

Molecular-length and chiral discriminations by β -structural poly(L-alanine) on silica

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Abstract

Poly(L-alanine)-grafted porous silica (Sil-Ala₂₂) was prepared by polymerization of *N*-carboxyanhydride of L-alanine initiated by 3-aminopropylated silica. Its selective interaction with aromatic guest molecules was evaluated by the retention time in liquid chromatography using the column packed with Sil-Ala₂₂. Sil-Ala₂₂ showed a specific selective retention with discriminating molecular shapes, such as molecular length, linearity and planarity. This selectivity can be explained by a multiple π - π interaction with the carbonyl groups one dimensionally-aligned on the rigid β -form structure in the peptide main chain. Chiral separation with Sil-Ala₂₂ was also described.

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1. Introduction

Host-guest chemistry has been attracting much attention, because it is strongly related to various biofunctions and important in understanding biofunctions at molecular level. Many researchers have developed various host compounds, showing molecular recognition. Especially, in recent year, the molecular imprinting methods have been applied as usable technique to prepare the host compounds with selective cavity for guest molecules. Molecular recognition with imprinted polymers was often demonstrated in liquid chromatography and applied to chemical sensing [1,2], catalysis [3,4] and chiral separation [5–7]. Since, imprinted polymers are allowed to possess specific cavity with exact placement of functional groups through templates, they are often regarded as artificial enzyme analogues [8]. On the other hand, it is well-known that excellent molecular recognition in biofunctions can be realized by inter- or intramolecular orientations of functional

groups in non-cavity-shaped structure like antibodies. Therefore, molecular recognition with non-cavity-shaped structure can also be realized through highly oriented functional groups.

Based on these facts, we have evaluated the molecular recognition ability due to non-cavity-shaped linear polymers, such as poly(alkyl acrylate) [9,10] on porous silica gels by using as stationary phase in HPLC. Through this work, it was found that the carbonyl groups in side chains worked as an interaction source for π -containing compounds. Especially, the carbonyl groups aligned on the highly ordered side chains yielded the multiple carbonyl- π interaction, which was accompanied by specific molecular-shape selectivity of polycyclic aromatic hydrocarbons [11–13]. Therefore, secondary structural polypeptides are especially interesting because the carbonyl groups in the peptide main chain can orient through rigid structures, such as α -helices or β -sheets. In this paper, we describe how poly(L-alanine) on silica (Fig. 1) behaves as a specific host molecule for polycyclic aromatic hydrocarbons (PAHs) through the carbonyl groups on the β -structural conformation.

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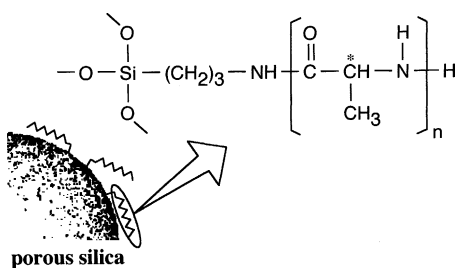


Fig. 1. Schematic illustration of poly(L-alanine)-grafted silica.

2. Experimental

2.1. Materials

L-Alanine (Ala; Wako, Japan) was used after crystallized from water. 3-Aminopropyltrimethoxysilane (APS) as an initiator was purchased from Chisso, Japan. YMC 120-S5 (diameter 5 μm , pore size 120 \AA , specific surface area 330 $\text{cm}^2 \text{g}^{-1}$) was used as a porous silica. Silica-supported poly(L-alanine) (Sil-Ala_n, where *n* is the average of polymerization degree) was prepared by polymerization of *N*-carboxyanhydride of Ala (Ala-NCA) initiated by 3-aminopropylated silica (Sil-APS). Typical preparation procedure is as follows: (1) APS (0.19 mmol for silica 1 g) was readily introduced by mixing in toluene at 60 °C for 24 h. The resulting particles were washed successively with toluene and ether and dried in vacuo. (2) Ala (1.23 g) was dispersed in tetrahydrofuran (40 ml) and then triphosgene (1.4 g) was added slowly to the dispersion. After the mixture became clear, the solution was concentrated in vacuo and white powders were precipitated by a large excess of *n*-hexane. Ala-NCA was obtained by filtration and dry in vacuo. (3) Ala-NCA (4.4 mmol for Sil-APS 1 g) was added in the suspension of Sil-APS (2.23 g) in dimethyl sulfoxide (25 ml) and stirred at room temperature for 3 days. The immobilization was confirmed by IR spectroscopy. The specific absorptions due to amide bonds (amide I and II) were detected at 1633 and 1540 cm^{-1} , respectively. Successive washing with 3,3,3,3',3',3'-hexafluoro-2-propanol as a good solvent for Ala_n showed no significant change in the elemental analysis. The amount of Ala_n immobilized was determined to be 22.5 wt.% by elemental analysis. The average of polymerization of Ala_n on silica was estimated to be 22 by the molar ratio between Ala_n and APS, which were used.

2.2. Measurements

Sil-Ala₂₂ was packed into a stainless steel column (i.d. 150 mm \times 4.6 mm, using a hexanol–chloroform mixture. The interaction between Ala₂₂ and guest molecules was evaluated by the retention time in HPLC. The chromatograph included a Jasco 980 pump and a Jasco MD-910 UV–vis photodiode array detector. Ten μl of sample was injected through a Reodyne Model 7125 injector. Liquid chromatography was carried out with a methanol–water mixture as mobile phase

at flow rate 0.5 ml min^{-1} . The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where *t_e* and *t₀* were retention time of guest molecules and methanol, respectively. The separation factor (α) was given by the ratio of retention factors.

Water–1-octanol partition coefficient ($\log P$) was determined by retention factor with octadecylated silica, ODS (Inertsil ODS, i.d. 250 mm \times 4.6 mm, GL Science): $\log P = 3.759 + 4.207 \log k$ ($r = 0.99997$) [14].

The conformation of Ala₂₂ on silica was evaluated by FT-IR measurements with attenuated total reflection (ATR) method. IR spectra were obtained, using 32 scans at a resolution of 4.0 cm^{-1} with a Spectrum One spectrometer attached ATR accessory (Perkin-Elmer, Japan).

2.3. Calculations

The structures of poly(L-alanine) and poly(L-leucine) were estimated by PEPCON [15]. The structures of PAHs were estimated by HyperChem version 5.1 with molecular mechanics (until the energy changes were below 0.001 kcal mol^{-1} ; 1 cal = 4.184 J) and following semi-empirical AM1 method.

3. Results and discussion

3.1. Secondary structure of poly(L-alanine) on silica

It is known that the absorption band ascribed to an amide V in IR spectroscopy confirms the conformation of polypeptides. However, it was difficult to detect the amide V of Sil-Ala₂₂, because of the strong absorption due to silica. Therefore, the conformation of Ala₂₂ on silica was estimated by the absorptions at amides I and II. As shown in Fig. 2a, the IR spectrum for Sil-Ala₂₂ provided an amide I absorption at 1633 cm^{-1} with a shoulder at 1655 cm^{-1} and an amide II absorption at 1540 cm^{-1} . Since, it was reported that amide I at 1630 and amide II at 1530 cm^{-1} were characteristic of a β -form structure [16], the Ala₂₂ moiety can be assumed to be mainly immobilized in a β -form structure. This result is quite different from the fact that poly(L-alanine) is usually α -helical in a solution state [12]. This difference should be due to that of polymerization condition. Probably, the solid silica as an initiator for polymerization induced a β -form conformation.

In order to estimate the conformation of Ala₂₂ under HPLC conditions, Sil-Ala₂₂ was dispersed in a methanol–water (6:4) mixture at 30 °C, which is the same condition used in HPLC in the present study, for the certain periods (0.5, 12 and 72 h) and then IR measurement was carried out after collecting by filtration. The IR spectroscopy showed almost no significant change in the absorption at 1633 cm^{-1} , although the absorbance of a shoulder band at 1655 cm^{-1} decreased slightly with increase of the dispersion time (Fig. 2b–d). These results imply that Ala₂₂ on silica

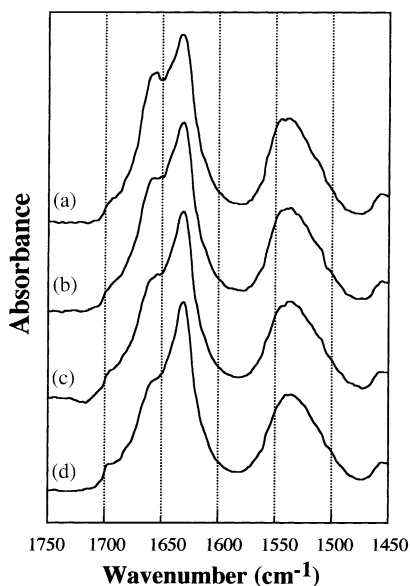


Fig. 2. FT-IR spectra of Sil-Ala₂₂ after dispersed in a methanol–water (6:4) at 30 °C for the certain periods: 0 h (a), 0.5 h (b), 48 h (c) and 72 h (d).

keeps the original β -form structure under the HPLC condition. In this study, Sil-Ala₂₂ which was allowed to stand in a methanol–water (6:4) for more than 72 h was used for HPLC.

3.2. Retention mode for alkylbenzenes in Sil-Ala₂₂

The comparison between the retention behaviors of alkylbenzenes and polycyclic aromatic hydrocarbons (PAHs) may be used to evaluate the retention mode of packing materials in HPLC [17]. Fig. 3 shows the relationship between $\log k$ and $\log P$ with Sil-Ala₂₂ and ODS.

As shown in Fig. 3, Sil-Ala₂₂ showed extremely small retention for alkylbenzenes. When a mobile phase was a methanol–water (9:1) mixture, k of octylbenzene was only 0.1. Therefore, a methanol–water (6:4) mixture was selected as a mobile phase for Sil-Ala₂₂. However, interestingly, the $\log P$ – $\log k$ plots showed a similar slope to that in ODS: for example, the separation factors between octyl- and hexylbenzenes were 1.79 in Sil-Ala₂₂ and 1.80 in ODS. This resembles a reversed-phase mode. However, it is no doubt that Sil-Ala₂₂ has few hydrophobic sites. This unusual coincidence with the retention mode of ODS is due to the fact that the propyl spacer used for amination can work as a hydrophobic organic phase.

3.3. Retention behavior for polycyclic aromatic hydrocarbons

Uniqueness of the Ala₂₂ phase can be seen in the retention behaviors for (PAHs). As shown in Fig. 3, Sil-Ala₂₂ showed higher retention factors for PAHs than for alkylbenzenes. For example, $\log P$ of naphthacene (5.71) is smaller than that of octylbenzene (6.30) but k of the former is much higher (9.75)

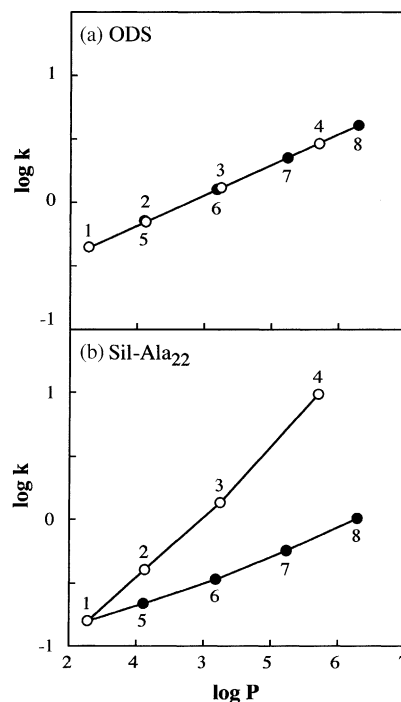


Fig. 3. Relationships between $\log k$ and $\log P$ with ODS (a) and Sil-Ala₂₂ (b). Mobile phase: (a) methanol–water (6:4) at 30 °C; (b) methanol–water (9:1) at 25 °C. Elutes: 1, benzene; 2, naphthalene; 3, anthracene; 4, naphthacene; 5–8, ethyl-, butyl-, hexyl- and octyl-benzenes.

than that of the latter (1.02). The increase of k for PAHs was accompanied by the selectivity enhancement. For example, $\alpha = 5.74$ was obtained for naphthacene/triphenylene in Sil-Ala₂₂ while $\alpha = 1.27$ in ODS. These results indicate that the Ala₂₂ phase provides specific interactive sites for PAHs. The driving force for the selectivity is discussed with a π – π interaction mechanisms later.

3.4. Molecular-length recognition

Both of triphenylene and *o*-terphenyl possess the same numbers in carbon atoms and π -electrons, but the molecular planarity is absolutely different as schematically illustrated in Fig. 4. Therefore, the separation factor for them has used as a good indicator to evaluate the molecular-planarity selectivity [18,19]. Sil-Ala₂₂ showed a small separation factor ($\alpha = 1.40$) similarly to ODS ($\alpha = 1.54$). It seems that the Ala₂₂ phase is not sensitive to the molecular shape of PAHs. This is quite interesting because carbonyl-containing organic phases on highly ordered structures, such as Sil-ODA_{*n*} [9,10,20] show much better molecular-planarity selectivity ($\alpha = 4.6$ in triphenylene/*o*-terphenyl at 15 °C with Sil-ODA_{*n*}). To understand this difference, the selectivity for various structural and geometrical isomers was investigated. Table 1 shows the selectivity for the isomers of stilbenes and terphenyls. Sil-Ala₂₂ showed remarkably higher selectivity for *p*- and *o*-terphenyls ($\alpha = 10.4$) while $\alpha = 1.47$ in ODS. As indicated in the CPK models of Fig. 4, *p*-terphenyl is twisted likely to

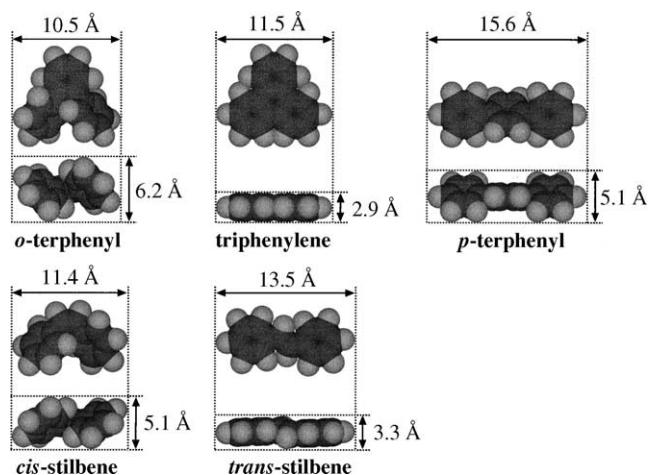


Fig. 4. CPK models of stilbenes and terphenyls estimated by HyperChem version 5.1 with molecular mechanics and following semi-empirical AM1 method.

Table 1
Retention and separation factors for the isomers of terphenyls and stilbenes

	Sil-Ala ₂₂		ODS	
	<i>k</i> ^a	α^b	<i>k</i> ^a	α^b
<i>o</i> -Terphenyl	1.24	1.37	10.4	1.49
Triphenylene	1.70			2.30
<i>p</i> -Terphenyl	12.92			2.19
<i>cis</i> -Stilbene	0.77	1.84	1.03	1.20
<i>trans</i> -Stilbene	1.42			1.24

^a The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where t_e and t_0 were retention time of samples and methanol, respectively.

^b The separation factor (α) was given by the ratio of retention factors. Mobile phases: methanol–water (6:4) at 30 °C in Sil-Ala₂₂, methanol–water (9:1) at 25 °C in ODS.

o-terphenyl. Therefore, the high selectivity of the Ala₂₂ phase cannot be explained by the difference of molecular planarity but it seems that the molecular length works as a more important factor. This assumption is applicable to explain for the higher selectivity for *cis*- and *trans*-stilbenes ($\alpha = 1.84$) than that in ODS (1.04).

Table 2 shows the retention and separation factors for benzene, naphthalene, anthracene, naphthacene and pentacene as

Table 2
Retention and separation factors for polycyclic aromatic hydrocarbons

	Sil-Ala ₂₂		ODS	
	<i>k</i> ^a	α^b	<i>k</i> ^a	α^b
Benzene	0.19	2.11	0.44	1.61
Naphthalene	0.40		0.71	
Anthracene	1.36		1.31	
Naphthacene	9.75		2.91	
Pentacene	Not eluted		6.75	

^a The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where t_e and t_0 were retention time of samples and methanol, respectively.

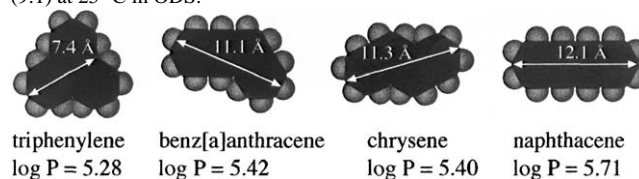
^b The separation factor (α) was given by the ratio of retention factors. Mobile phases: methanol–water (6:4) at 30 °C in Sil-Ala₂₂, methanol–water (9:1) at 25 °C in ODS.

Table 3
Retention and separation factors for four-ring polycyclic aromatic hydrocarbons

	Sil-Ala ₂₂		ODS	
	<i>k</i> ^a	α^b	<i>k</i> ^a	α^b
Triphenylene	1.70	5.74	2.30	1.27
Chrysene	3.24		2.45	
Benz[<i>a</i>]anthracene	2.44		2.48	
Naphthacene	9.75		2.91	

^a The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where t_e and t_0 were retention time of samples and methanol, respectively.

^b The separation factor (α) was given by the ratio of retention factors. Mobile phases: methanol–water (6:4) at 30 °C in Sil-Ala₂₂, methanol–water (9:1) at 25 °C in ODS.



planar and linear compounds with Sil-Ala₂₂ and ODS. The remarkably high separation factors were obtained in Sil-Ala₂₂. For example, the separation factor between naphthacene and anthracene reached $\alpha = 7.17$, while $\alpha = 2.22$ in ODS.

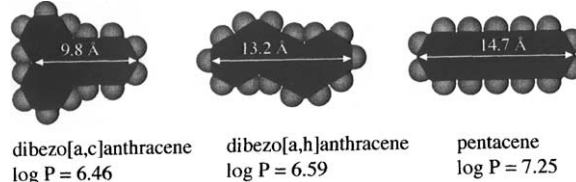
Similar specificity was also observed for structural isomers of four-ring PAHs, such as triphenylene, benz[*a*]anthracene, chrysene and naphthacene. All of them are planar compounds with the same numbers of carbon atoms and π -electrons. As it is clear in Table 3, the retention factors in ODS can be understood by log *P*'s of elutes but those in Sil-Ala₂₂ do not obey log *P*'s. It seems that the Ala₂₂ phase recognizes the molecular length because the longer the molecular length, the larger the retention factor. As supporting this assumption, similar results were obtained for five-ring PAHs as shown in Table 4.

Table 4
Retention and separation factors for five-ring polycyclic aromatic hydrocarbons

	Sil-Ala ₂₂		ODS	
	<i>k</i> ^a	α^b	<i>k</i> ^a	α^b
Dibenzo[<i>a,c</i>]anthracene	3.32	2.92	∞	4.40
Dibenzo[<i>a,h</i>]anthracene	9.69			4.72
Pentacene	Not eluted			6.75

^a The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where t_e and t_0 were retention time of samples and methanol, respectively.

^b The separation factor (α) was given by the ratio of retention factors. Mobile phases: methanol–water (6:4) at 30 °C in Sil-Ala₂₂, methanol–water (9:1) at 25 °C in ODS.



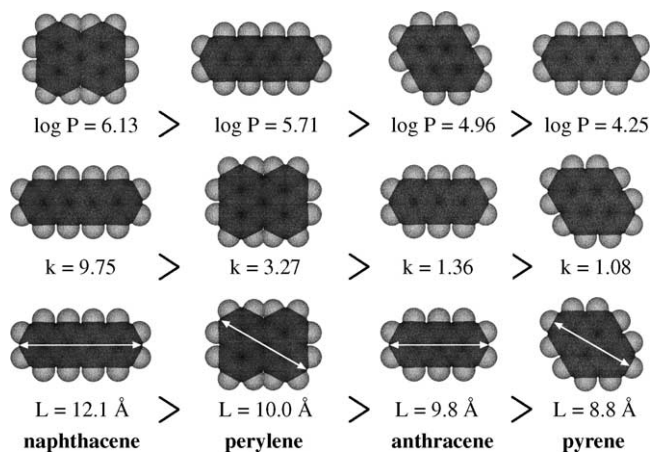


Fig. 5. Relationships between retention factor (k) in Sil-Ala₂₂ and the molecular length (L) and hydrophobicity ($\log P$) of PAHs. Mobile phase: methanol–water = 6:4 at 30 °C.

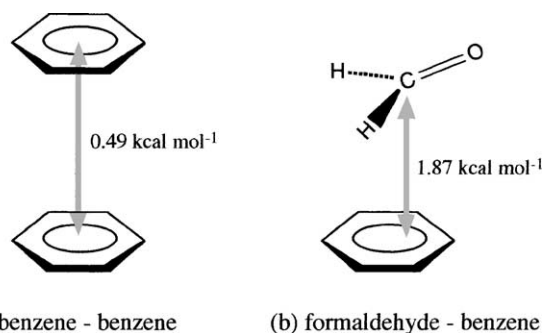


Fig. 6. Proposed structures of benzene–benzene (a) and formaldehyde–benzene (b) complexes estimated by ab initio MO/MP2 calculations.

3.5. Discussion on discrimination mechanism

As above-mentioned, Sil-Ala₂₂ shows the disagreement in the elution order for PAHs with ODS. The typical example can be seen in Fig. 5: $k = 9.75$ for naphthacene ($\log P = 5.71$) $> k = 3.27$ for perylene ($\log P = 6.13$) and $k = 1.36$ for anthracene ($\log P = 4.25$) $> k = 1.08$ for pyrene ($\log P = 4.96$). Another example is shown in the retention behaviors for pentacene and coronene: when methanol was used as a mobile phase, the k values for them were 1.67 and 0.32 in Sil-Ala₂₂ but 1.13 and 1.43 in ODS. These elution orders in Sil-Ala₂₂ cannot be explained by hydrophobic effect.

To explain the unique selectivity of the Ala₂₂ phase, we apply a multiple carbonyl π –benzene π interaction mechanism on highly ordered structures for it. A carbonyl group is polarized to be δ^+ (carbon) and δ^- (oxygen), respectively. These polarized atoms enable to work as an electrostatic source for a π – π interaction. For example, it has been reported that a carbonyl π –benzene π interaction is comparatively larger (1.87 kcal mol⁻¹) [21] than a benzene π –benzene π interaction (0.49 kcal mol⁻¹ in the plane-to-plane stacking) [22] as shown in Fig. 6. We have also described that the Hammett plots of the separation factors to the substituent effect showed

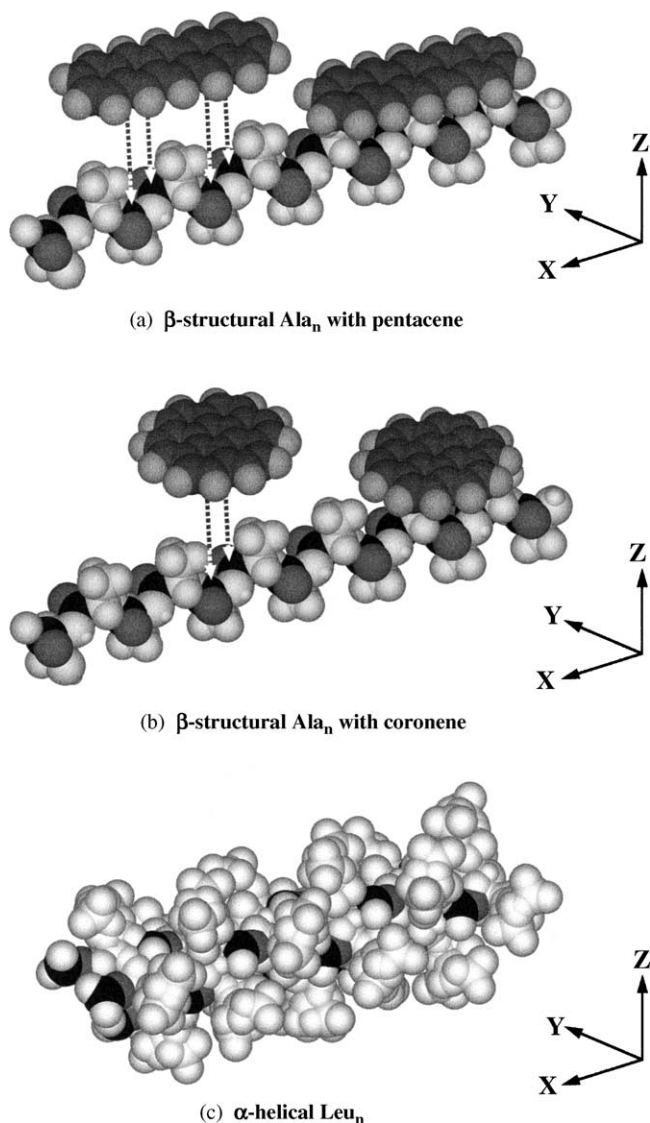


Fig. 7. (a and b) CPK models of β -structural Ala_n and (c) α -helical Leu_n derived from PEPCON [15]. The black atoms present carbonyl carbons. A linear and planar PAH, such as pentacene (a) provides more effective interaction area with the carbonyl groups aligned on rigid main chain than disk-like PAH, such as coronene (b). On the other hand, the carbonyl groups of Leu₂₀ are covered with their bulky residual groups (c).

that a carbonyl group works as an electron-accepting group toward a polycyclic aromatic hydrocarbon [14]. Therefore, we focus on the carbonyl groups in the peptide main chain as a π -electron interaction source. In addition, the carbonyl groups should be well-organized, because the peptide main chain is in a rigid β -form structure as discussed in Section 3.1. On the basis of these facts, we propose the multiple π – π interaction mechanism as schematically illustrated in Fig. 7. This assumption can be defined as follow: (1) PAHs can interact with the carbonyl groups. The methyl groups of Ala₂₂ side chain does not prevent the electrostatic interaction. (2) On the contrary, poly(L-leucine) does not give a chance to do this interaction because the residual isobutyl groups are too bulky to approach each other (Fig. 7c). As supporting

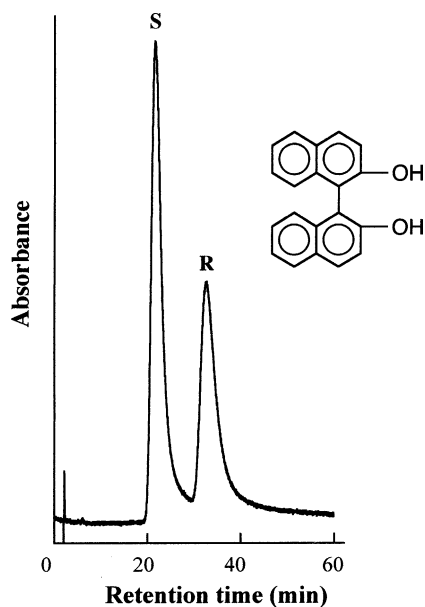


Fig. 8. Typical chromatographic separation of a racemic 1,1'-bi-2-naphthol with Sil-Ala₂₂. Mobile phase: 30 °C, methanol–water = 2:8. Flow rate: 1.0 ml min⁻¹.

this, we confirmed that poly(L-leucine)-grafted silica provides only α -helices and thus their carbonyl groups were absolutely covered with the bulky residual groups. As a result, the poly(L-leucine) phase showed only hydrophobicity recognition similar to ODS: $\alpha = 1.7$ for *p*- and *o*-terphenyls in silica-supported poly(L-leucine). This value is close to that in ODS ($\alpha = 1.5$). (3) The carbonyl groups in Ala₂₂ should be aligned one-dimensionally, because of its β -form structure. This conformation promotes the multiple carbonyl– π interaction, which works more effectively with longer PAHs than shorter ones. Fig. 7a and b show that a longer and planar PAH, such as pentacene yields higher contact points with Ala₂₂ than disk-like PAH, such as coronene.

3.6. Application for chiral separation

The Ala₂₂ phase is applicable for chiral separation because of its chirality. The primary application was carried out using 1,1'-bi-2-naphthol. A typical chromatogram is presented in Fig. 8. The separation factor was 1.6. The detail is under investigation.

4. Conclusion

β -Structural poly(L-alanine) was grafted onto porous silica and applied to molecular-shape selective HPLC for PAHs.

The attractive features of the Ala₂₂ phase were summarized as follows: (1) The Ala₂₂ phase showed higher retention and selectivity for PAHs than for alkylbenzenes. (2) The Ala₂₂ phase showed a specific selectivity, discriminating the molecular shape, such as planarity and length. (3) Probably, these molecular discrimination is derived from the highly-oriented carbonyl groups on the rigid β -form structure. (4) Sil-Ala₂₂ is also applicable for chiral separation of π -containing enantiomers.

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