Chiral Recognition Ability of Oligopeptide Derivatives Consisting of Glutamyl Residues

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ABSTRACT: The effect of the constituting amino acid residue [Glu(OBzl)] number on the chiral recognition ability was investigated. Chiral recognition sites were prepared from oligopeptide derivatives (constituting amino acid residue number = three–five) by adopting alternative molecular imprinting. It was made clear that with a constituting amino acid residue number of four, the tetrapeptide derivative of Glu(OBzl) is the best candidate material to generate a chiral recognition site among eight types of oligopeptide derivatives in the study. The affinity constant between Ac-L-Trp and a chiral recognition site ranged from 3.4×10^3 to 1.08×10^4 mol^{-1} dm^3, depending on the number of Glu(OBzl) residues in an oligopeptide derivative. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 95: 1302–1309, 2005

Key words: chiral; molecular imprinting; molecular recognition; peptides; selectivity

INTRODUCTION

Molecular imprinting, which was first proposed by Wulff and Sarhan,[1] has been studied intensively because this technique is a facile way to generate molecular recognition sites in connection with chromatography, membranes, sensors, catalysts, and so forth.[2–8] Even though molecular imprinting can be classified into two categories, which are covalent molecular imprinting and noncovalent molecular imprinting,[7] molecular recognition materials are prepared from low molecular weight compounds having polymerizable functional moieties; in other words, they are prepared by a bottom-up technique. On the contrary, molecular recognition materials are also generated by a top-down technique: they are produced from high molecular weight compounds, which are polymeric materials,[9,10] such as oligopeptide derivatives,[11] derivatives of natural polymers,[12] and synthetic polymers.[13] When attention is paid to only molecularly imprinted materials from oligopeptide derivatives, they are affected by factors such as the difference in shape between a print molecule and its target molecule,[14] the absolute configuration of oligopeptide derivatives,[15] the sequence of oligopeptide derivatives,[16] the species of amino acid residues consisting of oligopeptide derivatives,[17] the polarity of the environment around the generated recognition sites,[18] the constituting amino acid residue number of the oligopeptide,[19] and so forth. As for the effect of the constituting amino acid residue number, we made it clear that chiral recognition sites were constructed from oligopeptide derivatives, of which the number of constituting amino acid residues was three to six in a recent study.[19] At that time, the oligopeptide derivatives that were adopted were prepared from three types of amino acid residues, such as Asp(OcHex), Ile, and Glu(OBzl). In that study, the number of constituting amino acid residues and their sequence were indeed variables. It is interesting and indispensable to investigate the effect of the constituting amino acid residue number of oligopeptide derivatives, which were prepared from one type of amino acid residue, on the chiral recognition ability. To this end, various oligopeptide derivatives, which are candidate materials forming chiral recognition sites, were prepared from just one type of amino acid residue of Boc-L-Glu(OBzl) and their chiral recognition ability was investigated.

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EXPERIMENTAL

Materials

A protected amino acid [Boc-L-Glu(OBzl)], aminomethylated polystyrene (1% divinylbenzene; aminomethyl content = 0.83 mequiv/g), and dicyclohexylcarbodiimide (DCC) were purchased from Peptide Institute, Inc. (Osaka, Japan) and used without further purification. Dichloromethane,[20] triethylamine (TEA),[20] diisopropylethylamine (DIEA),[21] trifluoroacetic acid (TFA),[20] and 2-propanol[20] were purified by the usual methods. The copolymer of acrylonitrile and styrene (AS), in which the weight fraction of the acry-

TABLE I
Conditions for Anhydride Coupling

Step	Operation and reagent[a]	Mixing time (min)
1	CH_2Cl_2, 25 cm^3 (5 times)	1
2	5% TEA/CH_2Cl_2, 20 cm^3 (3 times)	2
3	CH_2Cl_2, 25 cm^3 (3 times)	2
4	5% TEA/CH_2Cl_2, 20 cm^3 (3 times)	2
5	CH_2Cl_2, 25 cm^3 (5 times)	1
6	Premix reaction mixture (once)	15
7	0.5 mol dm^{-3} DIEA/CH_2Cl_2, 1 cm^3 (once)	15
8	CH_2Cl_2, 25 cm^3 (3 times)	2
9	2-PrOH, 20 cm^3 (2 times)	1
10	CH_2Cl_2, 25 cm^3 (5 times)	1
11	5% DIEA/CH_2Cl_2, 20 cm^3 (3 times)	2
12	Repeat steps 5–9	

[a] The percentages are expressed as volume/volume ratios. TEA, triethylamine; DIEA, diisopropylethylamine.

lonitrile unit was 0.33, was kindly supplied by Ube Cycon, Ltd. Boc-L-Trp, Ac-D-Trp, Ac-L-Trp, tetrahydrofuran (THF), sodium azide, and ethanol were used without purification. Distilled water was employed.

Synthetic procedure for oligopeptide derivatives

Six types of oligopeptide derivatives were prepared by solid-phase peptide synthesis.[22–24] Aminomethylated polystyrene resin (1.000 g, 8.30×10^{-4} mol of aminomethyl moiety) was placed in a manual reactor similar to one previously reported[23] and carried out through the schedule[25] shown in Tables I and II. The first amino acid residue, which was directly attached to the aminomethylated polystyrene resin, was introduced following the procedure summarized in Table I. The second to eighth amino acid residues for each oligopeptide derivative were introduced following the method in Table II. The premix reaction mixture used in all coupling reactions was prepared as follows: 2.10 g (6.22×10^{-3} mol) of Boc-L-Glu(OBzl) in 15.0 cm^3 of CH_2Cl_2 was cooled at 0°C and mixed with 0.642 g (3.11×10^{-3} mol) of DCC. After the mixture was stirred for 30 min at 0°C, the precipitate was filtered at ambient temperature and washed with 5.0 cm^3 of CH_2Cl_2. The combined filtrate and washing were immediately added manually to the resin. The completeness of coupling was monitored by a qualitative ninhydrin (Kaiser) test.[26] Acylation was judged complete in the coupling of symmetrical anhydride of Boc-L-Glu(OBzl) after two or three cycles. Following the schedule mentioned above, the polystyrene resin bearing the oligopeptide derivatives Boc-Glu(OBzl)—NH—CH_2— (Boc-E1-resin), Boc-[Glu(OBzl)]$_2$—NH—CH_2— (Boc-E2-resin), Boc-[Glu(OBzl)]$_5$—NH—CH_2— (Boc-E5-resin), Boc-[Glu(OBzl)]$_6$—NH—CH_2— (Boc-E6-resin), Boc-[Glu(OBzl)]$_7$—NH—CH_2— (Boc-E7-resin), and Boc-[Glu(OBzl)]$_8$—NH—CH_2— (Boc-E8-resin) were obtained. The polystyrene resin bearing H—Glu(OBzl)—NH—CH_2— (E1-resin),H—[Glu(OBzl)]$_2$—NH—CH_2—(E2-resin),H—[Glu(OBzl)]$_5$—NH—CH_2— (E5-resin), H—[Glu(OBzl)]$_6$—NH—CH_2— (E6-resin), H—[Glu(OBzl)]$_7$—NH—CH_2— (E7-resin), and H—[Glu(OBzl)]$_8$—NH—CH_2— (E8-resin) were derived from corresponding Boc-protected resins by treatment with TFA in CH_2Cl_2,[25] respectively. For convenience, the chemical structures of the oligopeptide derivatives are given in Figure 1 together with the tripeptide derivative H—[Glu(OBzl)]$_3$—O—CH_2— (E3-resin)[27] and tetrapeptide derivative H—[Glu(OBzl)]$_4$—NH—CH_2— (E4-resin).[17]

From the hydrolysis of the polystyrene resin and derivatization with dimethylamino azobenzenesulfonyl chloride,[28] the contents of the oligopeptide derivative thus introduced into the aminomethylated polystyrene resin were determined to be 2.7×10^{-4} mol g^{-1} for the E1-resin, 2.6×10^{-4} mol g^{-1} for the E2-resin, 3.2×10^{-4} mol g^{-1} for the E5-resin, 2.1×10^{-4} mol g^{-1} for the E6-resin, 3.0×10^{-4} mol g^{-1} for the E7-resin, and 2.8×10^{-4} mol g^{-1} for the E8-resin, as summarized in Table III.

In the present study, chiral recognition ability is investigated in aqueous ethanol solution. To prevent structural deformation of recognition sites and retain the "molecular memory" in the imprinted polymers, the protecting groups of the side-chain carboxyl group of the benzyl ester (OBzl) was not removed.

Preparation of molecularly imprinted polymers

Our research group has previously studied the chiral recognition of imprinted molecular recognition sites as a form of membrane in connection with the study on the optical resolution with imprinted polymeric membranes. Thus, in the present study the effect of the constituting amino acid residue number on the chiral recognition ability was investigated in the form of a membrane.

TABLE II
Conditions for Performed Anhydride Coupling

Step	Operation and reagent[a]	Mixing time (min)
1	CH_2Cl_2, 25 cm^3 (5 times)	1
2	20% TFA/CH_2Cl_2, 20 cm^3 (once)	2
3	20% TFA/CH_2Cl_2, 25 cm^3 (once)	20
4	CH_2Cl_2, 25 cm^3 (5 times)	1
5	5% DIEA/CH_2Cl_2, 20 cm^3 (3 times)	2
6	CH_2Cl_2, 25 cm^3 (5 times)	1
7	Premix reaction mixture (once)	90
8	0.5 mol dm^3 DIEA/CH_2Cl_2, 1 cm^3 (once)	15
9	CH_2Cl_2, 25 cm^3 (3 times)	2
10	2-PrOH, 20 cm^3 (3 times)	1
11	Repeat steps 4–10	

[a] The percentages are expressed as volume/volume ratios. DIEA, diisopropylethylamine.

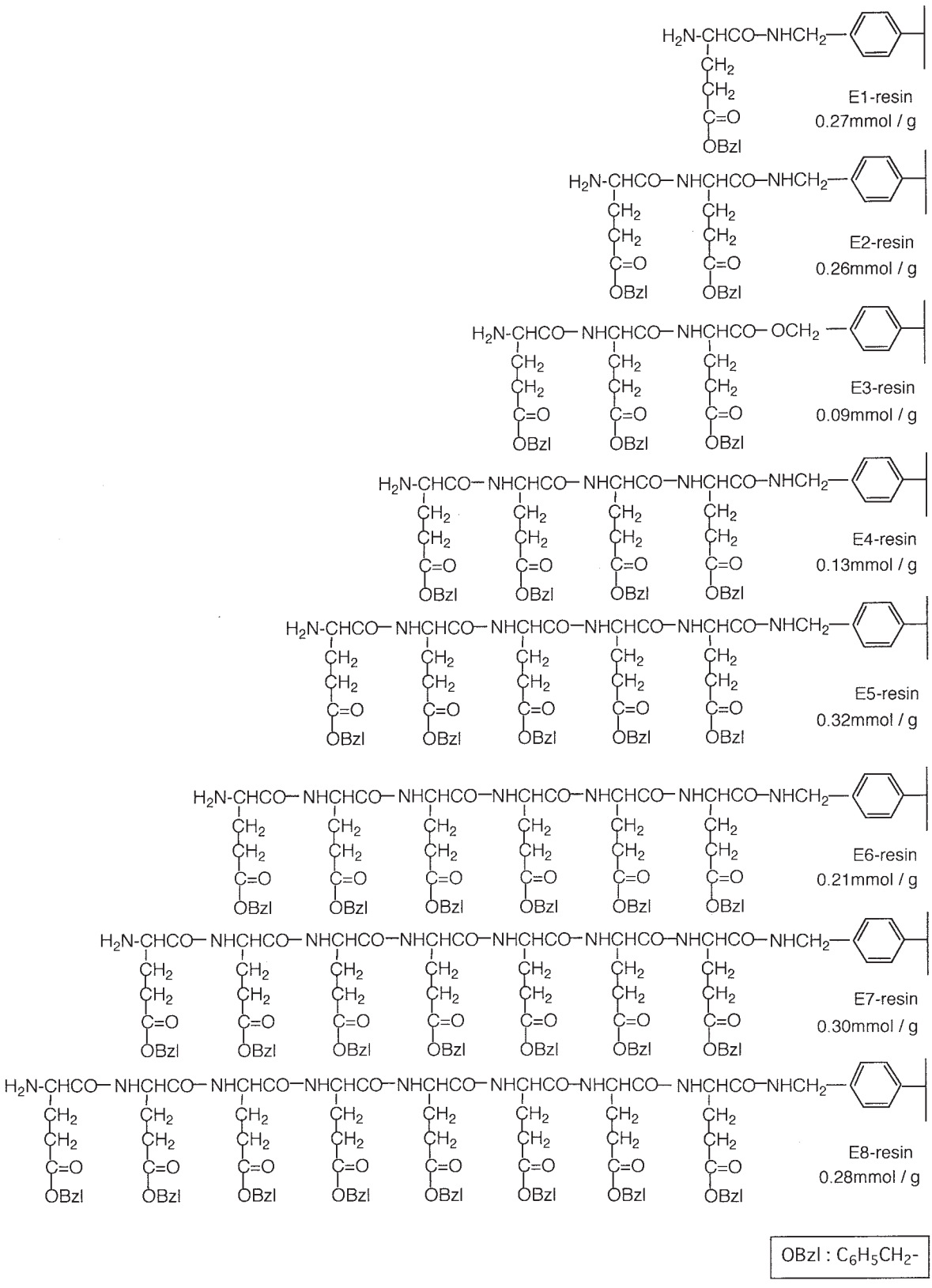

Figure 1 The chemical structures of the oligopeptide derivatives from monomer to 8-mer.

Each polymeric membrane was prepared from THF solution containing corresponding components. The polystyrene resin bearing the oligopeptide derivative does not form self-standing membranes by itself. Therefore, an AS copolymer, in which the weight fraction of the acrylonitrile unit was 0.33, was adopted as a membrane matrix.

TABLE III
Content of Oligopeptide Derivatives Introduced into Aminomethylated Polystyrene Resin

Resin	Obsd.[a] (mol g^{-1})	Calcd.[b] (mol g^{-1})	Boc-E-Resin (obsd.)[c] (mol g^{-1})
E1-resin	2.7×10^{-4}	—	2.7×10^{-4}
E2-resin	2.6×10^{-4}	2.4×10^{-4}	2.7×10^{-4}
E5-resin	3.2×10^{-4}	3.2×10^{-4}	3.3×10^{-4}
E6-resin	2.1×10^{-4}	2.4×10^{-4}	2.7×10^{-4}
E7-resin	3.0×10^{-4}	3.1×10^{-4}	3.6×10^{-4}
E8-resin	2.8×10^{-4}	2.8×10^{-4}	3.0×10^{-4}

[a] The content of oligopeptide derivative introduced into the aminomethylated polystyrene resin.
[b] The calculated content of oligopeptide derivative introduced into the aminomethylated polystyrene resin on the basis of that for the first Glu(OBzl) residue, which was directly attached to the resin.
[c] The content of the first Glu(OBzl) residue, which was directly attached to the aminomethylated polystyrene resin.

A typical membrane preparation process is described using that for the E5-resin membrane (mole ratio of the print molecule/E5 derivative = 1.0) from E5-resin and AS: 2.93 mg of the print molecule of Boc-L-Trp (same amount as that of the E5 derivative in the resin) was dissolved in 3.0 cm^3 of THF with 30.0 mg of E5-resin; 170 mg of AS was dissolved in the previous THF solution. The THF solution thus prepared was poured into a flat laboratory dish (8.9-cm diameter) and the solvent allowed to evaporate at 25°C for 24 h. The obtained membrane was dried at 50°C for an additional 2 h. After drying, the print molecule was extracted from the resultant membranes by a known, large volume of methanol until the print molecule was hardly detected in the methanol by UV analysis. In the present case, 92.1% of the added print molecule was recovered. Other membranes were prepared in a similar manner. The membrane preparation conditions are summarized in Table IV.

Adsorption of racemic mixtures to imprinted polymers

The molecularly imprinted polymers were immersed in a 50 vol % aqueous ethanol solution containing the racemic Ac-Trp (1.0×10^{-3} mol dm^{-3}), and the membrane was allowed to equilibrate at 40°C. Sodium azide (0.02 wt %) was added as a fungicide. Aliquots of the solution at the initial stage and after equilibrium were used for quantitative estimation by an HPLC (JASCO UV 1580) apparatus equipped with a UV detector (JASCO UV 1570) and a CHIRALPAK MA(+) column (50 × 4.6 mm i.d., Daicel Chemical Ind., Ltd.) with aqueous copper sulfate solution as an eluent.

The amount of amino acid in the supernatant subtracted from the initial amount in the solution gave the amount of amino acid adsorbed by the membrane. The adsorption selectivity ($S_{A(L/D)}$) is defined as

$$S_{A(L/D)} = ((\text{Ac-L-Trp})/(\text{Ac-D-Trp}))/([\text{Ac-L-Trp}]/[\text{Ac-D-Trp}])$$

where (Ac-L-Trp) and (Ac-D-Trp) are the amounts of Ac-Trp adsorbed in the imprinted polymer and [Ac-L-Trp] and [Ac-D-Trp] denote the concentrations in the solution after equilibrium was reached.

Adsorption isotherms of Ac-D-Trp and Ac-L-Trp

The imprinted polymers were immersed in solutions of various concentrations of pure Ac-D-Trp or Ac-L-Trp and allowed to equilibrate at 40°C. Sodium azide (0.02 wt %) was added as a fungicide. The quantitative analyses were performed as described above.

RESULTS AND DISCUSSION

Preparation of candidate materials forming chiral recognition sites

In the present study, all oligopeptide derivatives were prepared from one type of amino acid residue,

TABLE IV
Conditions for Preparation of Molecularly Imprinted Materials[a]

	Resin (mg)	Boc-L-Trp (mg)	AS (mg)	THF (cm^3)	Recovery[b] (%)	Thickness (μm)
E1-resin	29.9	2.43	170	3.0	88.0	146
E2-resin	30.0	2.34	170	3.0	73.1	133
E3-resin[c]	30.0	0.82	170	2.0	95.8	145
E4-resin[d]	30.0	1.19	170	3.0	90.0	95
E5-resin	30.0	2.93	170	3.0	92.1	73
E6-resin	30.0	1.92	170	3.0	86.5	65
E7-resin	30.0	2.69	170	3.0	71.3	87
E8-resin	30.0	2.52	170	3.0	84.3	88

[a] The mole ratio of the print molecule, Boc-L-Trp, to the oligopeptide derivative in the membrane preparation process was fixed at 1.0.
[b] Of the print molecule, Boc-L-Trp, after extraction with methanol.
[c] Cited from Yoshikawa et al.[27]
[d] Cited from Yoshikawa et al.[17]

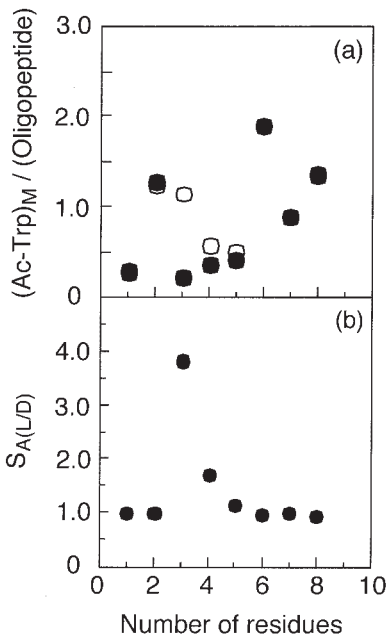

Figure 2 The effect of the number of constituting amino acid residues on (a) the Ac-Trp adsorption and (b) the adsorption selectivity toward Ac-L-Trp in oligopeptide membranes; (Boc-L-Trp)/(oligopeptide) = 1.0.

Glu(OBzl). In order to confirm the determined content of oligopeptide derivative introduced into the aminomethylated polystyrene resin, the determined values for the final oligopeptide derivative given in Figure 1 were compared with the calculated ones based on those for the first amino acid residue, which was directly attached to the aminomethylated polystyrene resin. The data are summarized in Table III. The determined oligopeptide content for each resin is in fair agreement with the calculated one. From those, it was concluded that the desired oligopeptide derivatives were obtained. Chiral recognition materials were constructed from those six types of resins bearing various oligopeptide derivatives, and their chiral recognition ability was investigated.

Adsorption selectivity of racemic Ac-Trps

The dependence of the adsorption of Ac-D-Trp and Ac-L-Trp on the number of constituting amino acid residues of Glu(OBzl) is shown in Figure 2. In the present study, the molecular imprinting conditions of the mole ratio of the print molecule Boc-L-Trp to the oligopeptide derivative for all membranes were fixed at 1.0. The amounts of amino acids adsorbed in the imprinted polymers are given as relative ones, which were converted to an oligopeptide derivative basis. The seven kinds of oligopeptides, except that in the E3-resin, were attached to polystyrene resins through amide linkages, whereas the tripeptide derivative of Glu(OBzl) in the E3-resin was attached to the resin through ester linkage. Here, we can assume that the difference between the amide and ester linkages hardly affects the ability of chiral recognition. As expected from the results reported previously,[19] the imprinted polymers (constituting amino acid residue number = three–five) showed adsorption selectivity toward the L-isomer of Ac-Trp. Other molecularly imprinted polymers from the E1-resin, E2-resin, E6-resin, E7-resin, and E8-resin hardly showed adsorption selectivity. The adsorption selectivity toward Ac-L-Trp gave the maximum value of 3.80 with the constituting number of three, whereas the maximum adsorption selectivity was given at the number of four in the previous study.[19] The adsorption selectivities for the E4-resin were determined to be 1.71 and 1.15 for the E5-resin. From this it can be said that the suitable constituting amino acid residue number for an oligopeptide derivative forming a chiral recognition site is three in the present study. Contrary to the adsorption selectivity, the amounts of adsorbed Ac-Trp are scattered. This might be due to the nature of the imprinted polymer itself. The oligopeptide derivatives with constituting amino acid residue numbers of one and two were too short to surround the print molecule during the imprinting process; as a result, chiral recognition sites were not generated from those two types of oligopeptide derivatives. The oligopeptide derivatives, (constituting amino acid residue number $>$ five) did not form chiral recognition sites as well. One plausible reasons is that oligopeptides having more constituting amino acid residues tend to be folded by self-association, such as the formation of an α helix and so forth. In addition, those oligopeptide derivatives cannot interact with the print molecule. Because of this reason, chiral recognition sites were not generated around the oligopeptide derivatives, in which the number of amino acid residues were six to eight in this study.

From the previously reported data[14,29–31] it is known that the oligopeptide membrane prepared in the absence of a print molecule does not produce chiral recognition ability.

Adsorption isotherms of Ac-D-Trp and Ac-L-Trp

As described in the previous section, it is expected that a chiral recognition site can be found in the membrane from the E5-resin, which showed adsorption selectivity toward Ac-L-Trp in Figure 2. It was already confirmed that chiral recognition sites were constructed from the E3-resin[27] and E4-resin.[17] In contrast, it is also anticipated that other oligopeptide resins, such as E1-resin, E2-resin, E6-resin, E7-resin, and E8-resin, did not generate chiral recognition sites in the present study. To this end, adsorption isotherms of Ac-D-Trp and Ac-L-Trp were studied. That is, each molecularly

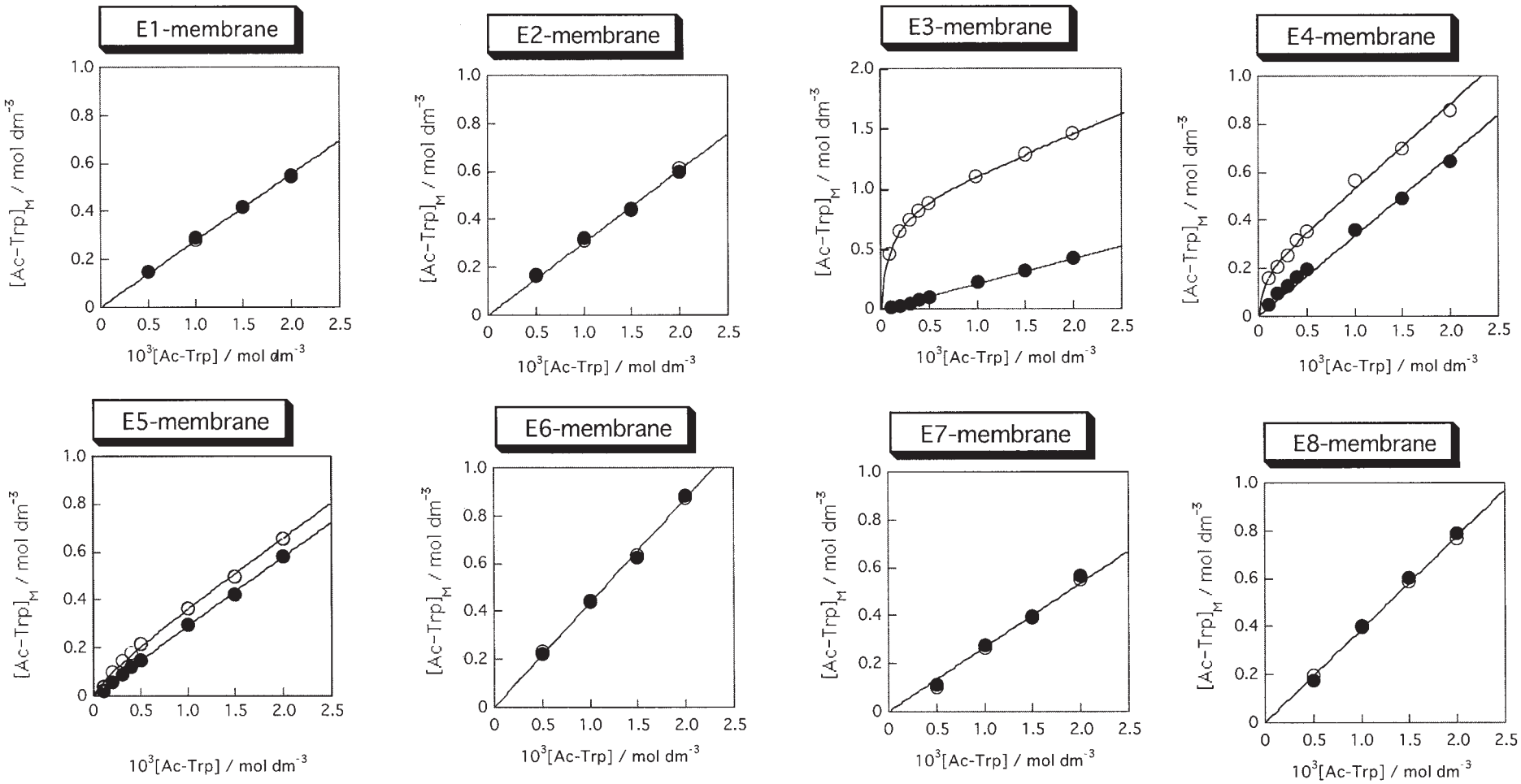

Figure 3 Adsorption isotherms of (●) Ac-D-Trp and (○) Ac-L-Trp on the molecularly imprinted polymeric membrane. The mole ratio of the print molecule, Boc-L-Trp, to the oligopeptide derivative in the membrane preparation process was fixed at 1.0.

imprinted polymer was in contact with individual optically pure Ac-D-Trp or Ac-L-Trp solution and was allowed to equilibrate. Figure 3 shows both adsorption isotherms for all membranes together with those for the E3-resin[27] and E4-resin.[17] As for the isotherms of the D-isomer for eight types of molecularly imprinted polymers, the isotherms are straight lines passing through the origin, implying that the D-isomer was adsorbed in the molecularly imprinted polymers without any specific interaction with the polymers. In this case, the concentration of Ac-D-Trp adsorbed in the imprinted polymer can be represented by the following equation:

$$[\text{Ac-D-Trp}]_P = k_A[\text{Ac-D-Trp}]$$

where $[\text{Ac-D-Trp}]_P$ is the concentration of Ac-D-Trp adsorbed in the imprinted polymer, k_A denotes the adsorption constant, and [Ac-D-Trp] is the Ac-D-Trp concentration in the outer solution equilibrated with the molecularly imprinted polymer.

Conversely, the adsorption isotherms of Ac-L-Trp for the E1-resin, E2-resin, E6-resin, E7-resin, and E8-resin are straight lines passing through the origin. It can be concluded that Ac-L-Trp was adsorbed in those polymers without any specific interaction like the adsorption of Ac-D-Trp. Their adsorption isotherms can be represented by the following equation:

$$[\text{Ac-L-Trp}]_P = k_A[\text{Ac-L-Trp}]$$

where $[\text{Ac-L-Trp}]_P$ is the concentration of Ac-L-Trp adsorbed in the imprinted polymer and [Ac-L-Trp] is the Ac-L-Trp concentration in the outer solution equilibrated with the molecularly imprinted polymer.

Those for Ac-L-Trp in the E3-resin, E4-resin, and E5-resin show dual adsorption isotherms, implying that adsorption consists of two types of populations of adsorption, such as nonspecific adsorption and adsorption on specific recognition sites toward Ac-L-Trp. The concentration of Ac-L-Trp in the imprinted polymer from the E5-resin can be represented by the following adsorption equation like those from the E3-resin[27] and E4-resin[17]:

$$[\text{Ac-L-Trp}]_P = k_A[\text{Ac-L-Trp}] + nK_S[\text{oligopeptide}][\text{Ac-L-Trp}]/(1 + K_S[\text{Ac-L-Trp}])$$

where n is the ratio of the maximum concentration of Ac-L-Trp adsorbed on the chiral recognition sites to the concentration of oligopeptide derivatives in the imprinted polymer; [oligopeptide] denotes the concentration of oligopeptide derivative in the imprinted polymer; and K_S is the affinity constant between Ac-L-Trp and the chiral recognition sites, which were constructed by the presence of the print molecule during the membrane preparation process. The three parameters in those adsorption equations for the imprinted polymers, which were determined to fit each adsorption isotherm in Figure 3 best, are summarized in Table V together with those for the E3-resin[27] and E4-resin.[17] The obtained adsorption isotherms summarized in Figure 3 support the results that the imprinted polymers from the E1-resin, E2-resin, E6-resin, E7-resin, and E8-resin hardly yielded adsorption selectivity as shown in Figure 2. Contrary to those five types of imprinted polymers, a chiral recognition site can be found in the imprinted polymer from the E5-resin like the E3-resin[27] and E4-resin.[17] As a result, the imprinted polymer from the E5-resin recognized Ac-L-Trp from racemic mixtures and the L-isomer was preferentially incorporated into the polymer. Among the three types of imprinted polymers, the imprinted polymer from the tetrapeptide derivative (E4-resin) gave a maximum affinity constant toward the target molecule.

TABLE V
Parameters for Adsorption Isotherms of Ac-D-Trp and Ac-L-Trp

	[Oligopeptide] (mol dm^{-3})	k_A	n	K_S (mol^{-1} dm^3)
E1-resin	0.38	27	—	—
E2-resin	0.41	30	—	—
E3-resin[a]	0.28	62	0.63	9.6×10^3
E4-resin[b]	0.36	35	0.57	10.8×10^3
E5-resin	0.42	29	0.21	3.4×10^3
E6-resin	0.36	43	—	—
E7-resin	0.31	27	—	—
E8-resin	0.44	39	—	—

$[\text{Ac-D-Trp}]_M = k_A[\text{Ac-D-Trp}]$
$[\text{Ac-L-Trp}]_M = k_A[\text{Ac-L-Trp}] + nK_S[\text{oligopeptide}][\text{Ac-L-Trp}]/(1+K_S[\text{Ac-L-Trp}])$
[a] Cited from Yoshikawa et al.[27]
[b] Cited from Yoshikawa et al.[17]

CONCLUSIONS

In the present study, the effect of the constituting amino acid residue [Glu(OBzl)] number on the chiral recognition ability was investigated. Chiral recognition sites were prepared from oligopeptide derivatives (constituting amino acid residue number = three–five) by adopting an alternative molecular imprinting. It was made clear that with the constituting amino acid residue number of four, the tetrapeptide derivative of Glu(OBzl) is the best candidate material to generate a chiral recognition site, because it gives the highest affinity constant toward the target molecule, among eight types of oligopeptide derivatives as previously reported.[19]

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