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-Ala 22 ) 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-Ala 22 . Sil-Ala 22 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-Ala 22 was also described.

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1. Introduction

Host–guest chemistry has been attracting much attention, because it is strongly related to various bionfunctions 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 multiple π-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.
2. Experimental

2.1. Materials

L-Alanine (Ala; Wako, Japan) was used after crystallized from water. 3-Aminopropylmethoxysilane (APS) was purchased from Sigma, USA. Silica 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 hexane. 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 at amides I and II. As shown in Fig. 2 a, the absorptions at amides I and II absorption at 1540 cm$^{-1}$ and an amide II absorption at 1630 cm$^{-1}$ were estimated by the absorptions at amides I and II. As shown in Fig. 2a, the IR spectrum for Sil-Ala$_{22}$ 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 Ala$_{22}$ 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 Ala$_{22}$ under HPLC conditions, Sil-Ala$_{22}$ 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 Ala$_{22}$ on silica
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 and thus the separation factor was also closed to that in ODS: for example, the separation factors between octyl- and hexyl-benzenes 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) than that of the latter (1.02). The increase of k for PAHs was accompanied by the selectivity enhancement. For example, α = 5.74 was obtained for naphthacene/triphenylene in Sil-Ala22 while α = 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 (α = 1.40) similarly to ODS (α = 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, show much better molecular-planarity selectivity (α = 4.6 in triphenylene/o-terphenyl at 15 °C with Sil-ODA). 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 (α = 10.4) while α = 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>
<thead>
<tr>
<th>Table 1</th>
<th>Retention and separation factors for the isomers of terphenyls and stilbenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sil-Ala22</td>
<td>ODS</td>
</tr>
<tr>
<td>α-Terphenyl</td>
<td>1.24 1.37</td>
</tr>
<tr>
<td>Triphenylene</td>
<td>1.70 1.04</td>
</tr>
<tr>
<td>β-Terphenyl</td>
<td>1.29 2.19</td>
</tr>
<tr>
<td>cis-Stilbene</td>
<td>0.71 1.64</td>
</tr>
<tr>
<td>trans-Stilbene</td>
<td>1.42 1.24</td>
</tr>
</tbody>
</table>

α-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 (α = 1.84) than that in ODS (1.04).

Table 2 shows the retention and separation factors for benzene, naphthalene, anthracene, naphthacene and pentacene as planar and linear compounds with Sil-Ala22 and ODS. Remarkably high separation factors were obtained in Sil-Ala22. For example, the separation factor between naphthalene and anthracene reached α = 7.17, while α = 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>
<thead>
<tr>
<th>Table 3</th>
<th>Retention and separation factors for four-ring polycyclic aromatic hydrocarbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sil-Ala22</td>
<td>ODS</td>
</tr>
<tr>
<td>Triphenylene</td>
<td>1.70 2.30</td>
</tr>
<tr>
<td>Chrysene</td>
<td>2.44 2.45</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>0.75 0.74</td>
</tr>
<tr>
<td>Naphthacene</td>
<td>2.91 2.42</td>
</tr>
</tbody>
</table>

α The retention factor (α) was determined by (τr − τ0)/τ0, where τr and τ0 were retention time of samples and methanol, respectively.

β 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 4 shows the retention and separation factors for five-ring polycyclic aromatic hydrocarbons.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Retention and separation factors for five-ring polycyclic aromatic hydrocarbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sil-Ala22</td>
<td>ODS</td>
</tr>
<tr>
<td>Dibenz[a]anthracene</td>
<td>3.32 2.92</td>
</tr>
<tr>
<td>Dibenzo[a]anthracene</td>
<td>9.69 9.07</td>
</tr>
<tr>
<td>Pentacene</td>
<td>Not eluted</td>
</tr>
</tbody>
</table>

α The retention factor (α) was determined by (τr − τ0)/τ0, where τr and τ0 were retention time of samples and methanol, respectively.

β 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.
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 \( P = 4.25 \)). 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–benzene \( \pi \) interaction mechanism on highly ordered structures for it. A carbonyl group is polarized to be \( + \) (carbon) and \( - \) (oxygen), respectively. These polarized atoms enable to work as an electrostatic source for a \( \pi-\pi \) interaction. For example, it has been reported that a carbonyl \( \pi \)-benzene \( \pi \) interaction is comparatively larger (1.87 kcal mol\(^{-1}\)) than a benzene \( \pi \)-benzene \( \pi \) interaction (0.49 kcal mol\(^{-1}\) in the plane-to-plane stacking) [22].

Fig. 7. (a and b) CPK models of \( \beta \)-structural Ala\(_n\) and \( \alpha \)-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 \( \pi \)-electron interaction source. In addition, the carbonyl groups should be well-organized, because the peptide main chain is in a rigid \( \beta \)-form structure as discussed in Section 3.1. On the basis of these facts, we propose the multiple \( \pi-\pi \) 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: α = 1.7 for p- and o-terphenyls in silica-supported poly(l-leucine). This value is close to that in ODS (α = 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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