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Short communication

Liquid chromatographic resolution of proton pump inhibitors including omeprazole on a ligand exchange chiral stationary phase

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ABSTRACT

A ligand exchange chiral stationary phase (CSP) developed previously in this laboratory by bonding (R)-phenylglycinol derivative, sodium N-[(R)-2-hydroxy-1-phenylethyl]-N-undecylaminoacetate, to silica gel was successfully applied to the resolution of proton pump inhibitors (PPIs) including omeprazole, pantoprazole, lansoprazole and rabeprazole. For example, the separation factors (α) for the resolution of omeprazole, pantoprazole, lansoprazole and rabeprazole were 4.27, 5.28, 2.77 and 4.06, respectively, and the resolutions (R_S) were 2.53, 2.55, 1.93, and 2.01, respectively, when 65% acetonitrile aqueous solution containing 0.5 mM $CuSO_4$ and 0.05 mM triethylamine was used as a mobile phase. Based on the chromatographic behaviors for the resolution of PPI analogues on CSP **1**, a chiral recognition mechanism utilizing the sulfoxide oxygen and the benzimidazole ring nitrogen of PPIs as bidentate coordination donors to form an enantioselective ternary complex with the central Cu(II) ion and the chiral stationary bidentate ligand was proposed.

1. Introduction

Proton pump inhibitors (PPIs) such as omeprazole, pantoprazole, lansoprazole and rabeprazole have been known to suppress gastric acid secretion via interaction with H^+/K^+ATPase in gastric parietal cells and consequently these drugs have been frequently prescribed to treat acid-related disorders such as gastric and duodenal ulcers, Zolinger–Ellison syndrome, and other hypersecretory diseases [1–3]. PPIs have a common chiral benzimidazole sulfoxide structure and consequently, they consist of two enantiomers. However, they have been used as racemic mixtures except omeprazole. Between the two enantiomers, the (S)-omeprazole has been known to show a greater and prolonged gastric acid suppression for the patients than the omeprazole racemate because of the different metabolism of the two enantiomers in the liver [4]. Enantioselective disposition of other PPIs has been revealed through the studies for the enantioselective metabolism or enantioselective pharmacokinetics of the two enantiomers [5–11]. In the studies on the enantioselective disposition of PPIs, the exact determination of the two enantiomers has usually been done by utilizing the method of liquid chromatographic enantiomer separation on chiral stationary phases (CSPs) based on amylose derivatives [5,6,8,12,13] or β-cyclodextrin derivatives [14]. However, ligand exchange CSPs have not been utilized in the separation of the two enantiomers of PPIs to the best of our knowledge.

Ligand exchange CSPs have been extensively studied and used in resolving racemic analytes, which can be used as bidentate ligands such as α-amino acids, since the pioneering works of Davankov [15–19]. We also developed ligand exchange CSPs by bonding (S)-leucinol derivative, sodium N-[(S)-1-hydroxymethyl-3-methylbutyl]-N-undecylaminoacetate, or (R)-phenylglycinol derivative, sodium N-[(R)-2-hydroxy-1-phenylethyl]-N-undecylaminoacetate, to silica gel [20,21]. The CSPs developed in our laboratory were quite successful in the resolution of α- and β-amino acids and α-hydroxycarboxylic acids [20–24]. Between the two CSPs, the one (CSP **1**, Fig. 1) prepared by bonding (R)-phenylglycinol derivative, sodium N-[(R)-2-hydroxy-1-phenylethyl]-N-undecylaminoacetate, to silica gel was found to show greater chiral recognition ability than the other [21,24].

In this study, we wish to extend the use of CSP **1** to the resolution of PPIs. The enantioselective formation of ternary complexes composed of chiral stationary bidentate ligand, central Cu(II) ion and racemic analyte has been known to be responsible for the chiral recognition by CSP **1** [21,24]. For the formation of ternary complexes with the central Cu(II) ion and the chiral stationary bidentate ligand, the analyte should be also bidentate. PPIs contain a sulfoxide functional group. In addition, PPIs contain a benzimidazole ring and a pyridine ring. The sulfoxide oxygen and the benzimidazole ring or pyridine ring nitrogen of PPIs can coordinate to the central Cu(II) ion. In this instance, PPIs are expected to be used as bidentate

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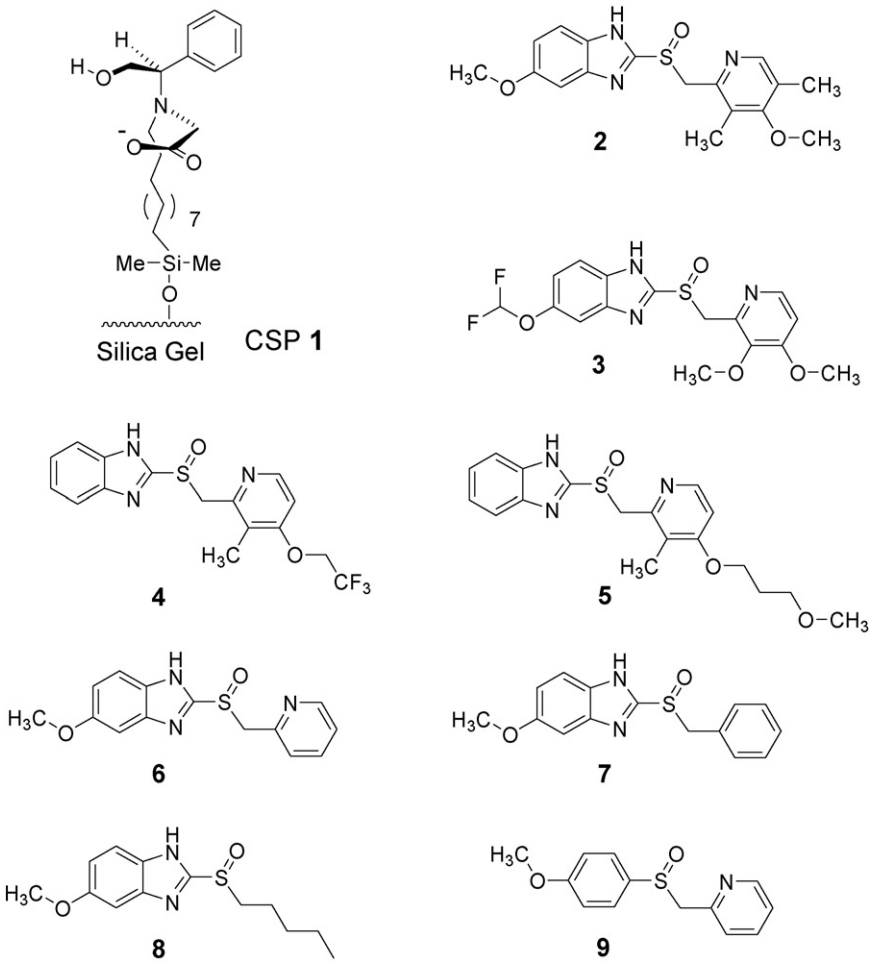

Fig. 1. Structures of CSP **1**, omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**), rabeprazole (**5**) and PPI analogues **6**, **7**, **8** and **9**.

ligands and, consequently, their two enantiomers are expected to be separated on CSP **1**.

2. Experimental

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump (Milford, MA, USA), a Rheodyne model 7725i injector (Rohnert Park, CA, USA) with a 20 μl sample loop, a Waters 2487 Dual absorbance detector and a YoungLin Autochro data module (Software: YoungLin Autochro WIN 2.0 plus). The temperature of the chiral column was set at 20 °C by using a Julabo F30 Ultratemp 2000 cooling circulator (Seelbach, Germany). The void volume was measured by injecting 2,6-lutidine.

Racemic PPIs [omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**), Fig. 1] and optically active omeprazole used in this study were generously donated to one of the authors (JSJ) by the Korea Food and Drug Administration (KFDA). Analytes **6**–**8** shown in Fig. 1 were prepared by treating 5-methoxy-2-benzimidazolethiol (1.0 equivalent, Aldrich) with 2-(chloromethyl)pyridine (1.0 equivalent, Aldrich), benzyl chloride (1.0 equivalent, Aldrich) or pentyl bromide (1.0 equivalent, Aldrich), respectively, in ethanol solution of NaOH (2.0 equivalent) at 0 °C and then by treating the resulting sulfides with 3-chloroperoxybenzoic acid (MCPBA, 1.0 equivalent, Aldrich) in methylene chloride at 0 °C. Analyte **9** was prepared similarly by treating 4-methoxythiophenol (1.0 equivalent, Aldrich) with 2-(chloromethyl)pyridine (1.0 equivalent) in ethanol solution of NaOH (2.0 equivalent) at 0 °C and then by treating the resulting sulfide with hydrogen peroxide (30% aqueous solution, 2.0 equivalent, Junsei Chemical Co., Japan) in methanol in the presence of amberlyst 15 (Aldrich) at room temperature. The ^{1}H NMR spectra of analytes **6**–**9** thus prepared were consistent with their structures. Injection samples were prepared by dissolving each analyte in ethanol at a concentration of 3.0 mg/ml. The usual injection volume was 0.1 μl.

3. Results and discussion

Resolution of bidentate analytes such as α- and β-amino acids and α-hydroxycarboxylic acids on CSP **1** has been performed with the use of aqueous methanol, ethanol or acetonitrile solution containing Cu(II) ion as a mobile phase [21,23,24]. Similarly, we tried to use aqueous methanol, ethanol or acetonitrile solution containing Cu(II) ion as a mobile phase in the resolution of PPIs on CSP **1**. However, the use of aqueous methanol or ethanol solution containing Cu(II) ion as a mobile phase was not suitable for the resolution of PPIs on CSP **1** because of the unstabilized base-line. In contrast, we found that the use of aqueous acetonitrile solution containing Cu(II) ion provided reasonable base-line resolutions. As an effort to find out the optimum content of acetonitrile and Cu(II) ion in aqueous mobile phase, the chromatographic resolutions of PPIs [omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**)] were performed with the variation of the content of acetonitrile and $CuSO_4$ in water and the results are summarized in Table 1.

When the content of acetonitrile in aqueous mobile phase containing 0.5 mM $CuSO_4$ was changed from 30% to 50% and then

Table 1
Resolution of PPIs [omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**)] on CSP **1** with the variation of the content of acetonitrile (ACN, %) and $CuSO_4$ (mM) in water as a mobile phase.[a]

	Mobile phase	**2**			**3**			**4**			**5**		
	ACN in water/$CuSO_4$ (pH)	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
a	30% ACN/0.5 mM $CuSO_4$ (4.92)	2.10	3.59	1.36	4.71	5.01	1.51	5.48	1.85	1.22	3.75	2.90	1.69
b	50% ACN/0.5 mM $CuSO_4$ (4.38)	1.37	3.10	1.98	1.32	3.88	2.09	1.58	2.06	1.34	1.32	3.07	1.80
c	65% ACN/0.5 mM $CuSO_4$ (3.89)	1.15	3.72	2.31	0.82	5.96	2.09	1.03	2.44	1.72	0.99	3.74	1.98
d	65% ACN/0.3 mM $CuSO_4$ (4.16)	1.34	4.07	2.66	1.20	4.76	1.94	1.21	2.57	1.27	1.59	3.69	1.67
e	65% ACN/0.1 mM $CuSO_4$ (4.51)	0.67	3.71	1.39	0.86	4.43	1.58	1.07	2.53	1.25	1.65	3.44	1.36

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. Column temperature: 20 °C. k_1: retention factor of the first eluted enantiomer. α: separation factor. R_S: resolution.

to 65%, the separation factors (α) and the resolutions (R_S) were usually improved (see entry a, b and c in Table 1). Increasing the content of acetonitrile in aqueous mobile phase further was not attempted with the consideration of the solubility problem of $CuSO_4$. As the content of acetonitrile in aqueous mobile was increased, the retention factors (k_1) decreased (see entry a, b and c in Table 1). In reversed-phase liquid chromatography, we assume that the lipophilic interaction of analytes with the stationary phase might be the major contributor for the retention of analytes especially when the analytes are relatively lipophilic. The lipophilic interaction of analytes with the stationary phase should decrease as the polarity of aqueous mobile phase decreases. In this instance, the retention of PPIs denoted by the retention factors (k_1) should decrease as the content of acetonitrile in aqueous mobile phase is increased.

When the content of $CuSO_4$ in 65% acetonitrile in water was decreased from 0.5 mM to 0.3 mM and then 0.1 mM, the general trends in the separation factors (α), the resolutions (R_S) and the retention factors (k_1) were not observed (see entry c, d and e in Table 1). In the resolution of omeprazole (**2**), the highest separation factor (α) and the resolution (R_S) were obtained when the content of $CuSO_4$ in 65% acetonitrile in water was 0.3 mM. However, in the resolution of pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**), the highest separation factors (α) and the resolutions (R_S) were obtained when the content of $CuSO_4$ in 65% acetonitrile in water was 0.5 mM. The longest retention times were observed when the content of $CuSO_4$ in 65% acetonitrile in water was 0.3 mM for the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) while 0.1 mM for the resolution of rabeprazole (**5**). Overall, the optimum mobile phase condition which can be equally applicable to the resolution of PPIs might be concluded to be 65% acetonitrile in water containing 0.5 mM $CuSO_4$.

As efforts to improve the resolutions of PPIs on CSP **1**, we performed the resolution of PPIs on CSP **1** by adding basic additive, triethylamine, and/or acidic additive, acetic acid, to the mobile phase consisting of 65% acetonitrile in water containing 0.5 mM $CuSO_4$ and summarized the chromatographic resolution results in Table 2. When 0.01 mM triethylamine was added to 65% acetonitrile in water containing 0.5 mM $CuSO_4$, much difference was not observed in the separation factors (α), the resolutions (R_S) and the retention factors (k_1) for the resolution of PPIs on CSP **1** (compare entry c in Table 1 with entry a in Table 2). When 0.05 mM triethylamine was added to 65% acetonitrile in water containing 0.5 mM $CuSO_4$, both of the separation factors (α) and the resolutions (R_S) improved for the resolution of omeprazole (**2**), lansoprazole (**4**) and rabeprazole (**5**) and the resolution (R_S) improved for the resolution of pantoprazole (**3**) (see entry c in Table 1 and entry b in Table 2). However, adding 0.10 mM triethylamine to 65% acetonitrile in water containing 0.5 mM $CuSO_4$ does not improve the separation factors (α) and/or the resolutions (R_S) further (see entry c in Table 2). In contrast, the retention factors (k_1) increased continuously as the amount of triethylamine in 65% acetonitrile in water containing 0.5 mM $CuSO_4$ is increased (see entry a, b and c in Table 2).

Addition of 0.01 mM acetic acid to 65% acetonitrile in water containing 0.5 mM $CuSO_4$ was found to improve the separation factors (α) and the resolutions (R_S) for the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) (see entry c in Table 1 and entry d in Table 2). However, addition of acetic acid further (0.05 and 0.10 mM) was found to diminish the separation factors (α), the resolutions (R_S) and the retention factors (k_1) for the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) (see entry d, e and f in Table 2).

Addition of both triethylamine and acetic acid to 65% acetonitrile in water containing 0.5 mM $CuSO_4$ generally improved the separation factors (α) and the resolutions (R_S) except for the resolution of rabeprazole (**5**) (see entries g–k in Table 2). When both triethylamine and acetic acid were added to 65% acetonitrile in water containing 0.5 mM $CuSO_4$, the retention factors (k_1) were also increased. By adding triethylamine and acetic acid to the mobile phase, the ionic strength of the mobile phase is expected to increase and consequently, the polarity of the mobile phase increases. In

Table 2
Resolution of PPIs [omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**)] on CSP **1** with the variation of the content of triethylamine(TEA, mM) and acetic acid (AcOH, mM) in 65% acetonitrile (ACN) in water containing 0.5 mM $CuSO_4$.[a]

	Mobile phase	**2**			**3**			**4**			**5**		
	ACN in water/$CuSO_4$/TEA/AcOH (pH)	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
a	65%/0.5 mM/0.01 mM/0.00 mM (4.35)	1.01	4.01	2.34	0.95	4.83	2.55	0.91	2.66	1.68	1.07	3.80	1.80
b	65%/0.5 mM/0.05 mM/0.00 mM (4.59)	1.07	4.27	2.53	0.94	5.28	2.55	0.97	2.77	1.93	1.25	4.06	2.01
c	65%/0.5 mM/0.10 mM/0.00 mM (4.82)	1.31	4.14	2.07	1.15	4.77	2.24	1.21	2.63	1.20	1.74	3.91	1.85
d	65%/0.5 mM/0.00 mM/0.01 mM (3.84)	1.13	4.30	2.55	1.08	5.39	2.75	1.21	2.76	1.97	1.52	4.01	2.34
e	65%/0.5 mM/0.00 mM/0.05 mM (3.81)	0.91	4.15	2.30	0.74	5.17	2.55	0.84	2.65	1.61	0.92	4.01	2.31
f	65%/0.5 mM/0.00 mM/0.10 mM (3.75)	0.94	3.97	2.09	0.76	4.98	2.51	0.82	2.60	1.58	0.94	3.80	2.20
g	65%/0.5 mM/0.01 mM/0.05 mM (4.29)	1.58	4.82	2.64	1.31	5.87	2.39	1.38	3.05	1.89	1.49	3.98	2.27
h	65%/0.5 mM/0.05 mM/0.05 mM (4.39)	1.67	5.01	2.52	1.43	5.80	2.93	1.48	3.14	2.22	1.35	4.04	1.80
i	65%/0.5 mM/0.10 mM/0.05 mM (4.45)	1.62	4.94	2.78	1.36	5.83	2.47	1.43	3.10	2.22	1.19	3.84	1.73
j	65%/0.5 mM/0.05 mM/0.01 mM (4.52)	1.65	5.10	2.66	1.41	5.99	3.01	1.45	3.20	2.11	1.88	3.43	1.90
k	65%/0.5 mM/0.05 mM/0.10 mM (4.37)	1.44	4.63	2.42	1.19	5.62	2.58	1.30	2.88	2.19	1.31	3.81	2.33

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. Column temperature: 20 °C. k_1: retention factor of the first eluted enantiomer. α: separation factor. R_S: resolution.

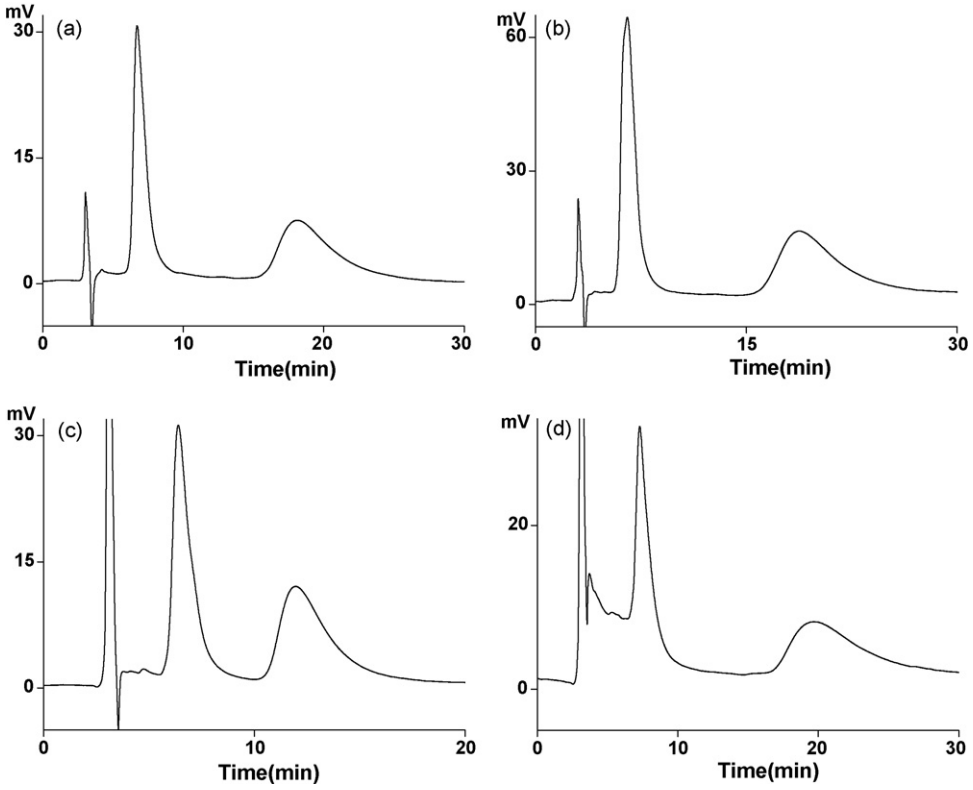

Fig. 2. Representative chromatograms for the liquid chromatographic resolution of (a) omeprazole (**2**), (b) pantoprazole (**3**), (c) lansoprazole (**4**) and (d) rabeprazole (**5**) on CSP **1**. Mobile phase: 65% acetonitrile in water containing 0.5 mM $CuSO_4$ and 0.05 mM triethylamine. Flow rate: 1.0 ml/min. Detection: 254 nm UV. Column temperature: 20 °C.

this instance, the lipophilic interaction of analytes with the stationary phase becomes more favorable and consequently, the retention factors (k_1) are expected to increase.

From the results shown in Tables 1 and 2, it is concluded that addition of triethylamine and/or acetic acid to 65% acetonitrile in water containing 0.5 mM $CuSO_4$ generally improves the separation factors (α) and the resolutions (R_S) for the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) on CSP **1** even though the reason is not clear yet. In every case, the base-line resolutions were observed. Practically, any mobile phase condition shown in Table 2 can be applied to the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) on CSP **1**. For example, Fig. 2 shows the representative chromatograms for the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) on CSP **1** with the use of 65% acetonitrile in water containing 0.5 mM $CuSO_4$ and 0.05 mM triethylamine as a mobile phase.

The pH values of the mobile phases used were also checked and included in Tables 1 and 2. The pH values of the mobile phases were found to be in the range of 3.75–4.92. However, we were not able to see any notable relationship between the pH values and the separation factors (α) and the resolutions (R_S) for the resolution of omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) on CSP **1**. PPIs have been known to be degraded to the corresponding sulfenamide in neutral or acidic environment [25]. However, even under the most acidic mobile phase condition (65% acetonitrile in water + 0.50 mM $CuSO_4$ + 0.1 mM acetic acid, pH = 3.75), PPIs were found to be stable enough to be resolved on CSP **1** within 120 min. After 120 min, PPIs were found to show some degradation. Therefore, there would be no difficulty in the application of CSP **1** to the resolution of PPIs under any mobile phase condition provided in Table 2 for the analytical purpose. However, the preparative scale resolution of PPIs on CSP **1** should be avoided because the long contact time of PPIs with the acidic mobile phase might induce the significant degradation of PPIs.

The chiral recognition mechanism for the resolution of PPIs on CSP **1** is not clear yet except that the enantioselective formation of ternary complexes with optically active chiral stationary bidentate ligand, central Cu(II) ion and racemic analyte is essential for the chiral recognition as mentioned in the introduction part. For the enantioseparation on CSP **1**, analytes should be used as bidentate ligands. In PPIs, the sulfoxide oxygen and the benzimidazole ring nitrogen of PPIs can coordinate to the central Cu(II) ion as a bidentate. Similarly, the sulfoxide oxygen and the 2-pyridyl ring nitrogen of PPIs can also coordinate to the central Cu(II) ion as a bidentate as shown in Fig. 3. The axial coordination of the hydroxyl group of the chiral stationary bidentate ligand shown in Fig. 3 has been proposed previously for the resolution of α-amino acids on CSP **1** [21,14]. In order to elucidate which mode of the two shown in Fig. 3 is utilized in the chiral recognition, we prepared PPI analogues such as compounds **6**–**9** shown in Fig. 1 and resolved them on CSP **1**. Compound **6** contains both of the benzimidazole ring and the 2-pyridyl ring in addition to the sulfoxide group as PPIs and consequently, it can form ternary complex with the chiral stationary bidentate ligand and the central Cu(II) ion by the two modes shown in Fig. 3. However, compounds **7** and **8** contain only the benzimidazole ring and the sulfoxide group and consequently, only the first mode shown in Fig. 3 is applicable for the formation of ternary complex from compound **7** or **8**, the chiral stationary bidentate ligand and Cu(II) ion. In contrast, compound **9** contains only the 2-pyridyl ring and the sulfoxide group and consequently, only the second mode shown in Fig. 3 is applicable for the formation of ternary complex from compound **9**, the chiral stationary bidentate ligand and Cu(II) ion. In this instance, comparison of the resolutions of compounds **6**–**9** on CSP **1** is expected to elucidate the importance of the two modes shown in Fig. 3 for the formation of ternary complexes.

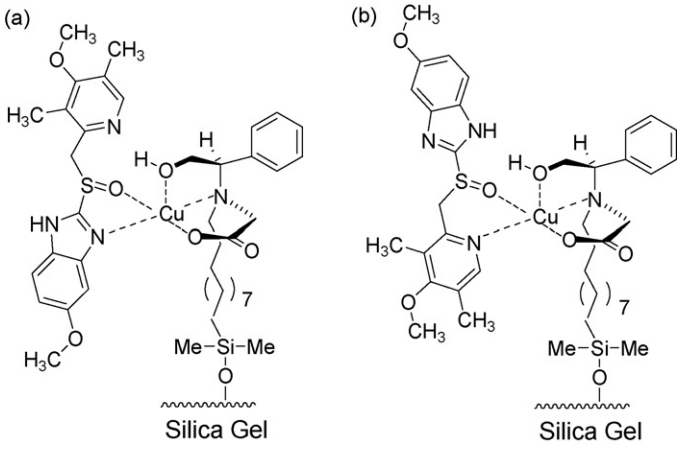

Fig. 3. Two modes for the formation of ternary complex (a) by utilizing the sulfoxide oxygen and the benzimidazole ring nitrogen of PPIs as bidentate donor atoms coordinating to the central Cu(II) ion and (b) by utilizing the sulfoxide oxygen and the 2-pyridyl ring nitrogen of PPIs as bidentate donor atoms coordinating to the central Cu(II) ion.

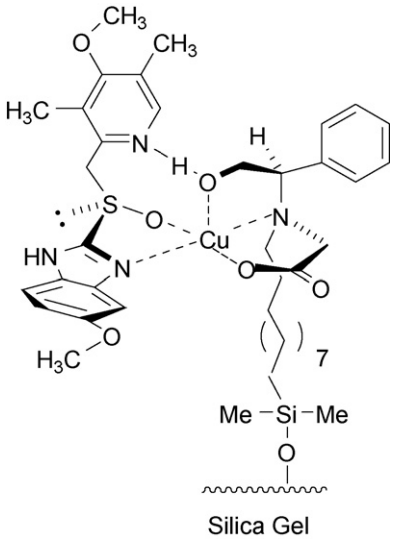

Fig. 4. A proposed chiral recognition mechanism for the resolution of PPIs on CSP **1**.

The chromatographic results for the resolution of compounds **6**–**9** on CSP **1** with the use of 65% acetonitrile in water containing $CuSO_4$ (0.5 mM), triethylamine (0.05 mM) and acetic acid (0.01 mM) as a mobile phase are summarized in Table 3. Resolution of compound **6** on CSP **1** is quite excellent, the separation factor (α) and the resolution (R_S) for the resolution of compound **6** on CSP **1** being even greater than those for omeprazole (**2**), pantoprazole (**3**), lansoprazole (**4**) and rabeprazole (**5**) (see entry j in Table 2 for the comparison of the chromatographic parameters under the identical mobile phase condition). When the 2-pyridyl group of compound **6** was replaced with a phenyl group in compound **7** or with a simple alkyl group in compound **8**, the separation factor (α) and the resolution (R_S) were decreased quite much even though the separation factors (α) were still high. However, in the resolution of compound **9**, which contains a 2-pyridyl ring, but does not contain an imidazole ring, the separation factor (α) and the resolution (R_S) were decreased significantly. In addition, the retention factors (k_1 and k_2) of the two enantiomers are very low, indicating that the ternary complex is not formed effectively. From these results, it is concluded that the first mode shown in Fig. 3 is utilized for the formation of the ternary complex for the chiral recognition of PPIs. Even though not involved in the coordination to the central Cu(II) ion, the-2-pyridyl ring is expected to play a significant role in the chiral recognition of PPIs based on the fact that the separation factors (α) and the resolutions (R_S) for the resolution of compound **6** are much greater than those for the resolution of compound **7** or **8**.

For the resolution of omeprazole (**2**) on CSP **1**, the (R)-enantiomer was found to be retained longer than the (S)-enantiomer by simply eluting optically active omeprazole. Based on the elution order of the two enantiomers and the fact that the first mode shown in Fig. 3 is applicable for the formation of the ternary complex and compound **6** is resolved better than compound **7** or **8**, we propose a chiral recognition mechanism for the resolution of PPIs on CSP **1** as shown in Fig. 4. In Fig. 4, the (R)-enantiomer of omeprazole is proposed to form a ternary complex with the chiral stationary bidentate ligand and the central Cu(II) ion by utilizing the sulfoxide oxygen and the benzimidazole ring nitrogen as bidentate donor atoms. The trans-(N,N)-configuration of the ternary complex shown in the proposed chiral recognition mechanism has been known to be more favorable than the cis-(N,N)-configuration [26] and successfully utilized in explaining the chiral recognition of α- and β-amino acids on CSP **1** [21,24]. In this instance, the 2-pyridyl ring of the (R)-enantiomer is directed upward and the nitrogen atom of the 2-pryidyl ring can form hydrogen bond with the hydrogen of the axially coordinating hydroxyl group of the stationary ligand. Even though we tried to support the proposed chiral recognition mechanism by molecular mechanics calculation or NMR study, it was not successful at the present time because of many variables to be considered.

In summary, in this study, we demonstrated that a ligand exchange chiral stationary phase, CSP **1**, is very successful in the resolution of PPIs including omeprazole, pantoprazole, lansoprazole and rabeprazole especially when 65% acetonitrile aqueous solution containing 0.5 mM $CuSO_4$ and a small amount of triethylamine and/or acetic acid as a mobile phase. Based on the elution order of the two enantiomers of omeprazole, the fact that the sulfoxide oxygen and the benzimidazole ring nitrogen of analytes are utilized as bidentate coordination atoms for the formation of the ternary complex with the central Cu(II) ion and the chiral stationary bidentate ligand and the fact that 2-pyridyl ring of analytes helps the chiral recognition, a chiral recognition mechanism was proposed. Our efforts to support the proposed chiral recognition mechanism by molecular mechanics calculation or NMR study were not successful yet, but the efforts are still underway in our laboratory.

Table 3
Resolution of compounds **6**–**9** on CSP **1** with the use of 65% acetonitrile in water containing $CuSO_4$ (0.5 mM), triethylamine (0.05 mM) and acetic acid (0.01 mM) as a mobile phase.[a]

Analytes	k_1	k_2	α	R_S
6	1.37	8.22	6.00	3.84
7	2.46	6.17	2.51	1.44
8	3.32	7.34	2.21	0.75
9	0.17	0.22	1.30	0.27

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature: 20 °C. k_1: retention factor of the first eluted enantiomer. k_2: retention factor of the second eluted enantiomer. α: separation factor. R_S: resolution.

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