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HOST-GUEST CHEMISTRY [Using α-Helical Poly(L-Lysine)]

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Many researchers have great interest in "host-guest" chemistry using synthetic compounds because of its important role in understanding biofunctions at the molecular level. These studies have also led to various developments in biomimetic applications such as artificial receptors for sensors, organic media for separation, and transducers for chemical signals. Although a great number of host compounds have been discovered during the past half-century, almost all of these compounds are restricted to low-molecular cyclic compounds (**Figure 1A**) such as cyclodextrins, calixarenes, crown ethers, criptands, cyclic polyamines, cyclophanes and cyclic dipeptides, or their polymer-supported materials (**Figure 1B**). In these cases, the polymers do not play a main role. However, we know that

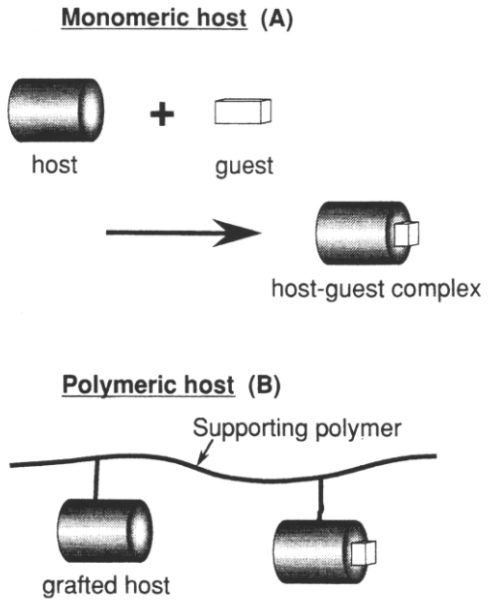

FIGURE 1. Schematic illustration of monomeric and polymeric host molecules.

specificities of biofunctions in enzymes and DNAs are derived from their three-dimensional configurations constructed by secondary structural polypeptides and polysaccharides, respectively.

Therefore, in this study, we aim to prove that α-helical synthetic polymers would be useful as host materials. As poly(L-lysine) is the simplest class of synthetic polymers that can produce chiral secondary structures spontaneously, we selected it as a host polymer and investigated its enantioselectivity in host-guest interaction. We focused especially on its α-helical conformation, in which the molecules are rather rigid and the residual amino groups assume identical position. However, random-coiled molecules are too heterogeneous to use for a molecular recognition. Unfortunately, we encountered two serious problems in this investigation: first, ionic property of the residual ammonium groups is useful as a driving force for selective binding, but charged poly(L-lysine) usually forms random coils in water. It is necessary to find a special condition in which charged poly(L-lysine) forms an α-helical conformation. Second, it is very difficult to detect enantioselective binding with chiral substances, because the interaction is not usually accompanied by a spectrophotometrically detectable response.

In this study, we avoided these problems by selection of methanol as a solvent, and by establishment of a new evaluating method for detecting the interaction, respectively. The latter technique is based on the fact that an achiral cyanine dye NK-2012 bound to polycations shows

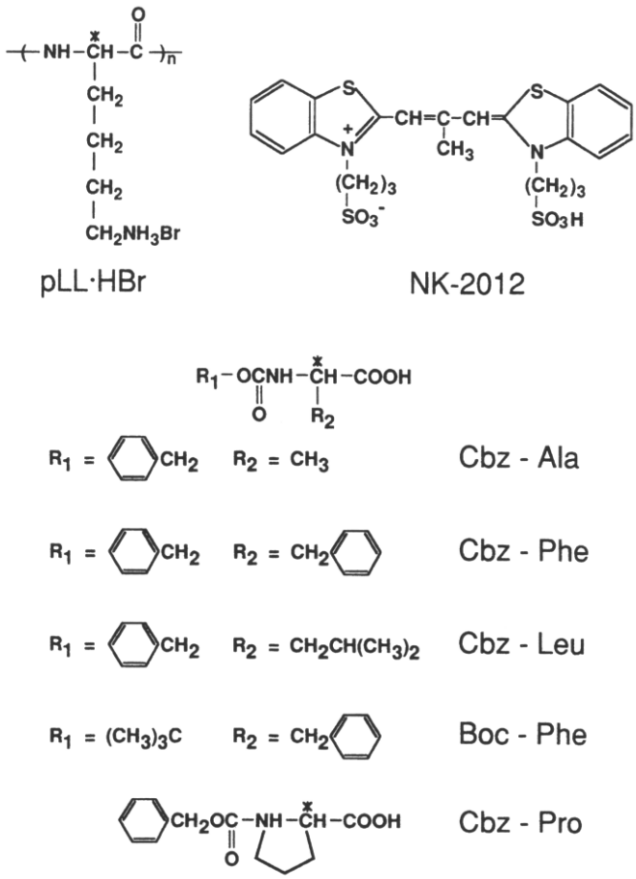

FIGURE 2. Chemical structures of pLL·HBr, NK-2012, and *N*-substituted α-amino acids.

remarkable changes in the visible and circular dichloism (CD) spectra due to dissociation of the polycation-dye complexes induced by the interaction between polycations and anionic guest molecules.[1–4]

Here we describe how α-helical poly(L-lysine) acts as an enantioselective host molecule for *N*–benzyloxycarbonyl α-amino acids as guest molecules and also on the molecular recognition mechanism. The chemical structures of the host polymer, cyanine dye, and *N*-substituted α-amino acids as guest molecules used in this study are given below, along with their abbreviations (**Figure 2**).

EXPERIMENTAL

Materials

Poly(L-lysine hydrobromide) was prepared by polymerization of N^{α}-carboxyanhydride of N^{ϵ}-benzyloxycarbonyl L-lysine and following debenzyloxycarbonylation with acetic acid saturated with hydrobromide. The average degree (n) of polymerization was determined to be 600 according

to the equation ($\log n = 0.79 \log \eta_{sp} + 2.46$) provided by Hatano et al.[5]

Cyanine dye NK-2012 was obtained from Nippon Kanko Shikiso Laboratories and was used without further purification.

We purchased *N*-benzyloxycarbonyl derivatives of L-phenylalanine, D-phenylalanine, L-proline, D-proline, L-leucine, D-leucine, L-alanine, and D-alanine from Wako Pure Chemicals Co., Ltd. We brought *N-tert*-Butyloxycarbonyl derivatives of L- and D-phenylalanines from Kokusan Kagaku Chemicals Co., Ltd.

Measurements

The pLL-NK-2012 complex solution was prepared by dissolving poly(L-lysine hydrobromide) (pLL·HBr) and NK-2012 in given ratios to methanol or water. We confirmed the formation of the complexes using both Shimadzu UV-160A and JASCO J-500C spectrophotometers. We monitored the interaction between pLL and *N*-substituted α-amino acids by detecting the spectral changes due to the dissociation of the complexes.

Computer Calculations

Right-handed α-helical structure in charged poly(L-lysine) was estimated using the PEPCON program.[6–8] The center-to-center distance between two nitrogen atoms of residual groups was also determined by the PEPCON conformation calculation.

We estimated possible molecular shapes of NK-2012 and *N*-substituted α-amino acids with the MOPAC program (MATERIA) using PM3 option.[9] We also determined the center-to-center distance between two sulfur atoms of NK-2012.

RESULTS

Formation of pLL-dye Complexes

Poly(L-lysine hydrobromide) (pLL·HBr) was dissolved in water (pH 7) to show a CD spectrum with 3000 deg cm^2 dmol^{-1} at 222 nm (a dotted line in **Figure 3**). This spectrum agrees with that in random coiled pLL.

Cyanine dye NK-2012 in water (pH 7) provided a visible spectrum with λ_{max}'s of 505 and 543 nm, respectively. We observed remarkable λ_{max} shifts to 463 nm in the presence of random-coiled pLL·HBr (**Figure 4a**). When the molar ratio of the residual ammonium groups to NK-2012 was between 2 and 10, the absorption due to the monomeric NK-2012 almost disappeared. This λ_{max}-shift behavior included induction of CD. As shown in **Figure 4b**, strong exciton coupling was observed at the absorption band around the new peak. Similar λ_{max} shifts and induced CD

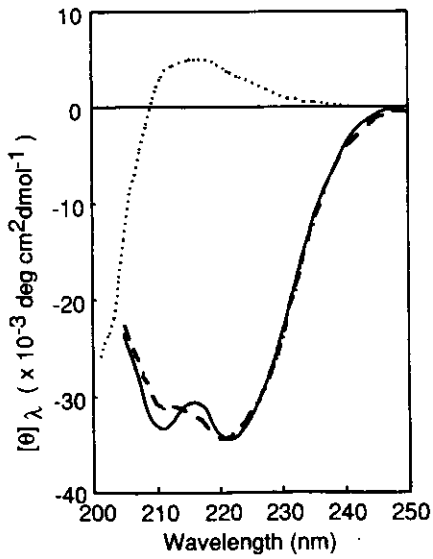

FIGURE 3. CD spectra of pLL in water at pH7 (· · · · · ·), in methanol (– – –) and in methanol with NK-2012 dyes. (——). (pLL·HBr) = 4.1×10^{-5} *M*.

have been reported in cyanine dyes bound to cationic and chiral lipid bilayer membranes in water and can be explained by formation of their chiral dimers on bilayer membranes.[10,11] In addition, we observed no blue shift in the absence of pLL·HBr, even when we added aqueous hydrobromide or sodium hydroxide to the NK-2012 solution. Therefore, the CD pattern (negative in the first cotton effect in Figure 4b) indicates that the complex contains S-chiral dimer formation of NK-2012. We will discuss the structure of this complex later.

However, we confirmed that 10 m*M* of pLL·HBr could be readily dissolved in methanol. This methanol solution provided a typical CD pattern (-34×10^3 deg cm^2 dmol^{-1} at 222 nm) belonging to right-handed α-helical conformation (the solid line, Figure 3). This CD strength indicates that the content of α-helix is almost 100%. The random coil-to-α-helix transition of the pLL main chain is due to lowering of electrostatic repulsion among the residual ammonium groups of pLL·HBr caused by using methanol as a solvent. This finding is very useful for investigation on the function of α-helical polymer because no additive is used.

We also observed a remarkable λ_{max} shift (to 455 nm) of NK-2012 dyes in the methanol solution in the presence of pLL·HBr (**Figure 5a**). This λ_{max} shift includes the induction of large CD to the dimeric NK-2012 dyes (**Figure 5b**). In addition, the CD pattern below 300 nm showed that the pLL maintained α-helical conformation in the presence of NK-2012 dyes. These results are also supported by the chiral dimer formation on α-helical pLL. However, the CD pattern (positive in the first cotton effect) in methanol solution

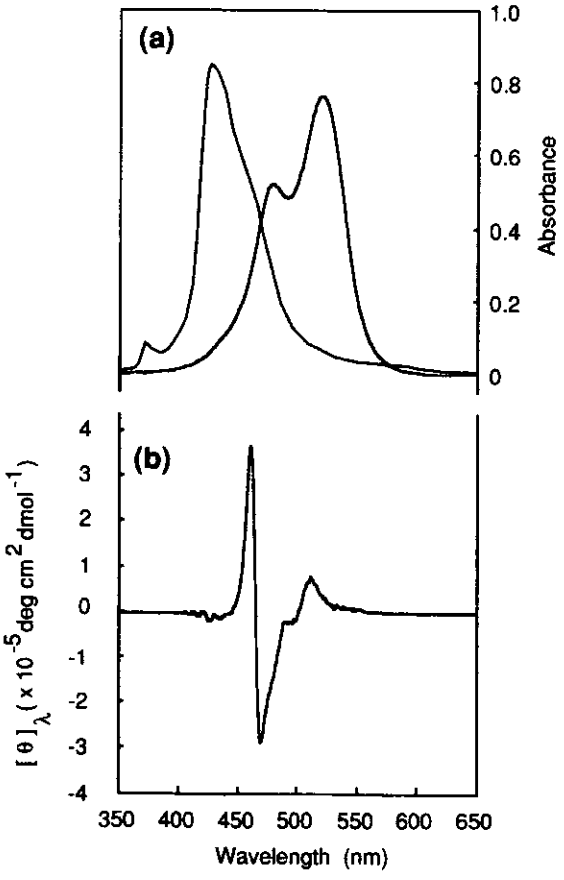

FIGURE 4. Visible (a) and CD (b) spectra of the pLL-NK-2012 complexes in water. (pLL·HBr) = 4.1×10^{-5} *M*, (NK-2012) = 8.2×10^{-6} *M*.

shows that the dimers are in R-chiral conformation, as opposed to the S-chiral dimers observed in the aqueous solution. This indicates that pLL·HBr can provide two kinds of chiral microenvironments depending on the kind of secondary structure.

The induced CD strength in methanol is about 10 times larger than the strength in water (Figure 4a and 4b). In addition, the CD spectrum in the aqueous solution includes R-chiral exciton coupling the wavelengths between 480 and 530 nm (Figure 4b). The visible spectrum also shows exciton couplings at wavelengths (around 500 nm) corresponding to this CD band. These results indicate that pLL lying in a random coil provides various chiral conformations for the complex formation with NK-2012 dyes. On the contrary, in the methanol solution, NK-2012 dyes show a single splitting in the CD spectrum and an almost symmetrical peak in the visible spectrum. This shows that α-helical pLL lying in a more ordered structure provides homogeneous binding sites against the dyes.

Enantioselective Dissociation of the Complexes by Amino Acid Derivatives

The pLL-dye complexes in a methanol solution provide extremely strong exciton couplings at the absorption band of dimeric dyes. We confirmed that the CD strengths ($[\theta]_{453}$ and $[\theta]_{463}$) were very sensitive to additional ions.[1,3,4] For example, the values decreased remarkably with addition of *N*-benzyloxycarbonyl L-phenylalanine triethylamine salt (Cbz-L-Phe) as shown in **Figure 6b**. The visible spectra showed that the decrease of the CD strength was accompanied by a λ_{max} shift from 455 to 543 nm (**Figure 6a**). The new peak agrees with that of a monomeric NK-2012 in a methanol solution. In addition, we observed no exciton coupling around the absorption band of the new peak. These results indicate that the pLL-dye complexes are dissociated by Cbz-L-Phe to produce monomeric dyes.

Figure 7a shows the dependency of absorbance (Abs_{455}) of dimeric NK-2012 on Cbz-L-Phe concentration. The Abs_{455} decreased progressively with increase of Cbz-L-Phe. Therefore, the interaction between pLL and Cbz-L-Phe can be evaluated as the dissociation ability of Cbz-L-Phe.

Figures 7b–7d show the dependency of Abs_{455} on the concentration of otfer *N*-benzyloxycarbonyl α-amino acids and **Table 1** shows the comparison of the dissociation ability, evaluated using the concentration which reduces the Abs_{455} value to half. It is clear that the dissociation ability of L-enantiomers is higher than that of D-enantiomers, although the enantioselectivity (the ratio of the dissociation ability between L- and D-enantiomers) is dependent on the kind of amino acids (the selectivity is progressively higher in derivatives of phenylalanine, proline, leucine, and alanine, respectively).

On the other hand, the enantioselectivity decreased remarkably in the following case: when *N*-tert-butyloxycarbonyl derivatives of phenylalanine (Boc-L-Phe and Boc-D-Phe) were examined instead of the Cbz derivatives, we observed no enantioselectivity. This result indicates that the Cbz group contributes to the enantioselective interaction.

DISCUSSION

Structure of pLL-dye Complexes

It is known that some ionic dyes show blue- or red-shifts in their λ_{max}s due to head-to-head[10,11] or head-to-tail[12–14] stackings, respectively. Such specific oriented structures have often been observed in dyes bound to highly oriented systems like lipid bilayer membranes. Similarly, it is assumed that the blue shift of NK-2012 dyes on pLL is due to head-to-head aggregation.

Why is α-helical pLL able to provide specific binding sites for NK-2012? To answer this question, we exam-

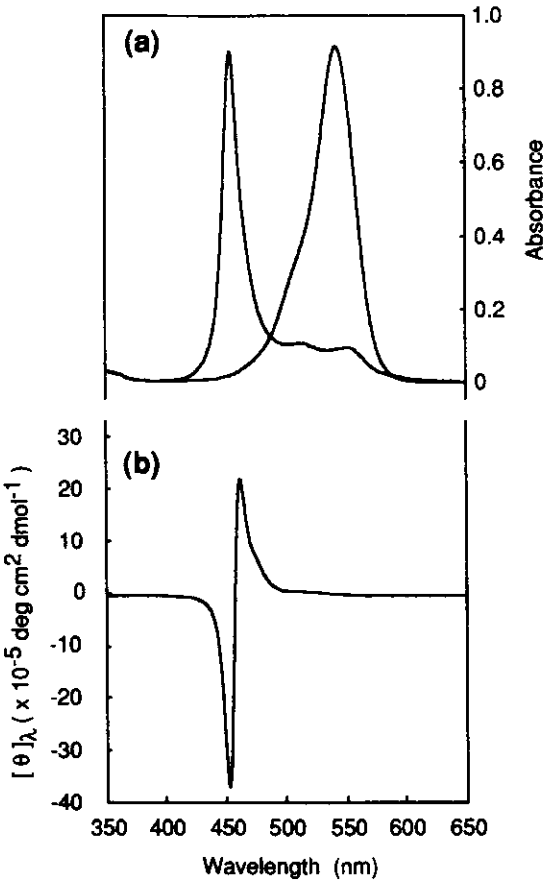

FIGURE 5. Visible (a) and CD (b) spectra of the pLL-NK-2012 complexes in methanol. (pLL·HBr) = 4.1×10^{-5} *M*, (NK-2012) = 8.2×10^{-6} *M*.

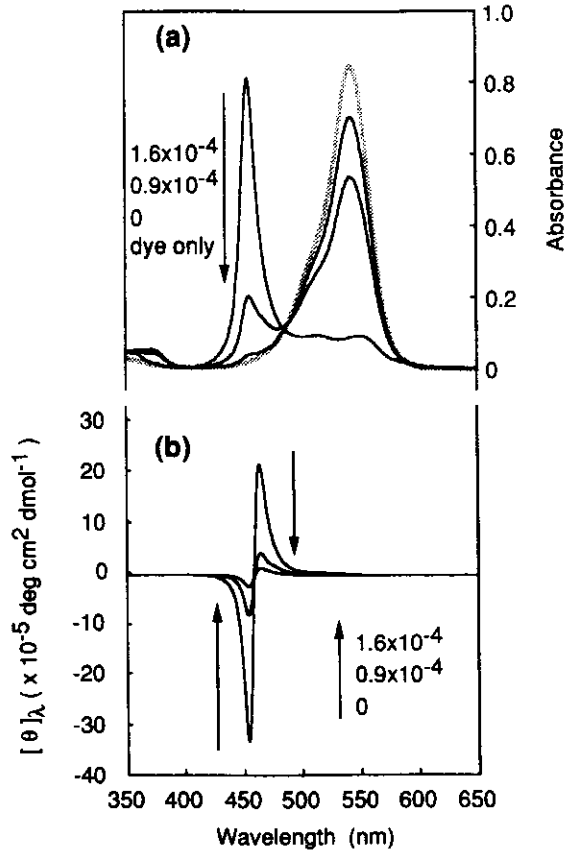

FIGURE 6. Visible (a) and CD (b) spectra of the pLL-NK-2012 complexes in methanol containing Cbz-L-Phe. The number in the figures shows the concentration (mol l^{-1}), of Cbz-L-Phe. (pLL·HBr) = 4.1×10^{-5} (NK-2012) = 8.2×10^{-6} *M*.

inedthe dissociation of the pLL-dye complexes with α,ω-diamines having different methylene length. The dissociation was spectrophotometrically detected according to the manner above. **Figure 8** shows the relationship between the relative dissociation ability and the methylene length. We obtained a bell-shaped correlation curve showing the highest value for 1,6-diaminohexane. In this case, the diamines do not interact with pLL because they are not anionic. Therefore, the dissociation of the complex is induced through electrostatic interaction between NK-2012 and the diamines. **Figure 9a** includes the proposed molecular shapes of NK-2012 and 1,6-diaminohexane, which we estimated by calculation with a MOPAC 6.00 program using the PM3 option.[9] Clearly in this simulation (Figure 9a), the center-to-center distance (12.6 Å) between two sulfur atoms in NK-2012 provides a cavity suitable to incorporate a 1,6-diaminohexane molecule having 8.8 Å of the center-to-center distance between two nitrogen atoms. Perhaps 1,4-diaminobutane (6.3 Å) is small and 1,8-diaminooctane (11.3 Å) is large compared to the cavity of NK-2012.

However, the three-dimensional molecular structure of right-handed α-helical pLL can be simulated by the PEPCON program.[6–8] **Figure 9b** includes the simulated structure of a charged L-lysine dodecamer. The center-to-center distances between two residual nitrogen atoms are shown in **Table 2**. According to this calculation, a value of 8.4 Å, which is very close to that of 1,6-diaminohexane, is obtained for the center-to-center distance of nitrogen atoms between

TABLE 1. Dissociation Ability Evaluated Using the Concentration which Reduces the Abs_{455} Value to Half

Amino acids	R_1	Selectivity (D/L)
Ala	Cbz	0.99
Leu	Cbz	1.20
Pro	Cbz	1.27
Phe	Cbz	1.61
Phe	Boc	1.13

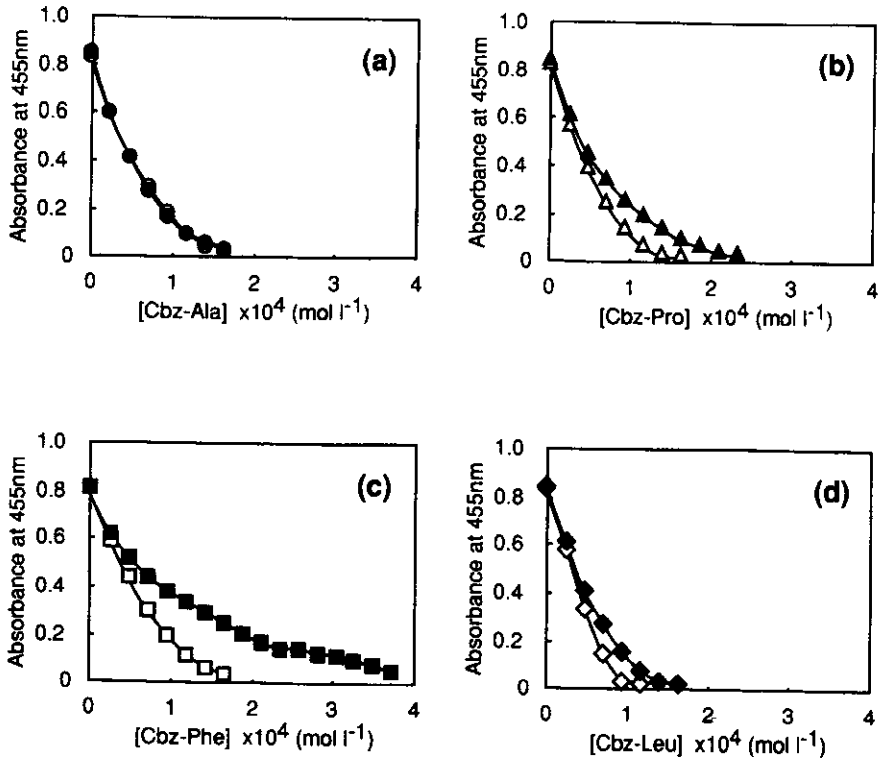

FIGURE 7. Relationships between the concentration of amino acid derivatives and the absorbance at 455nm in methanol. ([pLL·HBr]) = 4.1×10^{-5} *M*, ([NK-2012]) = 8.2×10^{-6} *M* ○ Cbz-L-Ala; △Cbz-L-Pro; □ Cbz-L-Phe; ◇ Cbz-L-Leu; ●— Cbz-D-Ala; ▲— Cbz-D-Pro; ■— Cbz-D-Phe; ◆— Cbz-D-Leu.

1- and 5-, 2- and 6-, 3- and 7-, 4- and 8-, 5- and 9-, 6- and 10-, 7- and 11-, and 8- and 12-positioned residual ammonium groups. On the basis of these calculations, we propose a possible structure of a pLL-dye complex as shown in Figure 9b: first, Sulfonic groups of NK-2012 interact with the residual ammonium groups. Second, NK-2012 is totally monoionic (composed of an ammonium and two sulphonic groups). Therefore, the dimer formation of NK-2012 is made by complex formation between two NK-2012 molecules and two residual ammonium groups that are at 1- and 5- (or 1- and 4-) positions and corresponding positions on pLL. Third, this dimer formation is a suitable conformation for head-to-head stacking rather than head-to-tail stacking. Therefore, the dimer formation induces blue-shift in its λ_{max}. Fourth, two NK-2012 molecules in a dimer state are twisted around each other. Perhaps R-chiral orientation is sterically preferable in right-handed α-helical conformation.

Recognition Mechanism

The dissociation of the pLL-NK-2012 complexes proceeds through replacement by *N*-Cbz α-amino acids. Therefore, the dissociation process can be followed by estimating the interaction between pLL and *N*-Cbz α-amino acids. As

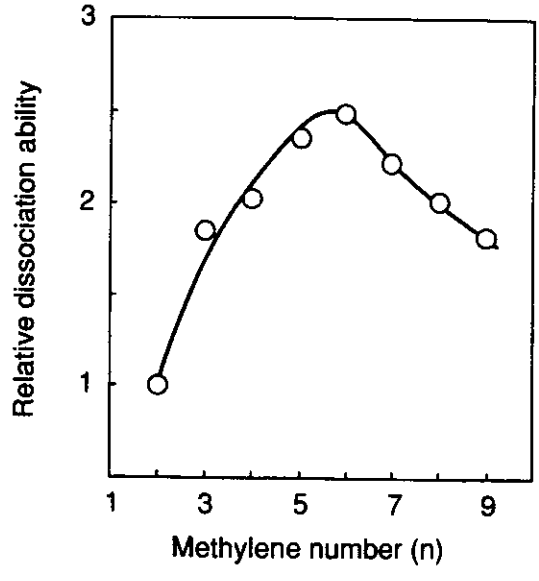

FIGURE 8. Relationship between the relative dissociation ability for the ethylene diamine and the methylene numbers of diaminoalkanes. ([pLL·HBr]) = 4.1×10^{-5} *M*, ([NK-2012]) = 8.2×10^{-6} *M*.

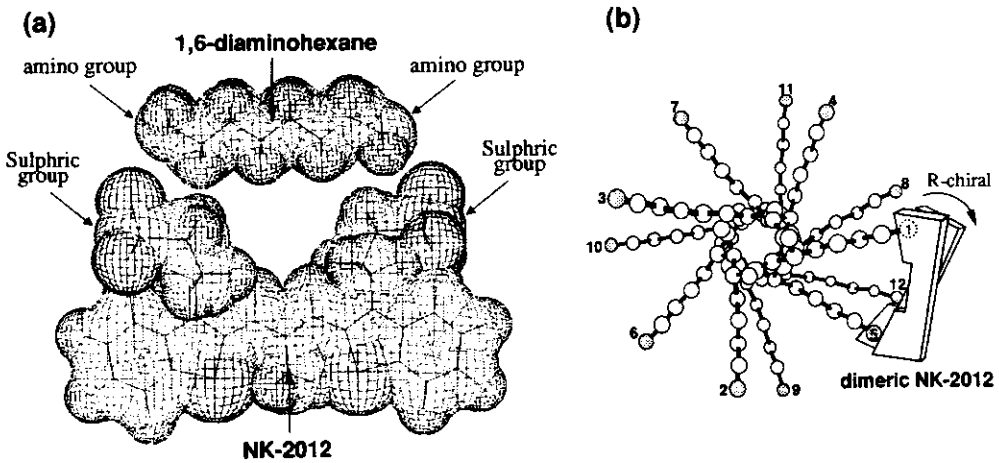

FIGURE 9. Proposed structure of a dimeric NK-2012 bound to 1- and 5-positioned ammonium groups on a right-handed α-helical deodecamer from L-lysines (a). Proposed structure of a one-to-one complex from NK-2012 and 1,6-diaminohexane (b).

clearly seen in Figure 7 and Table 1, the dissociation ability is remarkably dependent on the chirality and the chemical structures of residual groups and *N*-Cbz groups of α-amino acids: this indicates the presence of enantioselective binding behavior between pLL and α-amino acid derivatives.

In general, enantioselectivity is induced by multiple-interactions between host and guest molecules, although it is usually difficult to specify their driving forces. It is also estimated that pLL can provide multiple-interactive binding sites against *N*-Cbz α-amino acids. **Figures 10a** and **10b** illustrate schematically enantioselective interaction mechanisms between right-handed α-helical pLL and *N*-benzyloxycarbonyl L- or D-phenylalanines (Cbz-L- and D-Phe, respectively). This assumption explains the various facts observed in this study: the main driving force for interaction must be derived from a cationic property of pLL and an anionic property of Cbz-Phe. A π–π interaction will be effective as associating driving force for interaction. The carbonyl groups of pLL and phenyl groups of Cbz-Phe can play this role. In this case, the phenyl group of a Cbz moiety is more useful for interaction with carbonyl groups of pLL than that of a phenylalanine moiety, because the former provides a more suitable conformation for both electrostatic and π–π interactions, and because the enantioselectivity almost disappeared when *N-tert*-butyloxycarbonyl derivative of phenylalanine was added. If the enantioselective interaction between pLL and amino acid derivatives occurs through both electrostatic and π–π interactions, in the case of L-isomer the residual group orients outside with no effect on the interaction, but in the case of the D-isomers the sterical hindrance due to the residual group is not negligible. Supporting this estimation, the enantioselectivity is much smaller in alanine having a methyl group than in phenylalanine having a benzyl group. It is clear that the sterical hindrance due to the residual group is smaller in alanine than in phenylalanine.

TABLE 2. Distance (Estimated Using PEPCON) between Two Residual Ammoniums of a Right-handed α-Helical Dodecamer from L-lysines

To	From		
	$1_{(Å)}$	$2_{(Å)}$	$3_{(Å)}$
1	—	12.8	Impossible
2	12.8	—	12.8
3	Impossible	12.8	—
4	9.3	Impossible	12.8
5	**8.4**	9.3	Impossible
6	Impossible	**8.4**	9.3
7	Impossible	Impossible	**8.4**
8	10.6	Impossible	Impossible
9	Impossible	10.6	Impossible
10	Impossible	Impossible	10.6
11	17.9	Impossible	Impossible
12	16.4	17.9	Impossible

CONCLUSIONS

In this study we have proved that α-helical poly(L-lysine) is useful as a host polymer for molecular recognition and that the secondary structure plays in an important role. We have also clarified that poly(L-lysine) shows geometrical selectivity for dicarboxylic acids as reported elsewhere.[3,4] These successful findings have been supported by the establishment of a new method for detecting selective binding behavior between poly(L-lysine) and the guest molecule, using induced chirality due to bound cyanine dyes. This technique could have an extended range of

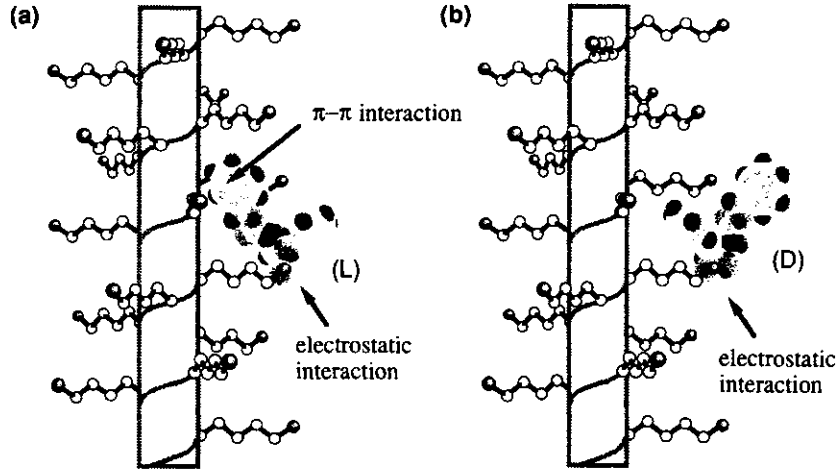

FIGURE 10. Schematic illustrations of Cbz-L-Phe (a) and Cbz-D-Phe (b) bound to right-handed α-helical pLL.

applications in various highly ordered systems, for example, lipid bilayer membranes[15] and ionic polysaccharides.[16]

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