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Immobilization of proteins on synthetic resins using supercritical carbon dioxide

Ryosuke Yamada, Yamato Suzuki, Masahiro Yasuda, Hiroyasu Ogino*

Department of Chemical Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

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ABSTRACT

Commercially available synthetic resins are durable and inexpensive. However, it is difficult to immobilize proteins on most commercially available synthetic resin plates, such as polypropylene (PP), polyethylene, polyurethane, and polyvinyl chloride, because these plates are durable and inactive. In this study, we attempted to immobilize bovine serum albumin (BSA) and lysozyme (LZM) on commercially available synthetic resin plates using supercritical carbon dioxide ($ScCO_2$). Immobilization was attained by incubating a solution containing synthetic resin plates and proteins under $ScCO_2$. BSA and LZM were successfully immobilized using this method; however, the amount of immobilized protein varied depending on the kind of protein and synthetic resin plate used as well as immobilization condition. The maximum amount of BSA immobilized on PP was 102 μg/g-resin. Although BSA conformation did not change after 24-h incubation under $ScCO_2$ at 40 °C and 8 MPa, it efficiently immobilized on PP plates. The protein immobilization mechanism by $ScCO_2$ could be speculated that synthetic resin plates were swollen and proteins were diffused into synthetic resin. Then, proteins could be entrapped when synthetic resins were deflated by decompression. Our results suggest that use of $ScCO_2$ may lead to new and improved methods for protein immobilization and surface modification of synthetic resins.

1. Introduction

Commercially available synthetic resins, such as polypropylene (PP), polyethylene (PE), and polyvinyl chloride (PVC), are used for many applications because they are durable, inexpensive, and easy to use. For example, in the biochemical field, synthetic resins can be used as carriers for immobilization of enzymes or microorganisms [1,2]. Efficient immobilization of proteins on synthetic resins could be a promising method for surface modification of synthetic resins and for immobilization of enzymes.

To immobilize enzyme has many industrial advantages such as improvement of enzyme stability and capability for using in repeated batch and continuous format [3]. Thus many immobilized enzymes such as glucose isomerase and lipase are industrially available. Enzyme immobilization methods can be categorized into three groups: binding to a carrier, encapsulation, and aggregation [4]. Binding to a carrier can be achieved by physical, ionic, or covalent bonding. However, physical and ionic bonds are generally too weak to keep enzymes fixed to a carrier under several conditions. Although covalent bonding is sufficiently strong to fix enzymes, chemical treatment under harsh conditions is often required for bond formation, which may result in denaturation of proteins and reduction or loss of enzyme activity. Regardless of the approach used, it is difficult to immobilize proteins on synthetic resins because resins are durable and inactive under mild conditions.

Supercritical fluid is defined as a substance that exists above its critical temperature and pressure. Supercritical fluids are highly compressed and have properties typical of both gases and liquids [5]. Among them, supercritical carbon dioxide ($ScCO_2$) is a particularly attractive supercritical fluid because it is inert, nonflammable, inexpensive, non-toxic, and its critical temperature (31.1 °C) and pressure (7.38 MPa) are low compared with those of other substances [6,7]. In addition, $ScCO_2$ can be easily removed from a system by decompression. Thus, $ScCO_2$ has been used for various purposes, such as drying [8], extraction [9], particle formation [10], and sterilization, in various fields, including chemical engineering, food science, and medicine [11,12]. Hirogaki et al. [5] reported that polyethylene terephthalate can be swollen under $ScCO_2$ reversibly. In addition, some reports indicated that $ScCO_2$ enhanced diffusion of dyes into synthetic resins [13,14]. Moreover, some enzymes were not denatured under $ScCO_2$, and enzymatic reactions using poor water soluble substrates could be achieved under $ScCO_2$ [15]. Thus, it can be speculated that synthetic

* Corresponding author. Tel.: +81 72 254 9296; fax: +81 72 254 9296.
E-mail address: ogino@chemeng.osakafu-u.ac.jp (H. Ogino).

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resin plates are swollen under $ScCO_2$. Then, proteins are diffused into synthetic resin and have some interactions with synthetic resin. Finally, proteins can be entrapped when synthetic resins are deflated by decompression.

In this study, we attempted to immobilize proteins on synthetic resins under $ScCO_2$. We evaluated effects of temperature, pressure, incubation period, and additives on protein immobilization under pressurized CO_2 and analyzed the surface of synthetic resin plates to which proteins were immobilized.

2. Materials and methods

2.1. Materials and reagents

PP (0.3-mm thickness; Shin-Kobe Electric Machinery, Tokyo, Japan), PE (0.3-mm thickness; Shin-Kobe Electric Machinery), polyurethane (PU, 0.5-mm thickness; Nihon Unipolymer, Tokyo, Japan), and PVC (0.3-mm thickness; Sekisui-seikei, Osaka, Japan) were purchased. Each synthetic resin was cut into circles of 6-mm diameter to form resin plates which were used as carriers for immobilization of proteins. Surface area and weight of each resin plate were PP; 62 mm^2 and 6.65 mg, PE; 62 mm^2 and 7.01 mg, PU; 66 mm^2 and 8.96 mg, and PVC; 62 mm^2 and 6.22 mg. Bovine serum albumin (BSA, Sigma–Aldrich Japan, Tokyo, Japan) and lysozyme (LZM, Wako Pure Chemical, Osaka, Japan) were obtained for immobilization on synthetic resins.

2.2. Instrumentation for preparation of pressurized CO_2

Pressurized CO_2 was prepared using instruments as shown in Fig. 1. CO_2 gas supplied from a CO_2 cylinder was cooled to 4 °C by a cooler (CCA-1100, Tokyo Rika Kikai Ltd., Tokyo, Japan), pressurized using a plunger pump (NP-D, Nihon Seimitsu Kagaku, Tokyo, Japan), and loaded into a high-pressure vessel (TSC-CO2-002, Taiatsu Garasu Kogyo, Osaka, Japan) placed in a temperature-controlled water bath (BK33, Yamato Kagaku, Tokyo, Japan).

2.3. Protein immobilization under pressurized CO_2

Ten synthetic resin plates and 150 μL of 100 μg/mL BSA solution (pH 6.4) or LZM solution (pH 6.8) were placed in a glass vial (7 mm in diameter × 32 mm in height) set in a high-pressure vessel and incubated under pressurized CO_2. The loading proteins were corresponding to PP; 226 μg/g-resin, PE; 214 μg/g-resin, PU; 167 μg/g-resin, and PVC; 241 μg/g-resin, respectively.

After incubation, synthetic resin plates were washed using 150 μL of deionized water by rotational mixing at 2800 rpm at room temperature for 10 min. Then, plates were subsequently washed in a solution containing 150 μL of 1 M NaCl and 150 μL of 1-propanol. The protein concentrations before and after incubation and washing were measured by the Lowry method [16] using a microplate reader (Benchmark Plus; Bio-Rad Laboratories, Inc., CA, USA) and protein standards. The amount of immobilized protein was estimated by subtracting the sum of non-immobilized protein remaining in the solutions after incubation/washing from the amount of protein (15 μg) initially present. Data represent averages of two independent experiments, and error bars and ± represent standard deviation.

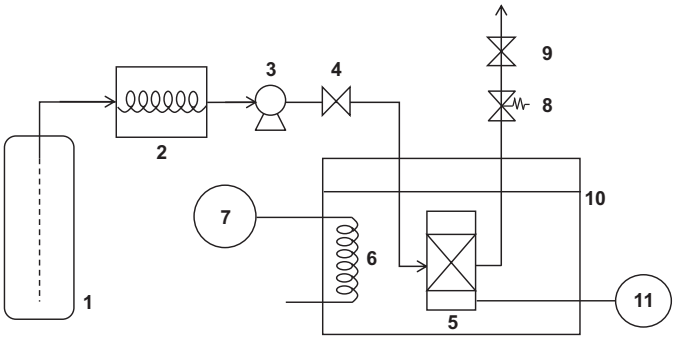

Fig. 1. Schematic diagram of a reactor. 1, CO_2 cylinder; 2, cooler; 3, plunger pump; 4, input valve; 5, high-pressure vessel; 6, heater; 7, temperature indicator and controller; 8, safety valve; 9, output valve; 10, water bath; 11, pressure indicator.

2.4. Circular dichroism spectral analysis

Five hundred microliters of 1 mg/mL BSA was incubated under pressurized CO_2 at 30–50 °C and 8–15 MPa for 24 h. Circular dichroism (CD) spectra of BSA before and after incubation were analyzed using a J-820 spectropolarimeter (JASCO, Tokyo, Japan) with a 0.1-cm path-length cell at 25 °C. Data represent averages from five scans.

2.5. FT-IR spectral analysis

The surface of PP plates incubated with 100 μg/mL BSA and 1 M NaCl under $ScCO_2$ was analyzed by FT-IR ATR spectroscopy with an FT/IR-410 Fourier transform infrared spectrometer (JASCO Co. Ltd., Tokyo, Japan). Data represent averages from 64 scans.

3. Results and discussion

3.1. Effects of incubation under pressurized CO_2 on protein conformation

For BSA, CD spectra before and after 24-h incubation under $ScCO_2$ at 40 °C and 8 MPa and BSA incubated at 95 °C for 30 min under non-pressurized air are shown in Fig. 2. The CD spectrum for BSA incubated at 95 °C showed denaturation and was drastically changed compared with that of BSA before incubation. On the other hand, for BSA incubated under $ScCO_2$, CD spectra were similar before and after incubation. Any CD spectra of BSA incubated under

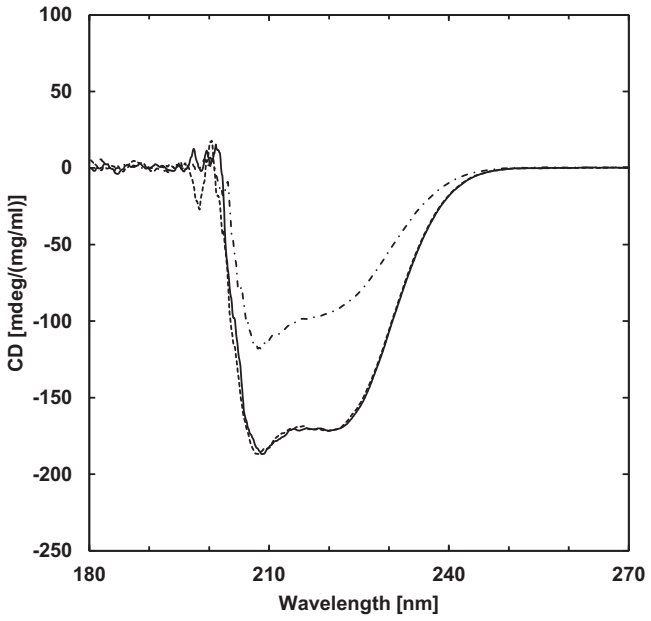

Fig. 2. CD spectra of BSA before and after incubation under $ScCO_2$. Solid line, BSA before incubation; dashed line, BSA after incubation; dashed dotted line, BSA denatured at 95 °C.