Biomimetic sequestration of CO_2 and reformation to $CaCO_3$ using bovine carbonic anhydrase immobilized on SBA-15

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생체모방공학을 이용한 bovine carbonic anhydrase를 SBA-15에 고정화하여 이산화탄소분리와 재구성된 $CaCO_3$ 연구

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ABSTRACT

The biocatalytic capture of CO_2,and its precipitationas $CaCO_3$, over bovine carbonic anhydrase (BCA) immobilized on a pore-expanded SBA-15 support was investigated. SBA-15 was synthesized using TMB as a pore expander, and the resulting porous silica was characterized by XRD, BET, IR, and FE-SEM analysis. BCA was immobilized on SBA-15 through various approaches, including covalent attachment (BCA-CA), adsorption (BCA-ADS), and cross-linked enzyme aggregation (BCA-CLEA). The immobilization of BCA on SBA-15 was confirmed by the presence of zinc metal in the EDXS analysis. The effects of pH, temperature, storage stability, and reusability on the biocatalytic performance of BCA were characterized by examining para-nitrophenyl acetate (p-NPA) hydrolysis. The K_{cat}/K_m values for p-NPA hydrolysis were 740.05, 660.62, and 680.11$M^{-1}s^{-1}$, respectively, where as K_{cat}/K_m for free BCA was 873.76$M^{-1}s^{-1}$. The amount of $CaCO_3$ precipitate was measured quantitatively using anion-selective electrode and was found to be 12.41, 11.82, or 11.28 mg $CaCO_3$/mg for BCA-CLEA, BCA-ADS, or BCA-CA, respectively. The present results indicate that the immobilized BCA-CLEA, BCA-ADS, and BCA-CA are green materials, and are tunable, reusable, and promising biocatalysts for CO_2sequestration.

1. INTRODUCTION

The most significant means of energy generation throughout the world is the combustion of fossil fuels, which results in the emission of the greenhouse gas CO_2. The accumulation of green house gases in the atmosphere leads to global warming; hence huge efforts are be ingtargeted at mitigating the accumulation of such gases. Natural carbonate minerals have regulated the atmospheric levels of CO_2 throughout earth's early history. Recent growth of economy and the increasing population have contributed to the ever-increasing atmospheric CO_2 levels. Hence,the necessity to control CO_2 concentrations in the atmosphere without disturbing the use of fossil fuels presents a challenge for researchers. Alargevariety of CO_2 sorbents have been reported in the literature, including oxides [1], zeolites [2], activated carbon [3], metal-organic frameworks [4,5], organo-silicas, surface-modified silicas [6-10], and amine or alkanolamines [11,12].

However, development of commercial CO2 capture technologies is expensive, energy-intensive, and results in equipment corrosion, thereby reducing plant efficiency [13].

Currently, novel biomimetic approaches to CO_2sequestrationbasedontheenzymebovinecarbonicanhydrase(BCA)asabiocatalyst have attracted attention, and a proof of principle has already been demonstrated [14,15]. The present study demonstrates the immobilization of the enzyme BCA onto mesoporous silica through various techniques, and the catalytic activity of BCA was tested by examining the hydrolysis of para-nitrophenyl acetate (p-NPA). This study was extended to test the biocatalytic activity of immobilized BCA measured by the quantity of precipitated $CaCO_3$ after hydration of CO_2.

2. Materials and methods

2.1 Materials

Bovine carbonic anhydrase (BCA), tetraethyl orthosilicate (TEOS) 3- aminopropyl triethoxysilane(APTES), P123 (triblock copolymer (poly(ethyleneoxide) - block-poly(propylene oxide) -block-poly(ethyleneoxide), EO_{20}-PO_{70}-EO_{20}, MW5800)), hydrochloricacid (HCl,37%), trimethyl benzene (TMB), glutaraldehyde (GA), 2-amino 2-(hydroxymethyl)-1,3-propanediol (Tris base), p-nitrophenol (p-NP), p-nitrophenyl acetate (p-NPA), acetonitrile, anhydrous calcium chloride, and protein assay reagents for producing the Bradford reagent were purchased from Aldrich and used without purification. All solutions were prepared with deionized water

2.2. Synthesis of amine-functionalized mesoporous SBA-15

Mesoporous silica SBA-15 was synthesized as per the reported procedure [16]. The molar composition of the reaction mixture was 1 TEOS: 0.017 P123: 5.87 HCl: 0.0025 TMB: 183 H_2O. Briefly,4g pluronic P123 was dissolved in 150mL 1.6MHCl at 40°C, 0.3g TMB and 9.2 mL TEOS were the nadded. The mixture was stirred at 40°C for 8 h then aged at 80°C for 24 h. The recovered as-synthesized sample was calcined in air at 550°C for 6 h with a heating rate of 1°C/min. Amine-functionalized SBA-15 was obtained by refluxing 1 g SBA-15 and 5 mmol APTES solution in dry toluene (100 mL) under a N_2 atmosphere for 24h. The product was then filtered, washed with ethanol, and dried over night at 80°C under vacuum. The obtained material was designated SBA-15/APTES.

2.3 Enzyme immobilization over the amine-functionalized SBA-15

BCA immobilization over the mesoporous silica was performed by three methods via cross-linked enzyme aggregation (CLEA), adsorption (ADS), and covalent attachment (CA) [17, 18]. Typically, CLEA of BCA was achieved by mixing 10 mg SBA-15 with BCA in a buffer (100 mM sodium phosphate buffer, pH 7.0) and incubating at 25°C with shaking. After 1 h incubation to allow for BCA adsorption, the samples were washed with minimum amounts of an aqueous buffer (100 mM sodium phosphate buffer, pH 7.0) then incubated in a sodium phosphate buffer containing 0.1% GA at 150 rpm with shaking. After GA treatment for 30 min, the samples were washed with copious amounts of Tris buffer (50 mM pH 8.0) then stored at 4°C. The final product was designated BCA-CLEA.BCA immobilization by the ADS method was performed by a procedure that was similar to that described for preparing CLEA-BCA without the GA treatment step, and the product was denoted BCA-ADS. Subsequently, the above-recovered product was treated with BCA by a procedure similar to that of the CLEA method to obtain BCA-CA. The amount of attached and leached BCA was calculated by the Bradfordmethod[19].

2.4. Hydrolysis of p-NPA using immobilized enzyme

The enzyme activities of BCA-CLEA, BCA-ADS, and BCA-CA were estimated spectrophotometrically using p-NPA as a substrate, as reported, with slight modification [20, 21]. Here, enzyme activities were measured at 25°C by monitoring the changes in concentration of p-NP, which is one of the hydrolysis products of p-NPA. For the free BCA, the activity assay

was performed in a 1 mL UV cuvette. The reaction mixture, composed of 0.8 mL Tris Buffer (50 mM, pH 8), 0.1 mL substrate solution (p-NPA dissolved in acetonitrile), and 0.1 mL enzyme solution, was mixed in the cuvette using a micropipette. The final mixture contained 10% acetonitrile. The enzyme activity was measured in an Optizen 2120F UV/vis spectrophotometer at 400nm. The free enzyme activity was determined by varying the concentration of the substrate while holding the enzyme concentration constant, or by varying the enzyme concentration while holding the substrate concentration constant. Blank experiments were also conducted to estimate the self-dissociation of p-NPA in each assay solution.

2.5. CO2 sequestration and evaluation of $CaCO_3$

$CaCO_3$ precipitation was carriedout after hydration of CO_2 in a fixedbed for medin a glasstube. Typically, 25 mg immobilized enzyme were packed in a glasstube equipped with an in fusion pump, and the catalyticbed temperature was maintained at 30°C. A saturated CO_2 solution was introduced at a flow rate of 0.9mL/min and mixed with 0.1mL/min 0.5 M Tris base before reaching the catalytic bed. The hydrated $CO_2(HCO_3^-)$ was collected in a beaker containing 40mL 4% $CaCl_2$ solution in 0.5M Trisbase and was subsequently precipitated as $CaCO_3$.

3. Results and Discussions

3.1. Characterization of SBA-15 and SBA-15/APTES

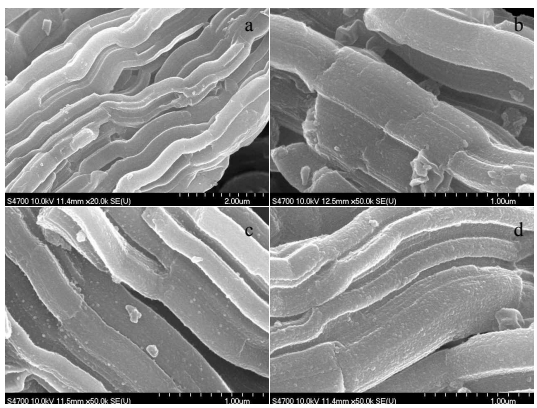
The XRD patterns of SBA-15 and SBA-15/APTES (data not shown) exhibited a well-ordered hexagonal mesophase. The (100), (110), and (200) silica reflections were observed at 0.92, 1.41, and 1.58° (2Θ), respectively. The intensities of the higher order peaks at 2Θ = 1.41 and 1.58° were lower in the parent SBA-15 due to the pore expansion achieved by addition of TMB during synthesis. SBA-15/APTES showed a decrease in peak intensity rather than a change or shift in the peak position due to scattering contrast between the silica wall and the pore channel upon grafting with APTES (data not shown). SBA-15 and SBA-15/APTES exhibited a well-defined hysteresis loop between the partial pressures of P/Po = 0.68 - 0.75, indicating a mesophase. The BET results suggested that SBA-15/APTES showed a decrease in the specific area corresponding to the pore volume and pore diameter relative to SBA-15. Such a significant decrease in the textural properties of SBA-15/APTES was due to pore filling and/or structural construction of APTES, which was in line with the XRD results [22].

[Table 1] Textural properties and zeta potential of nanocatalyst

Samples	S_{BET} (m^2/g)	V_{total} (cm^3/g)	D_A (nm)
SBA-15	633	1.42	15.2
BCA - ADS	286	0.76	6.2
BCA - CA	327	0.67	7.2
BCA - CLEA	213	0.63	5.6

* S_{BET} -surfacearea; V_p - meanporevolume; D_p - porediameter

Figure. 1 shows the surface morphologies of the parent SBA-15, BCA-ADS, BCA-CA, and BCA-CLEA materials obtained by FE-SEM analysis. The parent SBA-15 displayed ropelike hexagonal rods. The surface morphologies of the immobilized BCA-ADS, BCA-CA, and BCA-CLEA (Figure. 1 (b,c,d)) also appeared as hexagonal rods, which provided evidence for the preservation of the hexagonal structure (mesoporosity) even after enzyme immobilization.



[Figure 1] FE SEM images of (a) SBA-15, (b) BCA-ADS, (c) BCA-CA and (d) BCA-CLEA

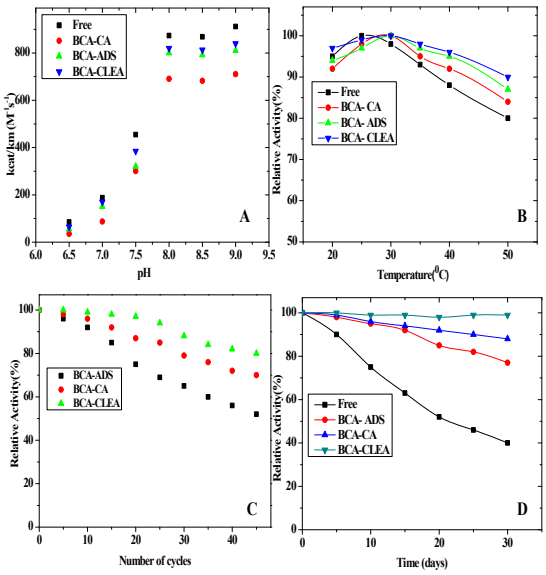
3.2. Biocatalytic activity of free and immobilized enzyme for hydrolysis of p-NPA

It has been reported that the enzymatic hydrolysis of p-NPA for the production of p-NP and acetic acid proceeds through an acyl-enzyme intermediate [23]. The acyl-enzyme intermediate of CA is actually a zinc-acetate complex that is less stable and dissociates to release BCA, p-NP, and acetic acid. This latter step is the rate-determining step, which releases BCA for further acetylation [24]. The enzymatic activities of the free and immobilized BCA were determined by while keeping the enzyme or/and p-NPA concentrations constant. The concentration of p-NPA was varied up to 2.5 mM. Figure. 3 shows the kinetics study of the free and immobilized BCA in Tris buffer (50 mM) in the presence of 10% acetonitrile. The K_{cat}/K_m was maximized at pH8 for the free and BCA-CLEA, BCA-ADS, and BCA-CA, and was found to be 873.76, 820.06, 800.11, and 690.50 $M^{-1}s^{-1}$, respectively(fig 2). The kinetic parameters for the hydrolysis of pNPA were estimated using the Michaelis - Menten equation (eq.1) and the Lineweaver - Burke quation for the double-reciprocal(eq.2):

$$V = \frac{K_{cat}[p-NPA][E_0]}{K_m + [p-NPA]} \longrightarrow (1)$$

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\frac{1}{[p-NPA]} \longrightarrow (2)$$

where V is the rate of p-NP formation, V_{max} is the maximum rate, k_{cat} is the catalytic rate constant,$[E_0]$ is the enzyme concentration, [p-NPA] is the substrate concentration, K_m is the substrate concentration when the rate is equal to $V_{max}/2$, which also shows the affinity of the enzyme for the substrate, and k_{cat}/K_m is the kinetic constant.



[Figure 2] Biocatalytic activities of free BCA, BCA-CLEA, BCA-ADS and BCA-CA (a) hydrolysis of p-NPA, (b) temperature, (c) reusability, and (d) storage stability.

3.3. Sequestration of CO_2 into Ca^{2+}over BCA-CLEA, BCA-ADS, and BCA-CA

$CaCO_3$ precipitation of the hydrated CO_2 collected through the fixed bed reactor using BCA-CLEA, BCA-ADS, and BCA-CA was carried out at pH=8.0, temperature 30°C for a constant volume of CO_2(aq).The results indicated that the amount $CaCO_3$ precipitated over BCA-CLEAwas nearly equal to that over free BCA, where as slightly smaller values were measured for BCA-ADS and BCA-CA.This was due to the decreased amount of hydrated CO_2 produced by BCA-ADS and BCA-CA. The quantities of $CaCO_3$ precipitates were measured.The results suggest that both the free enzyme and BCA-CLEA gave nearly equal quantities of $CaCO_3$.

4. Conclusions

Various immobilization techniques such as covalent attachment (BCA-CA), adsorption (BCA-ADS) and cross-linked enzyme aggregation (BCA-CLEA) were adopted to immobilize BCA over SBA-15. Immobilization of enzyme on SBA-15 was verified by observing the presence of zinc in the EDXS. The SEM images show the retainment of hexagonal structure of SBA-15

even after functionalization and subsequent immobilization with BCA. The biocatalytic activities of BCA-CLEA, BCA-ADS, and BCA-CA were investigated for hydrolysis of p-NPA. The k_{cat}/K_m valuesfor BCA-CLEA were high compared to BCA immobilized by CA over a silica gel reported by another researcher. BCA-CLEA was observed to be thermally stable, reusable, tunable, and storage stability as determined by p-NPA hydrolysis. The precipitation of hydrated CO_2 to form $CaCO_3$ was performed in a fixed bed using each immobilized BCA, and precipitation was quantified by the CISEmethod. The amount of $CaCO_3$ precipitated over BCA-CLEA (12.41 mg $CaCO_3$/mg BCA-CLEA) washing than that precipitatedoverBCA-ADSorBCA-CA. In addition, the recycle runs of $CaCO_3$ precipitation through BCA-CLEA gaven early equal amounts of $CaCO_3$, even after 10runs. The XRD and FESEM analysis of $CaCO_3$ through BCA-CLEA indicated the presence of calcite phase. Thus, BCA-CLEA provides a green, stable, reusable and convenient biocatalyst for CO_2 sequestration and produces pure calcite phase $CaCO_3$.

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