Biochemical and Biophysical Research Communications 452 (2014) 147–150

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Biocatalytic carbon capture via reversible reaction cycle catalyzed by isocitrate dehydrogenase

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ARTICLE INFO

Article history:
Received 11 August 2014
Available online 22 August 2014

Keywords:
Carbon dioxide
Carbon capture and storage
Biocatalysis
Isocitrate dehydrogenase
Mesocellular silicon
Biochemical reaction equilibrium

ABSTRACT

The practice of carbon capture and storage (CCS) requires efficient capture and separation of carbon dioxide from its gaseous mixtures such as flue gas, followed by releasing it as a pure gas which can be subsequently compressed and injected into underground storage sites. This has been mostly achieved via reversible thermochemical reactions which are generally energy-intensive. The current work examines a biocatalytic approach for carbon capture using an NADP(H)-dependent isocitrate dehydrogenase (ICDH) which catalyzes reversibly carboxylation and decarboxylation reactions. Different from chemical carbon capture processes that rely on thermal energy to realize purification of carbon dioxide, the biocatalytic strategy utilizes pH to leverage the reaction equilibrium, thereby realizing energy-efficient carbon capture under ambient conditions. Results showed that over 25 mol of carbon dioxide could be captured and purified from its gas mixture for each gram of ICDH applied for each carboxylation/decarboxylation reaction cycle by varying pH between 6 and 9. This work demonstrates the promising potentials of pH-sensitive biocatalysis as a green-chemistry route for carbon capture.

1. Introduction

Carbon capture and storage (CCS) is widely viewed as a promising and practical approach to the abatement of carbon emission which has been causing global climate changes [1]. As most carbon emission takes place in form of gas mixtures (for example, flue gas of coal-fired power plants contains 13–15% CO_2), CCS requires a process that can efficiently separates CO_2 from its gaseous mixtures, so that it can be subsequently released as a pure gas which can be liquefied and injected into underground storage sites [2]. By controlling the temperature and pressure, pure water can be used as a reaction medium that can capture and reversibly release CO_2, although the equilibrium absorption capacity of water is generally regarded as too low to be efficient. The application of carbonic anhydrase (CA) in water could increase the reaction rate by speeding up the dissolution reaction of CO_2 into HCO_3^- [3], but it does not necessarily improve the equilibrium carbon capture capacity of water. In order to improve the equilibrium adsorption capacity, chemical sorbents including monoethanolamine (MEA) have been commonly applied with substantially improved carbon capture capacity. However, it requires high temperatures to reverse the reactions, thereby releasing the captured carbon dioxide into a pure gas for subsequent storage and at the same time regenerating the chemical sorbents, for which energy consumption is generally high [4].

Recent studies have shown that many CO_2-associated biotransformations could be reversibly conducted *in vitro*, thus realizing biosynthesis such as production of methanol and pyruvate from CO_2 [5,6]. Theoretical analysis also showed that the direction of such reactions could be controlled and manipulated by altering pH or ionic strength, instead of temperature, of the reaction media [7]. The reversibility of such biocatalytic processes promises energy-efficient carbon capture and release reaction cycles, which may offer a green-chemistry alternative to the energy-intensive thermochemical carbon capture strategy. In this work, a pH-dependent carbon capture and release strategy is examined by realizing carboxylation/decarboxylation reactions catalyzed by isocitrate dehydrogenase (ICDH).

2. Materials and methods

2.1. Materials and chemicals

α-Ketoglutaric, NADPH/$NADP^+$, D-isocitrate, sodium bicarbonate, 2-(N-morpholino) ethanesulfonic acid (Mes), monopotassium

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http://dx.doi.org/10.1016/j.bbrc.2014.08.058
0006-291X/

phosphate, dipotassium phosphate, isocitrate dehydrogenase from porcine heart (ICDH), mesostructured silica foam (MSF, 560979) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemical agents were purchased and used with the highest purity available.

2.2. Enzyme immobilization

Deionized water was used to dissolve the enzyme ICDH to a final concentration of 100 mg/ml, MSF (1 g) was then added for every 1 ml of the enzyme solution. After mild shaking at 200 rpm for 5 min, the solid was recovered by centrifugation and washed by 5 ml DI water for 3 times. The protein concentration in supernatant and washing solutions was detected by Bradford protein assay and was used to calculate the enzyme loading on MSF.

2.3. Enzyme activity assay

For decarboxylation reaction, the activity assay reaction mixture contained 10 mM isocitrate, 2 mM $NADP^+$, 40 mM $MgCl_2$, 0.1 mg free or 20 mg immobilized enzyme in 1 mL of buffer solution. (N-morpholino)ethanesulfonic acid (Mes) buffer was used for pH 5.0 solution, while phosphate buffer was used for pH 6.0–9.0. For free native enzyme, the reaction solution was monitored continuously with a UV–Vis spectrometer (50 Bio, Varian) for changes in the concentration of NADPH via absorbance at 340 nm. For assay of immobilized enzyme, the reaction mixture was shaken under room temperature at 200 rpm, with assay aliquots of 200 μl were periodically taken for detection of NADPH concentration after the reaction was stopped by centrifugation and the supernatant diluted with adequate amount of fresh buffer. One unit of decarboxylation activity was defined as 1 μmol of NADPH produced per min. The carboxylation activity was detected in the same way except the reaction mixture constituted 20 mM sodium ketoglutarate, 3 mM NADPH, 40 mM MgCl2, 35 mM $NaHCO_3$. One unit of carboxylation activity was defined as 1 μmol of NADPH consumed in one minute.

2.4. Reaction equilibrium of carboxylation/decarboxylation reaction

Reaction equilibrium was determined by finding a zero net reaction rate by varying concentrations of the reactants and products. Specifically, concentrations of ketoglutarate, isocitrate, $NaHCO_3$ were fixed as 8, 1, and 3.5 mM, respectively; while the concentrations of NADPH and $NADP^+$ were adjusted, thereby achieving manipulation of the reaction rate, as well as the direction of the reaction to allow either carboxylation or decarboxylation (as indicated by consumption or generation of NADPH). An equilibrium point was determined by extrapolating the reaction rate-NADPH concentration curve to zero reaction rate, whereas a shift between carboxylation and decarboxylation reaction takes place.

2.5. Enzyme stability tests

Free or immobilized ICDH was kept in phosphate buffer (pH 6 or 9) for predetermined time periods, and the remaining activity of the enzyme samples for decarboxylation was detected with the same methods as described for enzyme activity assay. Activity loss vs conditioning time was examined as the indicator of enzyme stability.

2.6. Carbon capture reaction cycles by pH shifting

Carboxylation and decarboxylation reaction cycle was repeated several times by adjusting the pH of the reaction medium back and forth between 6 and 9. Specifically, 10 ml pH 7 phosphate buffer (100 mM) that contained 20 mM sodium ketoglutarate, 1 mM NADPH, 40 mM $MgCl_2$, and 3.5 mM $NaHCO_3$ was prepared and mixed with 1 g immobilized ICDH (containing 20 mg enzyme) under shaken condition (200 rpm). For each reaction cycle, the pH of system was first adjusted to 6 by adding hydrochloric acid. The reaction was allowed to continue for 20 min before 200 μl of the supernatant was taken from the reaction solution for determination of NADPH concentration. The pH of the reaction system was adjusted to 9 at the same time by adding sodium hydroxide, and NADPH concentration was detected again after 20 min of reaction.

3. Results and discussion

3.1. The reversibility of the carbon conversion reaction

ICDH is an enzyme found both in the tricarboxylic acid (TCA) and reductive tricarboxylic acid (RTCT) cycles [8–10], indicating the ability of the enzyme to catalyze the following reaction for ether carboxylation and decarboxylation:

$$\alpha - \text{Ketoglutarate} + CO_2 + \text{NADPH} \leftrightarrow \text{D} - \text{isocitrate} + NADP^+ + H_2O$$

Generally speaking, the direction of such a reversible reaction system under given reaction conditions is determined by the availability of the reactants, driven thermodynamically to reach reaction equilibrium. Mostly due to the protonated nature of the chemical species involved in the reaction, the reaction equilibrium constant can be, however, pH-dependent [7]. That allows one to manipulate the reaction equilibrium constant to be more favorite to carboxylation (forward reaction of the above equation), or decarboxylation as needed. Achieving such carboxylation/decarboxylation reaction cycles will allow capture and purification of carbon dioxide from a gas mixture under ambient conditions, offering energy efficient alternatives to thermally-driven carbon capture chemical reactions [1,2].

To examine the pH-sensitivity of the reaction, specific activities of free ICDH for carboxylation and decarboxylation under different pH were first examined. As indicated by the activity data shown in Fig. 1, the enzyme could afford much faster reactions for decarboxylation than carboxylation, a reflection of the intrinsic nature of the enzyme and reactants involved. However, carboxylation and decarboxylation reactions showed different preference of pH. It appeared that the decarboxylation reaction activity peaked at pH 8, while highest carboxylation activity was observed at pH 6 (Fig. 1). This observation agreed well in principle with thermodynamic analysis of cofactor-mediated biocatalytic carbon conversion reactions [7], whereas lower pH is preferred for carboxylation reaction, while higher pH promotes decarboxylation reactions.

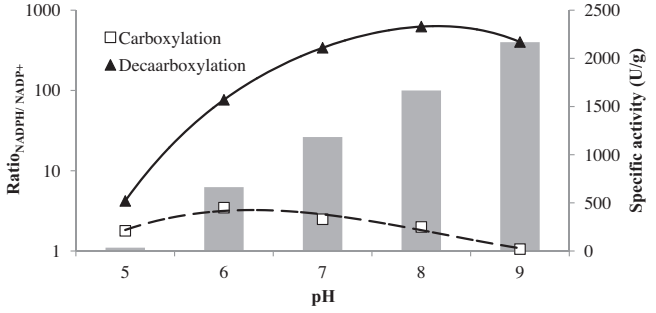

Fig. 1. Effect of pH on carboxylation and decarboxylation activity of free ICDH ($R_{[NADH]/[NADP+]}$: concentration ratio between NADPH to $NADP^+$ at equilibrium; solid line: specific activity of ICHD for carboxylation reaction; dotted line: specific activity of ICDH for decarboxylation reaction).

In addition to the pH-dependence of reaction rate, we further examined reaction equilibrium under different pH. The reaction equilibrium was determined by finding concentrations of reactants and products that afford zero reaction rate, either carboxylation or decarboxylation reaction. Specifically, the concentrations of species of both sides of the above reaction equation were fixed except those of the two forms of the cofactor, NADPH/$NADP^+$, which were manipulated to leverage the direction of reaction. The reaction was followed by monitoring the change of concentration of NADPH for either carboxylation (as indicated by decrease in NADPH concentration) or decarboxylation (increase in NADPH concentration). Initial reaction rates for either case were determined. Faster reaction rates indicate initial conditions are set far away from equilibrium; while when the initial reaction rate approaches zero, from either backward or forward directions, a reaction equilibrium is assumed realized. The ratio between the concentrations of NADPH an $NADP^+$ at equilibrium ($R_{[NADPH]/[NADP+]}$) was then calculated as an index of the reaction direction preference (data shown as columns in Fig. 1). Apparently, the value of $R_{[NADPH]/[NADP+]}$ increased almost linearly as the pH increased from 5 to 9, indicating again decarboxylation is preferred at higher pH, while carboxylation reaction is preferred for lower pH. By applying concentrations of all the chemical species involved in the reaction, reaction equilibrium constants were also calculated, with a value of 42.8 determined for pH 5 and 0.089 for pH 9. These results were comparable to the theoretical values reported for the reaction which predicted a value of 6.94 for pH 5, and 0.0015 for pH 9 [11]. The discrepancy between the measured and theoretical values may be attributed to the non-ideality of the chemical species involved, while the general trend in terms of pH-dependency of the reaction equilibrium is apparently in a good agreement.

3.2. Enzyme immobilization against pH inactivation

According to the above observation of reaction equilibrium constants, we may assume that larger carbon capture capabilities should be achieved with greater deviations of the pH's from the neutral point for the carboxylation/decarboxylation reactions. One potential concern for that is, of course, enzyme inactivation caused by structural denaturation under pH that is not native to the enzyme. Although it is not an intent of this work to a systematically study stabilization of ICDH, we examined the effect of enzyme immobilization via a simple adsorption method on the stability of ICDH as an attempt to minimize the effect of enzyme inactivation for the current evaluation on the carbon capture potentials of the enzyme. Mesocellular silica foam (MSF) was selected for enzyme immobilization due to its high surface area-volume ratio. The MSF has been reported previously for excellent ability for enzyme adsorption [12,13]. Enzyme loadings achieved under different pH were shown in Fig. 2 along with the specific activities of the immobilized ICDH. The immobilization appeared to be insensitive to pH in that both specific activities and enzyme loadings remained approximately unchanged, indicating enzyme immobilization should be stable even if the pH of the reaction gets shifted back and forth for carboxylation/decarboxylation reaction cycles. For a wide range of pH (5–9), 20–30 mg enzyme could be absorbed on 1 g of MSF within 5 min, with specific activity of decarboxylation in the order of 6 U per gram MSF (Fig. 2). Compared to free ICDH, the stability of the immobilized enzyme was apparently improved (Fig. 3). For example, at pH 6, the half lifetime ($t_{1/2}$) of immobilized ICDH was around 18 h, much higher than that of the free enzyme (8 h). For pH 9, $t_{1/2}$ of the enzyme was also improved from 1.5 to 3.5 as a result of immobilization.

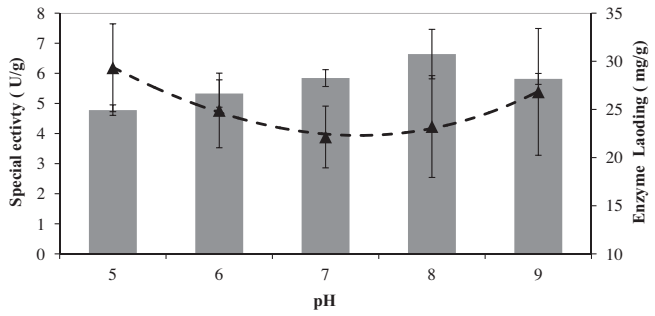

Fig. 2. Enzyme loading and specific activity for enzyme immobilization at different pH (dotted line: specific activity; column: enzyme loading).

3.3. Carbon capture reaction cycles

To demonstrate the carbon capture potentials of ICDH, the immobilized enzyme was applied to continuously catalyze both carboxylation and decarboxylation reactions by shifting the pH of the reaction medium between 6 and 9. As shown in Fig. 4, for the first cycle of pH shifting, ~45% of totally added cofactor remained as NADPH at pH 6, while ~95% of the added cofactor converted back to NADPH when the pH was adjusted to 9. That means that at least 50% of the added cofactor was involved in the carboxylation/decarboxylation reaction cycle. In other words, 1 mol of NADPH has the capability of capturing 0.5 mol of carbon dioxide for each pH shifting cycle. Considering the applied catalyst contained only ~20 mg of ICDH, each gram of ICDH can therefore be

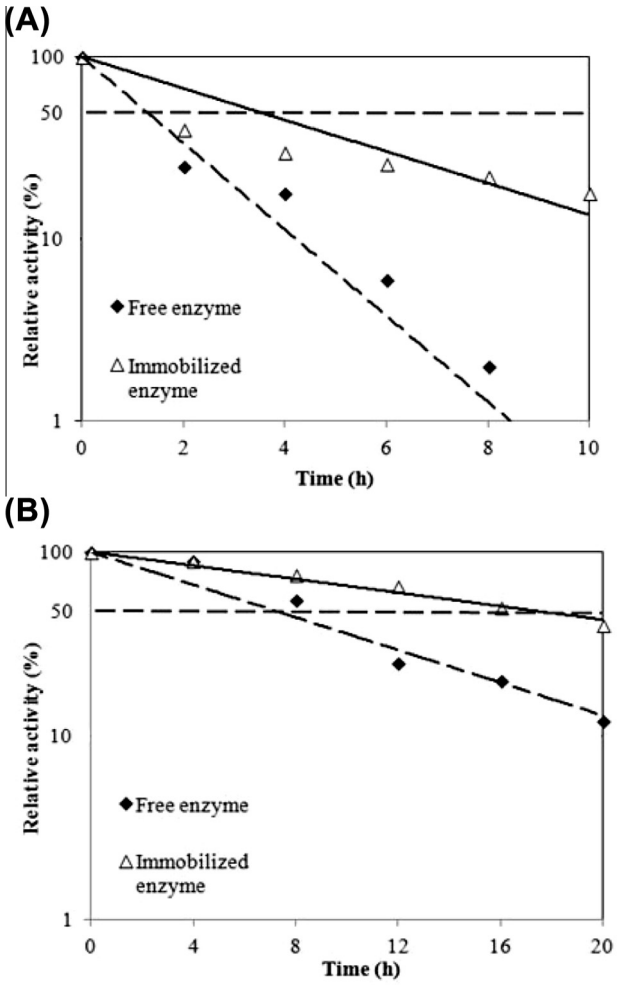

Fig. 3. Effect of immobilization on enzyme stability (diamond: free enzyme; triangle: immobilized enzyme. (A) pH 9; (B) pH 6).

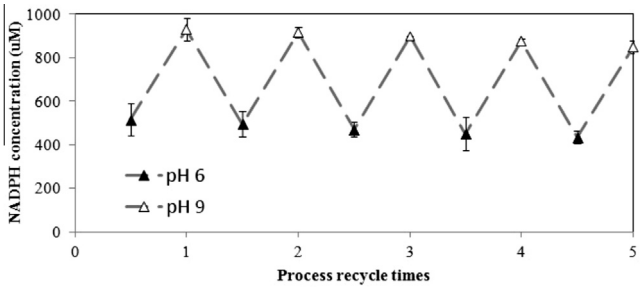

Fig. 4. Carbon capture-release cycles realized by pH shifting between 6 and 9 (filled triangle: concentration of NADPH at pH 6 at equilibrium; open triangle: concentration of NADPH at pH 9; the total added cofactor was 1000 μM).

assumed to have the capability of capturing 25 mol of CO_2. The reaction medium was then adjusted to pH 6, and then pH 9 for a second carbon capture cycle, and continued for subsequent reaction cycles from there. The carbon capture capacity of the system remained unchanged throughout the 5 capture-release cycles tested (Fig. 4).

In conclusion, a novel carbon capture strategy was demonstrated through ICDH-catalyzed pH-sensitive reversible carboxylation reaction. Carboxylation reaction was preferred under acidic conditions while decarboxylation reaction could be realized at basic conditions, as indicated by both peak reaction rates and reaction equilibrium constant. For each mole of added cofactor, one carboxylation/decarboxylation reaction cycle has the capability to capture 25 mol of carbon dioxide for each gram of applied enzyme. Obviously, the overall productivity of the enzyme will be eventually limited by its stability, which can be improved via either protein engineering and/or immobilization. In addition, previous studies have shown that ICDH from different sources may show different preference for carboxylation or decarboxylation [14,15]. For example, ICDH comes from green sulfur bacterium has a carboxylation activity higher than its decarboxylation (2000 vs 1200 U/g at pH 6). Accordingly, a combination of enzymes from different sources with optimal carboxylation and decarboxylation activities, as well as different cofactor preferences [15], may further improve the carbon capture capability of the system.

Acknowledgments

The authors thank supports from the Biocatalysis Initiative grant from UMN, and the 'International Collaborative R&D Program' of the Korea Institute of Energy Technology Evaluation and Planning (KETEP, #20118510020020).

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