Waste Biomass Valor (2011) 2:303–307
DOI 10.1007/s12649-011-9066-6

ORIGINAL PAPER

Transformation of Intact Chicken Feathers into Chiral Separation Membranes

Yuuki Sueyoshi · Takeshi Hashimoto · Masakazu Yoshikawa · Kunihiko Watanabe

Received: 10 November 2010 / Accepted: 3 February 2011 / Published online: 23 February 2011
© The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract Intact chicken feathers, most of which are currently discarded by incineration, were decayed by *Meiothermus ruber* H328 at the moderate aerobic conditions of 60°C so that they could be utilized as raw materials. The degraded chicken feather thus obtained was converted into membranes in the presence of reducing agent of 1-octanethiol. The membrane showed chiral separation ability; in other words, it transported the D-isomer of Glu and Phe, and the L-isomer of Lys from the corresponding racemic amino acid mixtures.

Keywords Chicken feather · Ciral separation · Keratin · *Meiothermus ruber* H328 · Membrane · Thermophile

Introduction

3Rs is promoted to create a sustainable society. “3Rs” consists of three actions, of which words start alphabet “R”; (1) “Reduce”, implying reducing the amount of waste, (2) “Reuse”, meaning using used items again and again, and (3) “Recycle”, implying using recyclable items as raw materials. Utilization of waste as raw materials is fascinating action to reduce the amount of waste, “Reduce”.

Most feathers generated by poultry processing are currently disposed by incineration [1], which has ecological disadvantages in terms of the energy loss and the emission of a large amount of carbon dioxide. Poultry proteins contain various potent proteins and amino acids. From this, a certain amount of poultry feather is converted to feather meal, which is used as animal feed stuff through extraordinary severe processes under high pressure and temperature [2]. Alternative methods, efficiently degrading intact feathers and overcoming the defects of prior processes, have been studied [3–7], though those were hardly applicable to practical degradation. One of the authors, KW, demonstrated the decomposition of hard-to-degrade animal proteins by moderately thermophilic bacteria showing optimum growth at around 60°C under aerobic conditions [8–11]. In the screening process of a novel thermophilic bacterium, KW and his research group newly found a thermophilic bacterium strain, *Meiothermus ruber* H328, that can efficiently decompose truly intact chicken feathers [12, 13].

Studies on utilization of naturally occurring or ‘green polymers’, their derivatives, and wastes from food industries as resources are important subjects to construct sustainable environment and society. To this end, the authors developed membranes from various raw materials, such as, egg shell membranes for chiral separation [14], agarose for pervaporation [15, 16], gelatin for vapor permeation [17], proteins from *Geobacillus thermodenifitricans* DSM465 for vapor permeation [18] and molecular recognition [19, 20], DNA for gas [21] and chiral separation [22], and chitosan for chiral [23] and vapor permeation [24]. The decomposed keratin by *Meiothermus ruber* H328 is an interesting and suitable material for membrane since intact chicken feathers are not in conflict with the feed supply chain. The degraded feathers also consist of various types of amino

Y. Sueyoshi · T. Hashimoto · M. Yoshikawa (✉)
Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo, Kyoto 606-8585, Japan
e-mail: masahiro@kit.ac.jp

K. Watanabe
Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo, Sakyo, Kyoto 606-8522, Japan

acid residue with L-configuration, which is expected to show optical resolution ability. Chirality plays a crucially important role in biological processes [25, 26]. As is well known, drug enantiomers with different chiral environments often show pharmacological activities depending on absolute configuration. From this, the production of enantiomerically pure compounds has gathered attention in chemical industries involving pharmaceuticals, agrochemicals, fragrances, food additives, and so forth. Membrane-based chiral separation is regarded as a promising and mighty method to obtain enantiomerically pure compounds [27–30], since it can be operated continuously under mild conditions and can be ecologically and economically competitive to other separation processes. To this end, degraded keratin from intact chicken feathers was converted into membrane and their membrane performance was investigated.

Experimental

Reagents and Materials

1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 1-octanethiol, D-glutamic acid (D-Glu), L-glutamic acid (L-Glu), D-phenylalanine (D-Phe), L-phenylalanine (L-Phe), D-lysine (D-Lys), L-lysine (L-Lys) were used as received. Water purified with an ultrapure water system (Simpli Lab, Millipores S. A., Molsheim, France) was used. Chicken feathers were kindly supplied by a local poulterer and extensively washed with tap water and air-dried prior to use.

Microorganism and Cultivation for Feather Degradation

The decomposition of intact chicken feathers by *Meiothernus ruber* H328 was carried out as reported previously [12, 13]. For feather degradation, cells aerobically cultured in 5 cm^3 of YS medium [0.5% (w/v) yeast extract, 0.5% (w/v) sucrose (pH 8.0)] at 60°C for 24 h were inoculated [1% (v/v) inoculum] to 50 cm^3 of a fresh YS medium containing 3% (w/v) of chicken feathers and 0.5% (w/v) $CaCO_3$ in 300 cm^3 Erlenmeyer flask and cultivated with rotary shaking at 190 rpm at 60°C for 6 days. After cultivation, the broth was centrifuged at 17,800×*g* for 10 min and the precipitates were recovered. They were resuspended in an appropriate amount of distilled water and centrifuged at 200×*g* for 2 min to remove bacterial cells. After repeating this step for cell removal twice, the obtained precipitates were resuspended in distilled water and recovered by centrifugation at 5,800×*g* for 2 min. The final precipitates were kept damp at 4°C and used as degraded chicken feathers. The molecular weight of keratin from intact chicken feathers was reported to be around 10 kDa [31]. That of degraded chicken feathers should be lower than that of starting feathers. However, the molecular weight of degradation products was not determined since it was not perfectly soluble in any solvents tested and the molecular weights of the degraded chicken feathers was anticipated to show a wide range of molecular weights.

Membrane Preparation

A 0.1 g of degraded chicken feathers was poured into 5.0 cm^3 of HFIP solution containing 0.15 cm^3 (ca. 8.64×10^{-4} mol) of 1-octanethiol and stirred for 24 h at an anaerobic condition. The HFIP solution thus obtained was poured into a TeflonPFA 50 mm diameter laboratory dish and the solvent was allowed to evaporate at ambient temperature for 24 h and then for additional 3 h at 50°C. The thickness of the membrane thus obtained was around 87 μm.

Enantioselective Transport

A membrane (area, 1.13 cm^2) was fixed tightly between two chambers of a permeation cell. The volume of each chamber was 5.0 cm^3. An aqueous solution of racemic mixture of amino acid was placed in the left-side chamber and an aqueous solution in the right-side chamber. Each concentration of racemic amino acid was fixed at 1.0×10^{-3} mol dm^{-3}. All experiments were carried out at 40°C. The amounts of the D- and L-isomers that transported through the membrane were determined by liquid chromatography (LC) [JASCO PU 1580, equipped with a UV detector (JASCO 1570)] employing a CHIRALPAK MA(+) column (50 mm × 4.6 mm (i.d.)) (Daicel Chemical Ind. Ltd.) for the analyses of racemic Glu's and Phe's and a CROWNPAK CR(+) column [250 × 4.0 mm (i.d.)] for the measurement of racemic Lys's. An aqueous copper sulfate solution was used as a mobile phase for Glu and Phe analyses and a perchloric acid solution as eluent for Lys analysis.

The flux, J (mol cm^{-2} h^{-1}), is defined as:

$$J = Q/At \quad (1)$$

where Q (mol) is the amount of transported amino acid, A (cm^2) is the effective membrane area, and t (h) denotes the time.

The permselectivity, $\alpha_{i/j}$, is defined as the flux ratio. J_i/J_j, divided by the concentration ratio ([i-AA]/[j-AA]):

$$\alpha_{i/j} = \left(J_i/J_j\right)/([\text{i - AA}]/[\text{j - AA}]) \quad (2)$$

The subscripts D and L refer to the D-isomer of amino acid and the L-isomer of amino acid, respectively.

Adsorption Selectivity

The membrane was immersed in a 1.0×10^{-3} mol dm^{-3} racemic amino acid solution and the mixture was allowed to equilibrate at 40°C. A 0.02 wt% sodium azide was added as a fungicide. The amount of amino acid in the supernatant subtracted from the amount initially in the solution gave the amount of the given amino acid adsorbed by the membrane. Quantitative analyses were done as above.

Adsorption selectivity, $S_{A(i/j)}$ is defined as

$$S_{A(i/j)} = ((\text{i - AA})/(\text{j - AA}))/([\text{i - AA}]/[\text{j - AA}]) \qquad (3)$$

where (i-AA) and (j-AA) are the amounts of enantiomer of amino acid adsorbed in the membrane and [i-AA] and [j-AA] denote the concentrations in the solution after equilibrium had been reached, respectively.

Results and Discussion

Membrane Preparation

Keratins are fibrous structural proteins that are composed of tightly packed chains. Due to large amounts of cysteine, the fibrous structures are stabilized by the high degree of cross-linking disulfide bonds. Intact chicken feather contains approximately 8% cysteine. In order to prepare HFIP solution dissolving degraded feather, it was required to transform disulfide linkage of cystine into cysteine residues by using reducing agents, various types of thiol. 2-Mercaptoethanol and dithiothreitol are often adopted as reducing agents [25, 26]. In the present study, thiol, which did not work as reducing agents in dissolving process and remained in the membrane thus obtained was required not only to be stably occluded in the membrane but also to work as plasticizers in the membrane to render the formed membrane flexible and stable. To this end, 1-octanethiol was adopted as a reducing agent. Even though 1-octanethiol, the amount of which was about ten times that of cystine, was added during the dissolution process of degraded keratin, insoluble parts were still found in the HFIP solution. This might be the reason that the durable membrane with wide area was not obtained in the present study. The membrane thus obtained was applied for the following study of membrane performance.

Enantioslective Transport of Racemic Amino Acids

In the present study, three types of racemic amino acid were adopted as model racemates, such as racemic glutamic acids (Glu's), which have very polar anionic side chains, racemic phenylalanines (Phe's), having aromatic side chains, and racemic lysines (Lys's) with very polar cationic side chains.

In the present study, concentration gradient was adopted as a driving force for the membrane transport of racemic amino acids. Time-transport curves of those three types of racemic amino acids through the membrane are shown in Fig. 1. D-Isomer of Glu and that of Phe were transported in preference to the corresponding L-isomers, while L-Lys was selectively transported through the membrane. Though racemic amino acids adopted in the present study are three of the twenty amino acids commonly found in proteins, the permselectivity of racemic Lys's was opposite to that of racemic Glu's or Phe's.

In order to elucidate the mechanism for the expression of permselectivity, adsorption selectivities of the membrane toward three types of racemic amino acids were studied. The results of adsorption experiments are summarized in Table 1. In the table, the amount of amino acid adsorbed in the membrane and adsorption selectivities are given. L-Glu was preferentially incorporated into the membrane, while the D-isomer of Phe or Lys was adsorbed in the membrane in preference to the corresponding L-isomer. From permselectivity determined by membrane transport and adsorption selectivity from adsorption experiment, the diffusivity selectivity ($S_{D(i/j)} = D_i/D_j$, where D_i and D_j are the diffusion coefficients of i-isomer

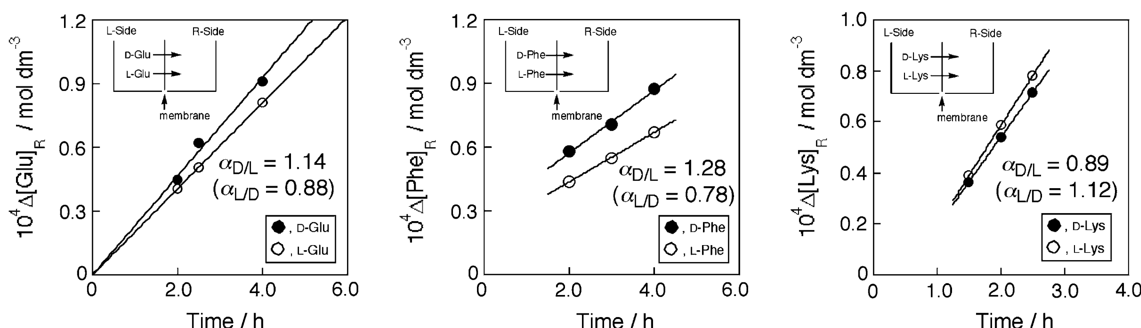

Fig. 1 Time-transport curves of three kinds of racemic mixture of amino acid through the degraded keratin membranes at 40°C. ($[\text{D-AA}]_0 = [\text{L-AA}]_0 = 1.0 \times 10^{-3}$ mol dm^{-3}; AA: Glu, Phe, or Lys.)

Table 1 Adsorption of racemic mixture of various amino acids in the Keratin membrane

Amino acid	Amino acid/mem. mol/g-mem.	$S_{A(D/L)}$	$S_{A(L/D)}$
D-Glu	7.60×10^{-5}	0.93	1.08
L-Glu	8.24×10^{-5}		
D-Phe	4.95×10^{-6}	2.20	0.45
L-Phe	2.23×10^{-6}		
D-Lys	1.86×10^{-5}	1.15	0.87
L-Lys	1.62×10^{-5}		

Table 2 Results of chiral separation with the Keratin membrane

Amino acid	$\alpha_{D/L}$ ($\alpha_{L/D}$)	$S_{A(D/L)}$ ($S_{A(L/D)}$)	$S_{D(D/L)}$ ($S_{D(L/D)}$)
Glu	1.14	0.93	1.23
	(0.88)	(1.08)	(0.81)
Phe	1.28	2.20	0.58
	(0.78)	(0.45)	(1.73)
Lys	0.89	1.15	0.77
	(1.12)	(0.87)	(1.29)

$\alpha_{i/j} = S_{D(i/j)} \times S_{A(i/j)}$

and j-isomer, respectively) can be determined. The diffusivity selectivities are summarized in Table 2 together with permselectivities and adsorption selectivities. Contrary to adsorption selectivity, the enantiomer, which was not preferentially incorporated into the membrane, was quickly diffused in the membrane. In other words, retarded diffusion of preferentially adsorbed enantiomer was observed. This might be due to relatively strong interaction between the preferentially incorporated enantiomer and the membrane. Such phenomena were often observed in chiral separation [22, 32–35].

Even though permselectivity observed in the present study was modest, the study described in the present paper is one of promising ways to reduce the waste from poultry (food) industries and utilize hard-to-degrade animal proteins as raw materials.

Conclusions

Intact chicken feathers, most of which are currently discarded by incineration, were decayed by *Meiothermus ruber* H328 at the moderate aerobic conditions of 60°C so that they could be utilized as raw materials. The degraded chicken feather thus obtained was converted into membranes in the presence of 1-octanethiol as reducing agent. The membrane showed chiral separation ability; in other words, it transported the D-isomer of Glu and Phe from the corresponding racemic mixtures while the L-isomer of Lys was preferentially transported from the racemic mixture of Lys's. The results obtained in the present study would open a door to novel materials.

Acknowledgments The authors thank Messrs. Tatsunobu Matsui and Goji Moromugi (Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University) for their assistance with the cultivation for feather degradation.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Deydier, E., Guilet, R., Sarda, S., Sharrock, P.: Physical and chemical characterization of crude meat and bone meal combustion residue: "waste or raw material?" J. Hazard. Mater. **121**, 141–148 (2005)
2. Dalev, P., Ivanov, I., Liubomirova, A.: Enzymatic modification of feather keratin hydrolyzates with lysine aimed at increasing the biological value. J. Sci. Food Agric **73**, 242–244 (1997)
3. Böckle, B., Galunsky, B., Müller, R.: Characterization of a keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530. Appl. Environ. Microbiol. **61**, 3705–3710 (1995)
4. Bressollier, P., Letourneau, F., Urdaci, M., Vemeuil, B.: Purification and characterization of a keratinolytic serine proteinase from S*treptomyces albidoflavus*. Appl. Environ. Microbiol. **65**, 2570–2576 (1999)
5. Riessen, S., Antranikian, G.: Isolation of *Thermoanaerobacter keratinophilus* sp nov, a novel thermophilic, anaerobic bacterium with keratinolytic activity. Extremophiles **5**, 300–408 (2001)
6. Allpress, J.D., Mountain, G., Gowland, P.C.: Production, purification and characterization of an extracellular keratinase from *Lysobacter* NCIMB 9497. Lett. Appl. Microbiol. **34**, 337–342 (2002)
7. Gupta, R., Ramnani, P.: Microbial keratinases and their prospective applications: an overview. Appl. Microbiol. Biotechnol. **70**, 21–33 (2006)
8. Okamoto, M., Yonejima, Y., Tsujimoto, Y., Suzuki, Y., Watanabe, K.: A thermostable colagenolytic protease with a very large molecular mass produced by thermophilic *Bacillus* sp strain MO-1. Appl. Microbiol. Biotechnol. **57**, 103–108 (2001)
9. Murai, A., Tsujimoto, Y., Matsui, H., Watanabe, K.: An Aneurinibacillus sp. Strain AM-1 produces a proline-specific aminopeptidase useful for collagen degradation. J. Appl. Microbiol. **96**, 810–818 (2004)
10. Watanabe, K.: Collagenolytic proteases from bacteria. Appl. Microbiol. Biotechnol. **63**, 520–526 (2004)
11. Itoi, Y., Horinaka, M., Matsui, H., Watanabe, K.: Characteristic features in the structure and collagen-binding ability of a thermophilic colagenolytic protease from the thermophile *Geobacillus collagenovorans* MO-1. J. Bacteriol. **188**, 6572–6579 (2006)
12. Matsui, T., Yanada, Y., Mitsuya, H.J., Shigeri, Y., Yoshida, Y., Saito, Y., Matsui, H., Watanabe, K.: Sustainable and practical degradation of intact chicken feathers by cultivating a newly isolated thermophilic *Meiothermus rubber* H328. Appl. Microbiol. Biotechnol. **82**, 941–950 (2009)
13. Shigeri, Y., Matsui, T., Watanabe, K.: Decomposition of intact chicken feathers by a thermophile in combination with an

acidulocomposting garbage-treatment process. Biosci. Biotechnol. Biochem. **73**, 2519–2521 (2009)

14. Kondo, Y., Yoshikawa, M.: Egg shell membranes for chiral separation. Membrane **26**, 228–230 (2001)
15. Yoshikawa, M., Yoshioka, T., Fujime, J., Murakami, A.: Pervaporation separation of MeOH/MTBE through agarose membranes. J. Membr. Sci. **178**, 75–78 (2000)
16. Yoshikawa, M., Masaki, K., Ishikawa, M.: Pervaporation of aqueous organic mixtures through agarose membranes. J. Membr. Sci. **205**, 293–300 (2002)
17. Yoshikawa, M., Higuchi, A., Ishikawa, M., Guiver, M.D., Robertson, G.P.: Vapor permeation of aqueous 2-propanol solutions through gelatin/Torlon poly(amide-imide) blended membranes. J. Membr. Sci. **243**, 89–95 (2004)
18. Yoshikawa, M., Kawamura, K., Ejima, A., Aoki, T., Watanabe, K., Guiver, M.D., Robertson, G.P.: Termostable natural protein polymers form *Geobacillus thermodenitrificans* DSM465 as membrane materials for vapor permeation. Membrane **29**, 384–387 (2004)
19. Yoshikawa, M., Kawamura, K., Ejima, A., Aoki, T., Sakurai, S., Hayashi, K., Watanabe, K.: Green polymers from Geobacillus thermodenitrificans DSM465–candidates for molecularly imprinted materials. Macromol. Biosci. **6**, 210–215 (2006)
20. Yoshikawa, M., Kawamura, K., Watanabe, K.: Evaluation of the recognition ability of molecularly imprinted proteind by syrface Plasmon resonance (SPR) spectroscopy. Membrane **32**, 40–44 (2007)
21. Matsuura, T., Sada, T., Yoshikawa, M., Ogata, N.: Oxygen permselective membranes from DNA-lipid complexes. Membrane **31**, 281–283 (2006)
22. Yoshikawa, M., Maruhashi, M., Iwamoto, Y., Ogata, N.: Optical resolution of racemic amino acids through DNA-poly(4-vinylbenzyl)trimethylammonium polyion complex membranes. Polym. J. **39**, 1193–1198 (2007)
23. Iwamoto, Y., Yoshikawa, M., Yamaoka, K., Ogata, N.: Optical resolution with membranes derived fropm marine polymers. Desalination Water Treat. **17**, 268–274 (2010)
24. Iwamoto, Y., Shimizu, T., Murai, Y., Yoshikawa, M., Ogata, N.: Vapor permeation of bioethanol with quaterinized chitosan-lipid complex membranes. Membrane **35**, 201–204 (2010)
25. Voet, D., Voet, J.G.: Biochemistry. Wiley, New York (1990)
26. McKee, T., McKee, J.R.: The molecular basis of life, 3rd edn. WCB/McGraw-Hill, Boston (2003)
27. Afonso, C.A.M., Crespo, J.G.: Recent advances in chiral resolution through membrane-based approaches. Angew. Chem. Int. Ed. **43**, 5293–5295 (2004)
28. Maier, N.M., Lindner, W.: Chiral recognition applications of molecularly imprinted polymers: a critical review. Anal. Bioanal. Chem. **389**, 377–397 (2007)
29. Xie, R., Chu, L.-Y., Deng, J.-G.: Membranes and membrane processes for chiral separation. Chem. Soc. Rev. **37**, 1243–1263 (2008)
30. Higuchi, A., Tamai, M., Ko, Y.-A., Tagawa, Y., Wu, Y.-H., Freeman, B.D., Bing, J.-T., Chang, Y., Ling, Q.-D.: Polymeric membranes for chiral separation of pharmaceuticals and chemicals. Polym. Rev. **50**, 113–143 (2010)
31. Presland, R.B., Gregg, K., Molloy, P.L., Morris, P., Crocker, L.A., Rogers, G.E.: Avian Keratin Genes I. A Molecular analysis of the structure and expression of a group of feather keratin genes. J. Mol. Biol. **209**, 549–559 (1989)
32. Masawaki, T., Sasai, M., Tone, S.: Optical resolution of an amino acid by an enantioselective ultrafiltration membrane. J. Chem. Eng. Jpn **25**, 33–39 (1992)
33. Aoki, T., Tomizawa, S., Oikawa, E.: Enantioselective permeation through poly{γ-[3-pentamethyldisiloxanyl]propyl}-L-glutamate} membranes. J. Membr. Sci. **99**, 117–125 (1995)
34. Tone, S., Masawaki, T., Eguchi, K.: The optical resolution of amino acids by plasma polymerized terpene membranes. J. Membr. Sci. **118**, 31–40 (1996)
35. Yoshikawa, M., Izumi, J.: Chiral recognition sites converted from tetrapeptide derivatives adopting racemates as print molecules. Macromol. Biosci. **3**, 487–498 (2003)