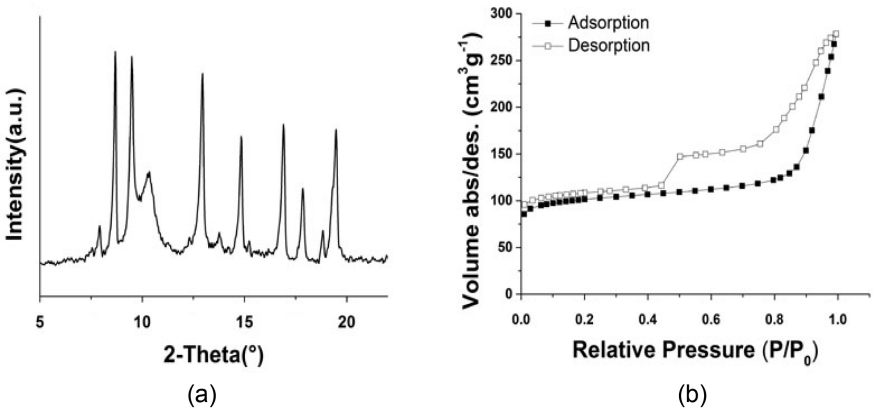

Figure 1. (a) XRD patterns of FJU-21. (b) N2 adsorption/desorption isotherm pore distribution of FJU-21.

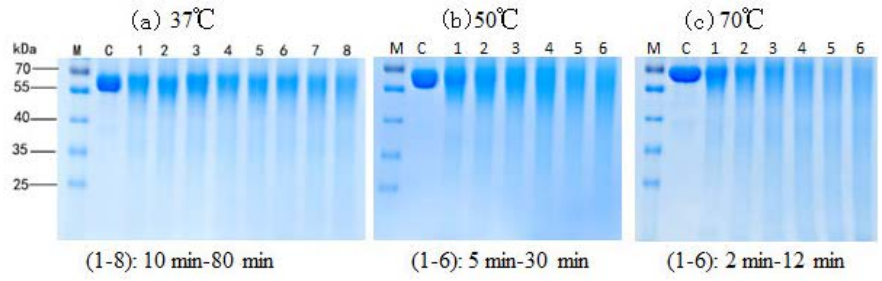

Figure 2. Results of SDS-PAGE performed on BSA (15 μM) incubated with and FJU-21 in boric acid buffer (pH 9.0). M: Mark. C: control groups (BSA incubated with boric acid buffer). (a) BSA (15 μM) incubated with FJU-21 (1.2 mM) for 0, 10, 20, 30, 40, 50, 60, 70, 80 min at 37˚C. (b) BSA (15 μM) incubated with FJU-21 (1.2 mM) for 5, 10, 15, 20, 25, 30 min at 50˚C. (c) BSA (15 μM) incubated with FJU-21 (1.2 mM) for 2, 4, 6, 8, 10, 12 min at 70˚C. Peptide solution was separated from the solid FJU-21 by centrifugation (10,000 rpm, 10 min). The results were measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

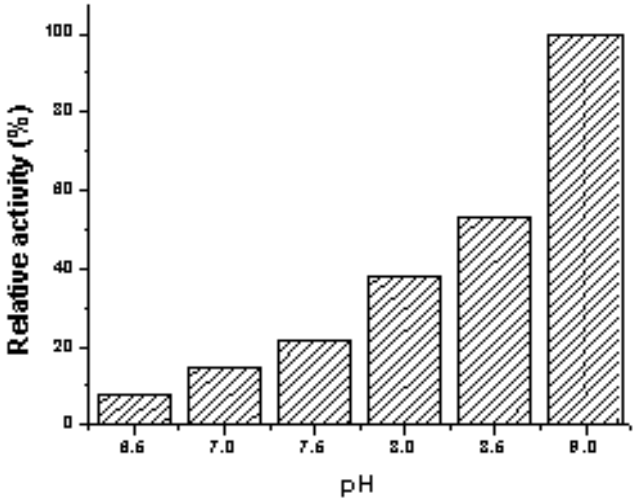

Figure 3. Effect of pH on the catalytic hydrolysis of BSA by FJU-21.

Michaelis-Menten curves were observed (50˚C in **Figure 4(a)** and 70˚C in **Figure 4(b)**) and the data were analyzed by a nonlinear regression program from which important kinetic parameters can be extracted (**Table 1**). The Michaelis constant, Km is a characteristic value irrelevant to the concentrations of substrate and enzyme, and is often associated with the affinity of the catalyst molecules for the substrate [23]. The greater the Km value is, the weaker the binding between the enzyme and substrate. The K_m value of FJU-21with BSA as the substrate under the optimum conditions was about 15-fold lower than that free trypsin (2.7×10^{-3} M^{-1}), and significantly lower than artificial metalloprotease Cu(II) complexes in **Table 1**. In particular, the K_{cat} value of FJU-21 is 110 times higher than that of soluble Cu(II) oxacyclen catalysts and which is slightly higher than Cu-MOF, interestingly, the value of K_{cat}/K_m is 3.72 s^{-1} M^{-1} at 50˚C and pH 9.0 for FJU-21, this is much higher than that for Cu(II) complexes obtained at 50˚C and

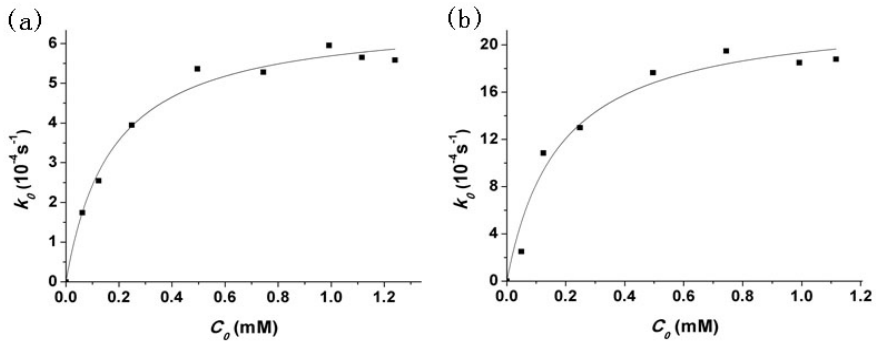

Figure 4. Kinetic data. The relative concentration of substrate was measured by analyzing the density of the electrophoretic bands. ((a), (b)) Dependence of k_0 on C_0 for the cleavage of BSA at 50˚C and 70˚C. Data are analyzed by a nonlinear regression program.

Table 1. Values of kinetic parameters for the cleavage of protein substrates by different catalysts.

Catalysts	pH	T(˚)	K_{cat}(10^{-4} s^{-1})	K_m(10^{-3} M)	K_{cat}/K_m(s^{-1} M^{-1})	Reference
FJU-21	9.0	50	6.69	0.18	3.72	This work
FJU-21	9.0	70	19.38	0.20	9.69	This work
Cu-MOF	9.0	50	6.28 ± 0.39	0.28 ± 0.04	2.23 ± 0.35	[19]
Cu-MOF	9.0	70	20.98± 0.65	0.27 ± 0.02	7.73 ± 0.68	[19]
Cu(II)A-PS	9.5	50	8.0	0.92	0.87	[24]
Cu(II)B-PS	9.5	50	8.7	1.2	0.73	[24]
Cu(II)oxacyclen	9.5	50	0.19 ± 0.003	0.51 ± 0.03	0.036 ± 0.003	[25]
Cu(II)cyclen	9.5	50	N/A	N/A	0.0005	[25]
Cu(II)C	9.5	50	3.89 ± 0.56	0.11 ± 0.03	3.61 ± 0.83	[26]
Cu(II)D	9.5	50	2.77 ± 0.277	0.52 ± 0.1	0.56 ± 0.11	[26]
Cu(II)E	9.5	50	1.89 ± 0.17	0.17 ± 0.03	1.14 ± 0.19	[26]
Cu(II)H	9.5	50	0.42 ± 0.06	0.14 ± 0.04	0.31 ± 0.083	[26]
Cu(II)I	9.5	50	0.47 ± 0.06	0.53 ± 0.16	0.09 ± 0.024	[18]

pH 9.5, suggesting that FJU-21 has a higher affinity for BSA than trypsin and most of the artificial metalloprotease Cu(II) complexes.

3.3. Reusability of FJU-21

As a novel mimic enzyme, since FJU-21 can be separated from the hydrolysate and used repeatedly, it could also be used as an effective heterogeneous proteolytic catalyst, which gives rise to the most prominent advantages. As shown in **Figure 5**, even after eleven times use, catalytic activity displayed no significant decrease and at least 80% of the original activity could be maintained. It could be concluded that FJU-21 exhibited both excellent catalytic ability and stability. Therefore, a pleasing prospect is that such artificial protease is safe for mass production in industrial applications.

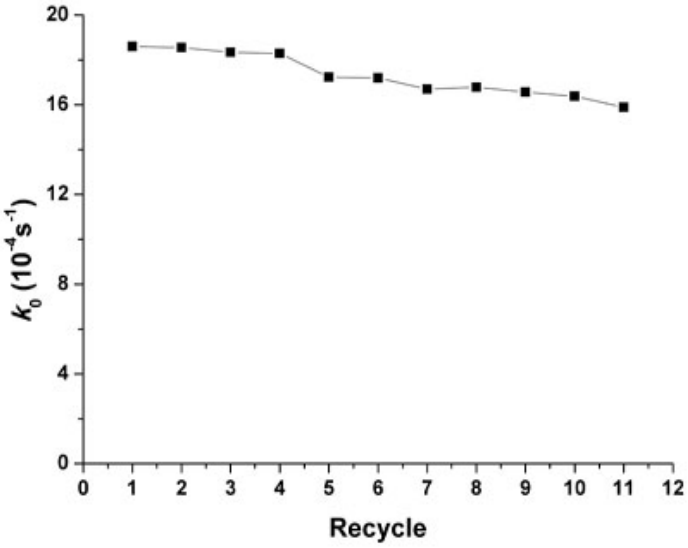

Figure 5. Reusable experiments of FJU-21 hydrolyzing activity. BSA (15 μM) incubated with FJU-21 (1.2 mM) at pH 9.0 and 70˚C for 12 min. FJU-21 was recollected by centrifugation and shaken in water for 5 min. Then the catalyst was washed with ethanol three times. The solvent was removed under vacuum at 85˚C for 3 h. The recycling was conducted eleven times.

4. Conclusion

FJU-21 has excellent hydrolysis intrinsic protease-like activity, catalyzing the proteins (BSA) and exhibited surprisingly high catalytic activity over a wide temperature range, event at 37˚C. It also exhibited good stability during hydrolyzing reaction and could be reused at least eleven times without losing a significant amount of its activity. Kinetic analysis indicates that the activity of FJU-21 was consistent with the typical Michaelis-Menten kinetics, the K_m value of FJU-21with BSA as the substrate under the optimum conditions was about 15-fold lower than that free trypsin (2.7×10^{-3} M^{-1}), and significantly lower than artificial metalloprotease Cu(II) complexes, indicating that FJU-21 is superior to trypsin and most of other peroxidase mimetics under the same conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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