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Thermostable Natural Protein Polymers from *Geobacillus thermodenificans* DSM465 as Membrane Materials for Vapor Permeation

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The vapor permeation of aqueous 2-PrOH mixtures was investigated by using the thermostable natural polymer, water soluble protein from thermophile *Geobacillus thermodenificans* DSM465, as one of the membrane components in a polymer blend. The cultured protein/Torlon poly(amide-imide) blended membranes preferentially permeated H_2O from aqueous 2-PrOH by vapor permeation. Membranes containing the proteins from *G. thermodenificans* DSM465 were applied to the dehydration of aqueous 2-PrOH.

Key words : *Geobacillus thermodenificans* DSM465／permselectivity／protein／thermostabilizing protein／vapor permeation

Introduction

It is of environmental interest to obtain polymeric membranes from naturally occurring or 'green' polymers. From this viewpoint, agarose was adopted as a membrane material and pervaporation of methanol/methyl *tert*-butyl ether (MeOH/MTBE) through agarose membranes[1] or hydrophilic polymer/agarose blended membranes[2, 3] and that of aqueous organic mixtures through agarose membranes[4, 5] were studied.

Proteins derived from microorganisms are abundant renewable resources and biodegradable, environmentally-friendly 'green' polymers. Moreover, proteins from thermophilic microorganisms show a unique property of high thermostability. Their thermostability is associated with a higher resistance to chemical denaturants such as organic solvents. To take advantage of these special properties, we investigated proteins from thermophilic microorganisms. A strain *Geobacillus thermodenificans* DSM465[6, 7] which can grow at relatively high temperature of 65 ℃ was selected in this study since the high cultivating temperature lessens the risk of other microbial contaminations. It can be expected that *G. thermodenificans* DSM465 is easily cultivated at the growing temperature of 65 ℃ and in addition, thermostable membranes could be obtained from this strain.

To this end, hydrophilic water soluble proteins were obtained from *G. thermodenificans* DSM465 as membrane materials and their performance was

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preliminarily investigated.

Experimental Section

Materials

Torlon grade® 4000T poly(amide imide) was kindly provided by BP Amoco Polymers Inc. (now Solvay Advanced Polymers). N, N-Dimethylformamide (DMF) and 2-propanol (2-PrOH) were purified by the usual method[8]. Deionized water was employed throughout the experiments.

Microorganism and Growth Conditions

G. thermodenificans DSM465 cells were grown in Luria broth (1% tryptone, 0.5 % yeast extract, 0.5 % NaCl, pH 7.2) at 65 ℃ with reciprocal shaking (95 rpm) for the liquid culture. Cultures were incubated 8 h in 2-dm^3 Sakaguchi flasks containing 200 cm^3 medium.

Protein Fractionation

After the cultivation, the grown cells were obtained by centrifugation (8,000 rpm, 10 min), washed with saline solution, and re-centrifuged at the same condition. The cells were suspended in phosphate buffer (50 mmol dm^{-3} KPB, 5 mmol dm^{-3} EDTA, pH 7.0) and disintegrated by sonication to prepare soluble cell-free extract. Supernatant as the extract was obtained by centrifugation (12,000 rpm, 20 min).

Crude protein fractionation was carried out by ammonium sulfate precipitation of the extract solution. To 100 cm^3 of the extract solution was added solid ammonium sulfate (35 % saturation), and the precipitates were collected by centrifugation. To supernatant was further added solid ammonium sulfate to give 85 % saturation and the resulting precipitates were obtained by centrifugation. Each precipitate was dissolved in the same phosphate buffer. Three kinds of protein solutions were named as Fraction 1, 2, and 3 for 0-35, 35-85, and 85- % saturation in the ammonium sulfate precipitation. The solutions were dialyzed against deionized water and lyophilized to obtain powders of the proteins.

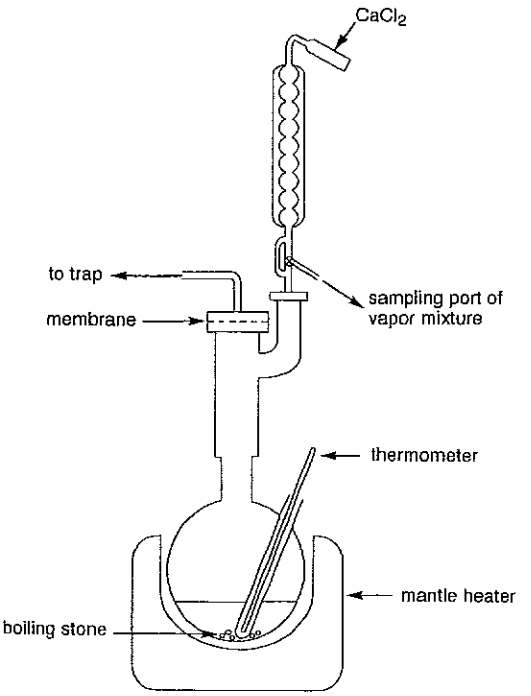

Fig. 1 Schematic diagram of apparatua for vapor permeation.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli[9].

Blended Membranes for Vapor Permeation

Torlon grade® 4000T poly(amide imide), which was produced and kindly provided by Amoco Performance Products, was adopted as a membrane matrix for blended membranes[10]. Blended membranes were prepared from DMF solution. 500 mg of Torlon and the prescribed amount of protein sample of Fraction 1 were dissolved in 5.0 cm^3 of DMF. Comparing the molecular weight distribution of Fractions 1 and 2 from SDS-PAGE patterns in Fig. 2, that of Fraction 1 is thought to be narrower than that of Fraction 2. From this, Fraction 1 was adopted as a protein sample in the present study. The resulting solution was cast onto a glass plate with an applicator (casting

thickness, 0.254 mm), and the solvent was allowed to evaporate at 50 ℃ for 2 d. The thickness of the membrane thus obtained was 33 −36 μ m.

Vapor Permeation

Vapor permeation (VP) experiments were carried out at a temperature of around 82 ℃, at which the liquid mixture of water and 2-PrOH was refluxed under atmospheric pressure (ca. 0.101 MPa (ca. 1.0 atm)). The schematic diagram of the VP apparatus is shown in Fig. 1. The apparatus was connected to a vacuum line. Permeate was condensed and trapped by liquid nitrogen, in the same manner as in pervaporation experiments[11]. The effective membrane area was 17.3 cm^2 and the downstream pressure was maintained at around 133.3 Pa (1.0 mmHg).

Separation analysis was carried out on Shimadzu GC-7APT with 3.0 m long column packed with polyethyleneglycol 6000 (Shimalite TPA).

The separation factor, α, is defined as

$$\alpha = (Y_{H_2O} / Y_{2\text{-PrOH}}) / (X_{H_2O} / X_{2\text{-PrOH}})$$

where Y_is are the weight fractions in permeate and X_is are those in vapors in feed, respectively.

Results and Discussion

From SDS-PAGE shown in Fig. 2 the molecular mass of the obtained protein Fraction 1 was estimated to be 108 − 10 kDa, that of Fraction 2 to be 115 − 10 kDa, and that of Fraction 3 to be 92 − 20 kDa, respectively. In one batch microorganism cultivation procedure, 132.0 mg of Fraction 1, 179.0 mg of Fraction 2, and 3.0 mg of Fraction 3 were obtained.

During the initial stages of the present study, the authors attempted to prepare membranes from only the obtained proteins and to study their performance. However obtained protein membranes from Fraction 1 and Fraction 2 were found to be

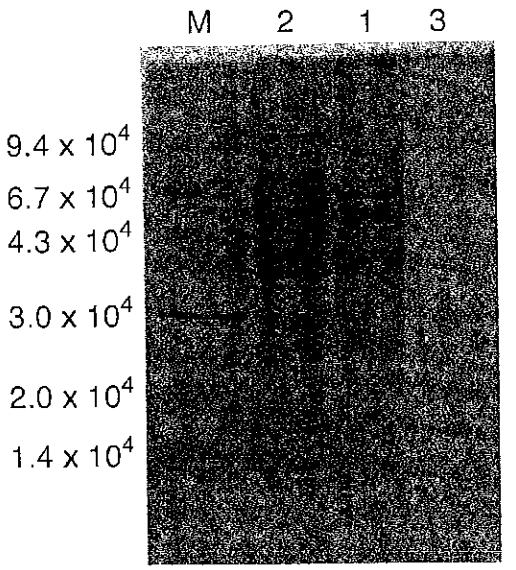

Fig. 2 SDS-PAGE pattern of fractionated proteins from *Geobacillus Thermodenificans* DSM465.
(M, molecular weight marker; 2, Fraction 2 ; 1, Fraction 1 ; 3, Fraction 3.)

mechanically weak so that vapor permeation could not be studied. Even crosslinked protein membranes, which were crosslinked with hexamethylene diisocyanate according to the method described in the literature[12, 13], did not show enough durability for vapor permeation. As a result, Torlon 4000T polyamide-imide was adopted as a membrane matrix to impart mechanical strength. Blended membranes were prepared from Fraction 1 and Torlon with protein contents in the range of 0 − 4.8 %, and their membrane performance was investigated.

The weight fraction of water in vapor was fixed at around 3.0×10^{-3} and the effect of protein content on membrane performance was studied (Fig.3). As expected from the fact that the incorporated protein Fraction 1 was water soluble, the blended membrane permeated H_2O in preference to 2-PrOH. Torlon membrane itself selectively permeated H_2O from aqueous 2-PrOH solution as well, showing a selectivity of over 100. Permselectivity toward water at a protein content of 2.0 − 4.0 wt.% was over that of Torlon membrane, with typi-

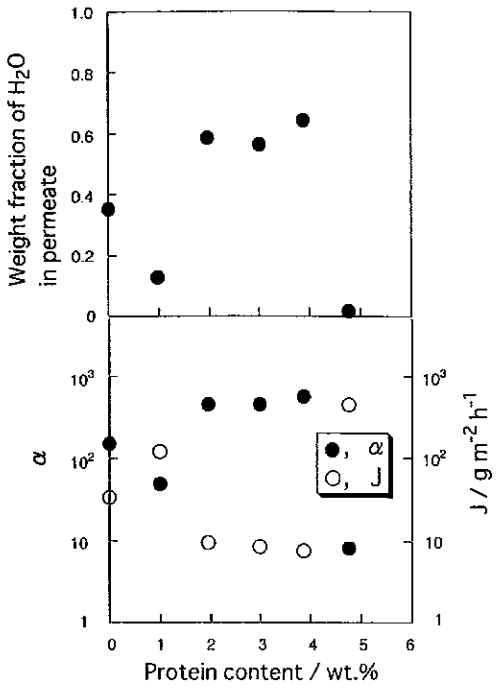

Fig. 3 Effect of protein content on vapor permeation of H_2O/2-PrOH mixtures through protein/Torlon blended membranes.
(Weight fraction of H_2O in vapor feed, ca. 3.0×10^{-3}; downstream pressure, 133.3 Pa(1.0 mmHg); operating temperature, ca.82℃.)

cal permselectivities of > 300 and increases in weight fraction of water in the permeate from ~ 0.35 to ~ 0.60. The permselectivity toward water of the blended membrane at the protein content of around 1.0 wt.% was below that of Torlon membrane. However, this is likely the result of a membrane defect, since the trend is quite different from the other membranes in the series. Above a protein content of ca. 4.0 wt.%, permselectivity toward water was drastically decreased and the flux value was increased because the effect of membrane swelling surpasses that of permselectivity caused by the incorporation of protein into Torlon.

Further investigations, such as whether the proteins from cultured *Geobacillus thermodenificans* DSM465 have potential for a candidate polymer of molecularly imprinted materials, will be reported shortly.

Conclusions

Novel vapor permeation membranes were obtained from the thermostable water soluble protein from *G. thermodenificans* DSM465 using Torlon as a membrane matrix. The blended membrane thus prepared preferentially permeated H_2O from aqueous 2-PrOH mixtures, with permselectivities of ~ 300 versus ~ 100 for the pure Torlon membranes. The present study showed that the protein from *G. thermodenificans* DSM465 could be applied successfully as membrane materials for the dehydration of water miscible organics.

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Erratum

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Throughout this article the word "*thermodenificans*" should be changed to "*thermodenitrificans*."