Effect of Carbonic Anhydrase on CO\textsubscript{2} Separation Performance of Thin Poly(amidoamine) Dendrimer/Poly(ethylene glycol) Hybrid Membranes

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Abstract: The effect of carbonic anhydrase (CA) on the separation performance of thin poly(amidoamine) (PAMAM) dendrimer/poly(ethylene glycol) (PEG) hybrid membranes was investigated. CA, a type of enzyme, was used to promote CO\textsubscript{2} hydration and dehydration reactions and to assess whether these reactions were the rate-limiting step in CO\textsubscript{2} permeation through the membrane. The relationship between the membrane thickness and the CO\textsubscript{2} permeance was evaluated in CO\textsubscript{2}/H\textsubscript{2} or CO\textsubscript{2}/He separation using PAMAM/PEG hybrid membranes (thickness: 10–100 µm) with and without CA. Without CA, the CO\textsubscript{2} permeance of PAMAM/PEG hybrid membranes was not inversely proportional to the membrane thickness. On the other hand, with CA, the CO\textsubscript{2} permeance was inversely proportional to the membrane thickness. It was implied that, without CA, the rate-limiting step of CO\textsubscript{2} transport was either the CO\textsubscript{2} hydration reaction at the feed side or the CO\textsubscript{2} dehydration reaction at the permeate side. On the other hand, with CA addition, the rate-limiting step of CO\textsubscript{2} transport was diffusion, and CO\textsubscript{2} permeance could be increased without sacrificing the selectivity by reducing membrane thickness. The effect of the position of CA (i.e., on the surface and/or reverse surface) on CO\textsubscript{2} separation performance was investigated to evaluate which reaction was the rate-limiting step of CO\textsubscript{2} permeation through the membrane. It was suggested that the rate-limiting step of CO\textsubscript{2} permeation was CO\textsubscript{2} dehydration reaction at the permeate side.

Keywords: CO\textsubscript{2} separation; poly(amidoamine) dendrimer; carbonic anhydrase (CA); rate-limiting step; membrane thickness

1. Introduction

CO\textsubscript{2} capture and storage (CCS) is widely accepted as an important option for mitigating climate change, and has been attracting worldwide attention [1]. CO\textsubscript{2} capture includes CO\textsubscript{2} separation from flue gas (post-combustion, CO\textsubscript{2}/N\textsubscript{2}), CO\textsubscript{2} separation from natural gas (CO\textsubscript{2}/CH\textsubscript{4}), and CO\textsubscript{2} separation from integrated gasification combined cycle (IGCC) processes (pre-combustion, CO\textsubscript{2}/H\textsubscript{2}) [2,3]. For practical application of the CCS technology, cost-effective methods for CO\textsubscript{2} capture are required. Many studies have focused on the development of effective CO\textsubscript{2} capture and separation technologies. Membrane separation would be one of the most promising approaches among them in terms of technological and economic perspectives. Polymeric membranes [4,5], inorganic membranes [6,7], ionic liquid membranes [8], and facilitated transport membranes [9,10] were studied for CO\textsubscript{2} separation. Most of the CO\textsubscript{2} selective membranes were developed for post-combustion. If CO\textsubscript{2} selective membranes are used for pre-combustion (high pressure gas), H\textsubscript{2} can be kept at high pressure in the retentate side and directly fed to gas turbine without compression, and CO\textsubscript{2} can transport though the membrane without using vacuum pump in the permeate side, so it is energy and cost saving to apply a CO\textsubscript{2} selective
membrane for pre-combustion. However, it is very difficult to separate CO\textsubscript{2} over H\textsubscript{2}, because the molecular size of CO\textsubscript{2} is greater than that of H\textsubscript{2}, and only limited numbers of CO\textsubscript{2} selective membranes were reported for this purpose [11–13].

It was reported that poly(amidoamine) (PAMAM) dendrimer showed excellent CO\textsubscript{2}/N\textsubscript{2} separation performance in a liquid immobilized membrane (ILM) under 200 kPa low pressure [14]. In our group, PAMAM dendrimer/crosslinked-polymer hybrid membranes, such as PAMAM dendrimer/poly(ethylene glycol) (PEG) hybrid membranes [11–13] have been developed by immobilizing PAMAM dendrimer into the cross-linked polymer matrix for use in high pressure CO\textsubscript{2} separation. We found that these membranes show high CO\textsubscript{2}/H\textsubscript{2} separation performance at pressurized conditions, and have the potential to be applied for pre-combustion. In our previous paper, we developed hybrid membranes composed of PAMAM dendrimer and polyethylene glycol dimethacrylate (PEGDMA) and a compatible cross-linker 4GMAP that enabled thickness less than 100 µm [13]. However, we found that CO\textsubscript{2} permeance (Q\textsubscript{CO\textsubscript{2}}) was not inversely proportional to thickness, and CO\textsubscript{2}/H\textsubscript{2} separation performance was reduced from ca. 30 µm to ca. 10 µm by reducing the membrane thickness. The experimental results were explained by the facilitated transport theory [15]. The permeances of both CO\textsubscript{2} and H\textsubscript{2} increased with decreasing thickness. However, since the membrane thickness dependence of Q\textsubscript{CO\textsubscript{2}} was lower than that of H\textsubscript{2}, selectivity of CO\textsubscript{2} over H\textsubscript{2} decreased with decreasing the membrane thickness.

In this paper, we investigated the effect of enzyme on the CO\textsubscript{2} separation performance, in order to find the solution to obtain both high Q\textsubscript{CO\textsubscript{2}} and selectivity for the thin PAMAM/PEG hybrid membranes. Carbonic anhydrase (CA) is a well-known enzyme to promote CO\textsubscript{2} hydration and dehydration reaction (CO\textsubscript{2} + H\textsubscript{2}O ⇌ H\textsubscript{2}CO\textsubscript{3}) [16,17]. CA is an efficient catalyst for CO\textsubscript{2} hydration and dehydration with a turn over number of 106 mol-CO\textsubscript{2}/(mol-CA s) at the maximum. The carbonic anhydrase active region is shown in Figure 1 [16]. The CO\textsubscript{2} hydration mechanism of carbonic anhydrase is shown in Figure 2 [16,17]. A carbon dioxide molecule is attached to the Zn\textsuperscript{2+} active site to form a meta-stable complex. The complex is then attacked by a Lewis base (OH\textsuperscript{−}) to produce bicarbonates (HCO\textsubscript{3}−). In this two-step process, CO\textsubscript{2} is converted to HCO\textsubscript{3}− and the active site in the CA is left un-reacted. Therefore, in this paper, in order to overcome the limitation of the CO\textsubscript{2} separation properties, the effect of CA on separation properties was investigated. As far as the authors know, this paper is the first to report the effects of CA on CO\textsubscript{2} separation performance of the facilitated transport membranes in detail, such as the relationship between Q\textsubscript{CO\textsubscript{2}} and membrane thickness, rate-limiting step of CO\textsubscript{2} permeation, etc.

![Figure 1. Carbonic anhydrase active region.](image)

2. Materials and Methods

2.1. Materials

PEGDMA (average Mn 750), 1-hydroxycyclohexyl phenylketone, PAMAM dendrimer in methanol (0th generation, 50 wt.%), and carbonic anhydrase (CA) from bovine erythrocytes, were obtained from Sigma-Aldrich (MO, USA). Other organic and inorganic materials were reagent grade and used without further purification. Polyethersulfone (PES) porous support membrane with 30 kDa NMWCO was
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purchased from Millipore Com (Tokyo, Japan). A compatible cross-linker, 4GMAP, was synthesized by the reaction between PAMAM dendrimer (G0) and glycidyl methacrylate, as shown in [13].

![Figure 2. CO2 hydration mechanism of carbonic anhydrase.](image)

2.2. Membrane Preparation

A polymeric membrane was fabricated by photopolymerization of PEGDMA in the presence of PAMAM dendrimer in water. The composition of precursor solution is PAMAM (50 wt %), PEGDMA (42.5 wt %) and 4GMAP (7.5 wt %) in water.

A schematic diagram of membrane fabrication of thin PAMAM/PEGDMA/4GMAP with or without CA hybrid membranes is shown in Figure 3. The hybrid membrane was prepared by casting precursor solution on a quartz plate, followed by the UV curing at 312 nm UV for 1.5 min and transferred onto PES support membrane. The membrane thickness was controlled by sandwiching the reaction mixture between quartz plates with stainless steel spacers (10–100 μm in thick).

![Figure 3. Membrane fabrication of thin poly(amide amine) (PAMAM) dendrimer/poly(ethylene glycol) (PEG) hybrid membranes with or without carbonic anhydrase (CA).](image)
To investigate the effect of CA additive, composite membranes with the selectivity layer PAMAM/PEGDMA/4GMAP shown were prepared with the reverse surface, with CA coated on the surface of the PES support membrane by spraying in advance (Figure 3 (3)). A schematic diagram of CA addition by spray method of CA onto the membranes is shown in Figure 4. The membrane thickness is determined by a KeyenceVHX-1000 digital microscope (Tokyo, Japan) [14].

![Figure 4](image-url)

**Figure 4.** CA addition by a spray method CA 0.5 wt % aqueous solution onto the membranes with 20 µm gap.

### 2.3. Gas Separation Experimental

A schematic diagram of the gas separation experiment setup is shown in [18]. A CO₂/H₂ or CO₂/He (80/20 by vol.) gas mixture was humidified at 80% relative humidity and then fed to a flat-sheet membrane cell at a flow rate of 100 mL/min. As we reported in our previous papers, our membrane needs relative humidity as high as 80% RH to show high separation performance [18]. The CO₂ partial pressures of the feed side was 560 kPa (total feed pressure 700 kPa). Dry Ar was supplied at a flow rate of 10 mL/min to the permeate side of the cell as a sweep gas. The test operating temperatures were at 40 °C. The CO₂ and He concentrations in both feed and permeate gas were measured by gas chromatography. Permeance, Q, and selectivity, CO₂/H₂ or CO₂/He were calculated as expressed in [18]. A CO₂/H₂ (80/20 by volume) gas mixture was used for the gas separation experimental of Section 3.1. A CO₂/He (80/20 by volume) gas mixture was used for the gas separation experimental of Sections 3.2 and 3.3. He was used instead of H₂ for the safety reason. The relationship between Q₄, He (permeance of He) and Q₄, H₂ (permeance of H₂) was as follows: Q₄, He ≈ 0.8 Q₄, H₂. On the other hand, Q₄, CO₂ was almost the same for both CO₂/He and CO₂/H₂ separation.

### 3. Results and Discussion

#### 3.1. Effect of Membrane Thickness on CO₂ Permeance and CO₂/H₂ Selectivity

The effect of membrane thickness on CO₂ permeance and CO₂/H₂ selectivity was studied at 560 kPa of CO₂ partial pressure and 80% RH at 40 °C with hybrid membranes of PAMAM/PEGDMA/4GMAP = 50/42.5/7.5 by wt %; thickness = 10–100 µm. It was found that thinner membranes gave higher CO₂ permeation properties, as shown in Figure 5. However, CO₂ permeance was not inversely proportional to membrane thickness (Q₄, CO₂ ∝ L⁻⁰.⁶²). On the other hand, H₂ permeance was inversely proportional to membrane thickness (Q₄, H₂ ∝ L⁻⁰.⁹⁵). As a result, selectivity of CO₂ over H₂ decreased as the membrane thickness decreased.

The amino group contributes to transport of CO₂ though membrane as a bicarbonate ion (HCO₃⁻) in the wet membrane, while H₂ is only transported by a solution–diffusion mechanism [16]. The rate-limiting step of CO₂ permeation was the reaction from CO₂ to a bicarbonate ion (HCO₃⁻) at the feed side, or the reaction from HCO₃⁻ to CO₂ at the permeate side. Since the membrane thickness dependence of Q₄, CO₂ is lower than that of H₂, selectivity of CO₂ over H₂ decreased as the membrane thickness decreased. The experimental results were explained by the facilitated transport theory [15,16].
To obtain high \( Q_{\text{CO}_2} \) and selectivity, CA was added into \( \text{CO}_2 \) carrier (PAMAM) to obtain much higher reactivity with \( \text{CO}_2 \) in next section.

\[
Q_{\text{CO}_2} \propto L^{-0.62} \\
Q_{\text{He}} \propto L^{-0.95}
\]

\[
1 \times 10^{-9} \\
1 \times 10^{-10} \\
1 \times 10^{-11} \\
1 \times 10^{-12}
\]

\[
\text{Membrane thickness, } L \text{ [\( \mu \text{m} \)]}
\]

**Figure 5.** Effect of membrane thickness (\( L \)) on \( \text{CO}_2 \) permeance and \( \text{CO}_2/\text{H}_2 \) selectivity.

### 3.2. Effect of CA Addition on the \( \text{CO}_2 \) Separation Properties

The effect of CA addition and membrane thickness on \( \text{CO}_2 \) permeance and \( \text{CO}_2/\text{H}_2 \) selectivity was studied at 560 kPa of \( \text{CO}_2 \) partial pressure and 80% RH at 40 °C with hybrid membranes of PAMAM/PEGDMA/4GMAP = 50/42.5/7.5 by weight ratio with addition 1 wt % CA (membrane thickness = 10–60 \( \mu \text{m} \)). As can be seen from Figure 6, \( \text{CO}_2 \) permeance was inversely proportional to membrane thickness (\( Q_{\text{CO}_2} \propto L^{-0.98} \)). He permeance was also inversely proportional to membrane thickness (\( Q_{\text{He}} \propto L^{-0.94} \)). As a result, \( \text{CO}_2/\text{He} \) selectivity kept constant. It was suggested that CA addition enhanced the reaction rate of \( \text{CO}_2 \) hydration at the feed side and the dehydration at the permeate side, and that the rate-limiting step of \( \text{CO}_2 \) transport rate became diffusion.

\( \text{CO}_2 \) permeance of as high as \( 2.47 \times 10^{-11} \text{ m}^3 (\text{STP})/(\text{m}^2 \text{ s Pa}) \) accompanied with a \( \text{CO}_2/\text{He} \) selectivity of 26.8 was achieved by the membrane with CA addition, ca. 55 \( \mu \text{m} \) thick. The enhancement in \( \text{CO}_2 \) permeance is 270% compared with the membrane without CA addition (\( Q_{\text{CO}_2} \): \( 6.71 \times 10^{-12} \text{ m}^3 (\text{STP})/(\text{m}^2 \text{ s Pa}) \), ca. 55 \( \mu \text{m} \) thick). \( \text{CO}_2 \) permeance of as high as \( 1.08 \times 10^{-10} \text{ m}^3 (\text{STP})/(\text{m}^2 \text{ s Pa}) \) accompanied with a \( \text{CO}_2/\text{He} \) selectivity of 28.7 was achieved by the membrane with CA addition, ca. 15 \( \mu \text{m} \) thick. The enhancements in \( \text{CO}_2 \) permeance is 490% compared with the membrane without CA addition (\( Q_{\text{CO}_2} \): \( 1.84 \times 10^{-11} \text{ m}^3 (\text{STP})/(\text{m}^2 \text{ s Pa}) \), ca. 15 \( \mu \text{m} \) thick). It was indicated that CA addition was effective to break though the limitation of \( \text{CO}_2 \) permeation of the thin facilitated transport membrane.

\[
1 \times 10^{-9} \\
1 \times 10^{-10} \\
1 \times 10^{-11} \\
1 \times 10^{-12}
\]

\[
\text{Membrane thickness, } L \text{ [\( \mu \text{m} \)]}
\]

**Figure 6.** Effect of CA addition on the \( \text{CO}_2 \) separation properties of PAMAM/PEG hybrid membranes.
3.3. Effect of Position of CA on CO₂ Separation Performance

The effect of position of CA on CO₂ separation performance was studied at 560 kPa of CO₂ partial pressure and 80% RH at 40 °C, using hybrid composite membranes of PAMAM/PEGDMA/4GMAP = 50/42.5/7.5 (weight %) with CA at different positions: (1) without CA; (2) S: surface; (3) RS: reverse surface; (4) S/RS. The thicknesses of these membranes were ca. 20 μm. The results are shown in Figure 7. CO₂ permeance of the membrane with CA at S position was not higher than that of membrane without CA. On the other hand, the membranes with CA at RS and S/RS positions showed CO₂ permeance and selectivity around twice as high as that of the membrane without CA or the membrane with CA at S position. From these results, it could be seen that the existence of CA at the RS position was more important than the S position. RS position is the place where CO₂ dehydration occurs at the permeate side, and the S position is the place where CO₂ hydration occurs at the feed side. Therefore, it was suggested that the rate-limiting step of CO₂ permeation was CO₂ dehydration reaction at the permeate side. These findings will be useful in the development of the facilitated transport membrane with high CO₂ separation properties. Detailed research on the CO₂ permeation mechanism is ongoing.

4. Conclusions

Effect of CA enzyme on CO₂ separation performance of thin poly(amidoamine) dendrimer/poly(ethylene glycol) hybrid membranes was investigated by examining the relationship between membrane thickness and CO₂ permeance using the membranes with or without CA. CO₂ permeance was inversely proportional to membrane thickness with CA, and not proportional to membrane thickness without CA. The addition of CA exhibited significantly enhanced CO₂ separation performances. The membrane with CA at the permeate side showed higher CO₂ separation performance than that of the membrane with CA at the feed side. Therefore, it was indicated that the rate-limiting step of CO₂ permeation was CO₂ dehydration reaction at the permeate side. These findings will be useful in the development of the CO₂ facilitated transport membrane with high CO₂ separation properties.

Author Contributions:
S.D. participated in its design, methodology, validation and formal analysis. S.D. carried out writing-original draft preparation and writing-review. T.K. participated in its design and helped to draft and writing-review the manuscript. S.-i.N. conceptualized and supervised project administration.

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Conflicts of Interest:
The authors declare no conflict of interest.
References
1. Liu, H.; Liang, X. Strategy for promoting low-carbon technology transfer to developing countries: The case of CCS. *Energy Policy* **2011**, *39*, 3106–3116. [CrossRef]
2. D’Alessandro, D.M.; Smit, B.; Long, J.R. Carbon Dioxide Capture: Prospects for New Materials. *Angew. Chem. Int. Ed.* **2010**, *49*, 6058–6082. [CrossRef] [PubMed]
3. Sharma, S.D.; Azzi, M. A critical review of existing strategies for emission control in the monoethanolamine-based carbon capture process and some recommendations for improved strategies. *Fuel* **2014**, *121*, 178–188. [CrossRef]
4. Swaidan, R.; Ghanem, B.S.; Litwiller, E.; Pinnau, I. Pure- and mixed-gas CO$_2$/CH$_4$ separation properties of PIM-1 and an amidoxime-functionalized PIM1. *J. Membr. Sci.* **2014**, *457*, 95–102. [CrossRef]
5. Wang, S.; Li, X.; Wu, H.; Tian, Z.; Xin, Q.; He, G.; Peng, D.; Chen, S.; Yin, Y.; Jiang, Z.; et al. Advances in high permeability polymer based membrane materials for CO$_2$ separations. *Energy Environ. Sci.* **2016**, *9*, 1863–1890. [CrossRef]
6. Huang, A.; Liu, Q.; Wang, N.; Caro, J. Organosilica functionalized zeolitic imidazolate framework ZIF-90 membrane for CO$_2$/CH$_4$ separation. *Microporous Mesoporous Mater.* **2014**, *192*, 18–22. [CrossRef]
7. Nafisi, V.; Hägg, M. Development of dual layer of ZIF-8/PEBAX-2533 mixed matrix membrane for CO$_2$ capture. *J. Membr. Sci.* **2014**, *459*, 244–255. [CrossRef]
8. Kasahara, S.; Kamio, E.; Ishigami, T.; Matsuyama, H. Effect of water in ionic liquids on CO$_2$ permeability in amino acid ionic liquid-based facilitated transport membranes. *J. Membr. Sci.* **2012**, *415–416*, 168–175. [CrossRef]
9. Deng, L.; Kim, T.; Hägg, M. Facilitated transport of CO$_2$ in novel PVAm/PVA blend membrane. *J. Membr. Sci.* **2009**, *340*, 154–163. [CrossRef]
10. Kim, T.; Vralstad, H.; Sandru, M.; Hägg, M. Separation performance of PVAm composite membrane for CO$_2$ capture at various pH levels. *J. Membr. Sci.* **2013**, *428*, 218–224. [CrossRef]
11. Taniguchi, I.; Duan, S.; Kazama, S.; Fujioka, Y. Facile fabrication of a novel high performance CO$_2$ separation membrane: Immobilization of poly(amideamine) dendrimers in poly(ethylene glycol) networks. *J. Membr. Sci.* **2008**, *322*, 277–280. [CrossRef]
12. Taniguchi, I.; Urai, H.; Kai, T.; Duan, S.; Kazama, S. A CO$_2$-selective molecular gate of poly(amideamine) dendrimer immobilized in a poly(ethylene glycol) network. *J. Membr. Sci.* **2013**, *444*, 96–100. [CrossRef]
13. Taniguchi, I.; Kai, T.; Duan, S.; Kazama, S.; Jinnai, H. A compatible crosslinker for enhancement of CO$_2$ capture of poly(amideamine) dendrimer-containing polymeric membranes. *J. Membr. Sci.* **2015**, *475*, 175–183. [CrossRef]
14. Kovvali, A.S.; Chen, H.; Sirkar, K.K. Dendrimer membranes: A CO$_2$-selective molecular gate. *J. Am. Chem. Soc.* **2000**, *122*, 7594–7595. [CrossRef]
15. Watari, T.; Huang, Q.; Teramoto, M. Effects of Support Membrane Properties on Facilitated Transport of CO$_2$ through Supported Liquid Membrane of Aqueous Amine Solution. *J. Chem. Eng. Jpn.* **1998**, *21*, 155–157.
16. Bao, L.; Trachtenberg, M.C. Facilitated transport of CO$_2$ across a liquid membrane: Comparing enzyme, amine, and alkaline. *J. Membr. Sci.* **2006**, *280*, 330–334. [CrossRef]
17. Zhang, Y.; Zhang, L.; Chen, H.; Zhang, H. Selective separation of low concentration CO$_2$ using hydrogel immobilized CA enzyme based hollow fiber membrane reactors. *Chem. Eng. Sci.* **2010**, *65*, 3199–3207. [CrossRef] [PubMed]
18. Duan, S.; Kai, T.; Saito, T.; Yamazaki, K.; Ikeda, K. Effect of cross-linking on mechanical and thermal properties of poly(amideamine) dendrimer/poly(vinyl alcohol) hybrid membranes for CO$_2$ separation. *Membranes* **2014**, *4*, 200–209. [CrossRef] [PubMed]