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# Molecular Recognition Using Cyanine-α-Helical Poly(L-Lysine) Complexes in Methanol

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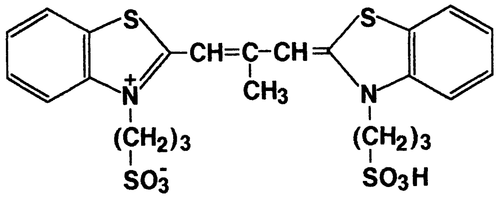
Cyanine dyes bound to α-helical poly(L-lysine·HBr) (PLL·HBr) in methanol provide extremely strong exciton couplings in CD spectra caused by their R-chiral dimer formation and that dyes-PLL complexes show varying degrees of remarkable strength change in the CD recognizing on their methylene numbers upon addition of α,ω-diaminoalkanes.

Poly(α-amino acid)s are very attractive as model compounds of enzymes because they can provide specific microenvironments for low molecular substances. For example, some anionic dyes combine to charged poly(L-lysine) (PLL) in water to produce specific optical activity.[1-3] This is explained by the chiral dimer formation of dyes in residual ammonium groups of PLL which are in random coils in the main chain.[1,3] However, PLL which lies in a random coil can provide various conformations for dye binding.[4] Therefore, it is considered that these complexes are too heterogeneous to use for a molecular recognition system. In this communication, we wish to report molecular recognition using dyes bound to α-helical PLL.

Poly(L-lysine·HBr) (PLL·HBr) generally forms a random coil in water. On the other hand, we have found in our previous study[4] that PLL·HBr (degree of polymerization, 600) was dissolved in pure methanol to provide a clear solution and PLL·HBr formed a right-handed α-helix in the main chain. In that conformation the molecule is rather rigid and the residual amino groups assume identical position. This could be useful for binding dye molecules to poly(L-lysine) since all dye molecules assume identical conformation.

Cyanine dye NK-2012 (Nippon Kanko Shikiso Laboratories) was selected as a guest dye for PLL because of its unique photochemical and photophysical properties. The $\lambda_{max}$ of cyanine dye in methanol was located at 543 nm. A $\lambda_{max}$ shift to 455 nm was induced by addition of PLL·HBr. The absorbance ratios at 455 and 543 nm were related to the molar ratio of NK-2012 to PLL·HBr.



NK-2012

$\lambda_{max}$ shifts of NK-2012 dyes have been observed on chiral lipid bilayer aggregates in water.[5-7] In these cases, the NK-2012 dyes can form either head-to-head[5] or head-to-tail[6,7] stacked dimers, depending on the physical states and chemical structures of the lipids. The CD spectra in the mixture of NK-2012 and PLL·HBr (1 : 5) showed extremely strong exciton coupling around 455 nm. The wave length (457 nm) corresponding to $[\theta]=0$ almost agree with the $\lambda_{max}$ (455 nm) of the NK-2012 dimers. The values of $[\theta]_{453}$ and $[\theta]_{463}$ reached -4000000 and 2600000 deg cm$^2$ dmol$^{-1}$, respectively. No exciton coupling was detected without PLL·HBr. The value of $[\theta]_{222}$ related to the conformation of the PLL main chain showed almost no change with addition of NK-2012. These results indicate that the NK-2012 dyes interact with right-handed α-helical PLL·HBr to produce head-to-head stacked dimers with R-chirality.

The values of $[\theta]_{453}$ and $[\theta]_{463}$ in the CD spectra were very sensitive to additional ions. For example, the value of $[\theta]_{453}$ decreased remarkably with addition of 1,6-diaminohexane (Fig. 1). The UV-visible spectra showed that the decrease of $[\theta]_{453}$ was accompanied by a $\lambda_{max}$ shift from 455 to 543 nm.

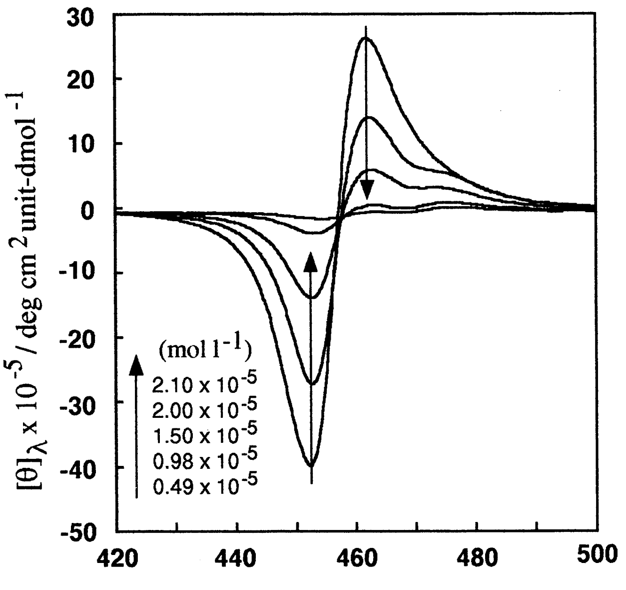


Fig. 1. CD spectra of the NK-2012-PLL complexes in methanol in the presence of 1,6-diaminohexane. The number in the figure shows the concentration of 1,6-diaminohexane.
[NK-2012] = 8.2 x 10$^{-6}$mol l$^{-1}$
[PLL·HBr] = 4.1 x 10$^{-5}$unit-mol l$^{-1}$

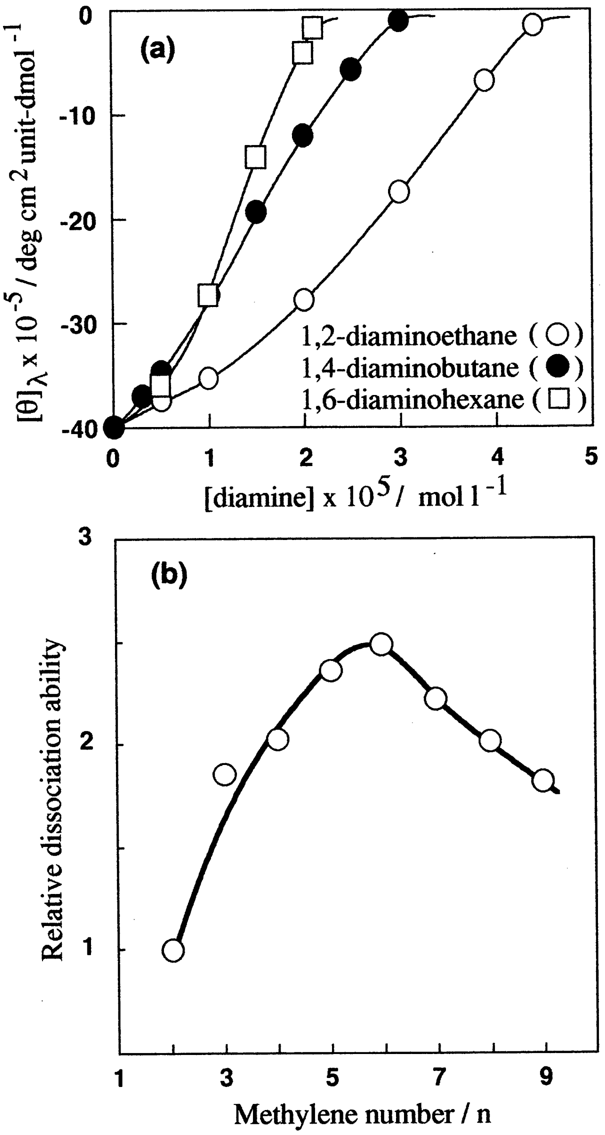
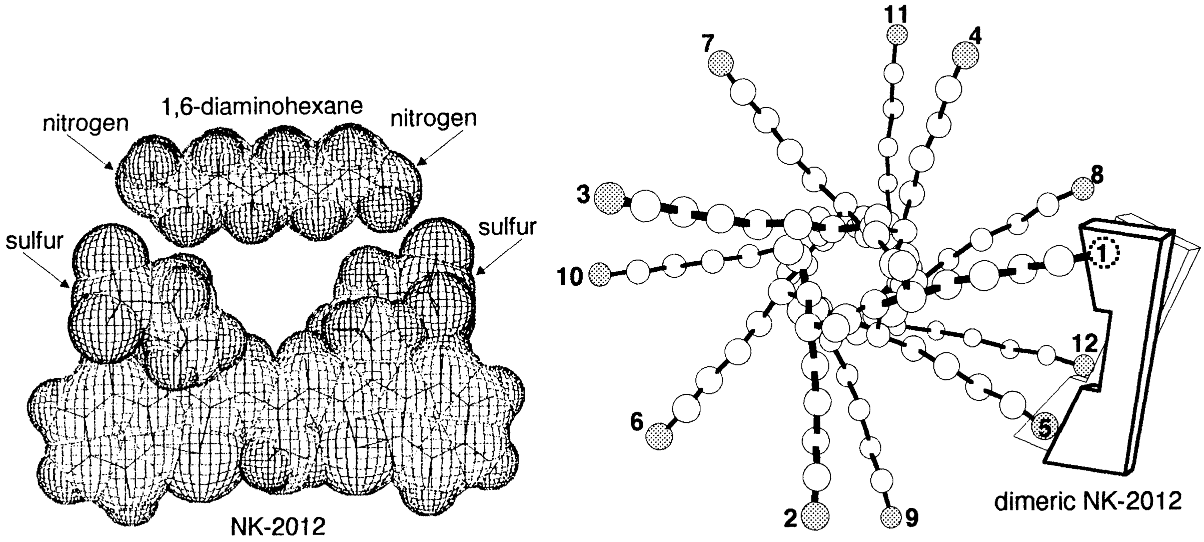


Fig. 2. Concentration dependencies of diamine on the $[\theta]_{453}$ (a) and relationship between the relative dissociation ability for ethylene diamine and the methylene number of diamines (b).
[NK-2012] = 8.2 x 10$^{-6}$mol l$^{-1}$
[PLL·HBr] = 4.1 x 10$^{-5}$unit-mol l$^{-1}$

The new peak agrees with that of a monomeric NK-2012 and no exciton coupling around the absorption of the new peak was observed. These results indicate that 1,6-diaminohexane interacts with NK-2012 and thereby dissociates the NK-2012-PLL complexes to produce monomeric NK-2012. In addition, it was found that the dissociation ability depended on the lengths of $\alpha,\omega$-diaminoalkanes. Figure 2-a shows the concentration dependencies of various diamines on the $[\theta]_{453}$ value. The dissociation ability by diamines was estimated as the concentration of diamines which reduced the $[\theta]_{453}$ value to half. Figure 2-b shows the relationship between the relative concentration of ethylene diamine and the methylene number (n) of diamines. A bell-shaped correlation curve showing the highest value for 1,6-diaminohexane was obtained. Figure 3-a includes the molecular shapes of NK-2012 and 1,6-diaminohexane, whose determination was carried out by calculation with the MOPAC 6.00 program (MATERIA) using the PM3 option.8) 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. Neither 1,4-diaminobutane (6.3 Å) nor 1,8-diaminooctane (11.3 Å) fits this cavity. On the other hand, the typical isosbestic point shown in Fig. 1 indicates that the mode of formation in the NK-2012-PLL complexes is single-binding. Figure 3-b shows a right-handed $\alpha$-helical structure of a dodecamer from charged L-lysines estimated using PEPCON.9-11) If it is assumed that an NK-2012 molecule interacts with two residual ammoniums of PLL according to a binding state similar to Fig. 3-b, the 1- and 4- or 1- and 5-positioned ammonium residues seem to provide the most satisfactory values for the center-to-center distance between their nitrogen atoms (9.3 and 8.4 Å).



(a) (b)

Fig. 3. Proposed structures of a one-to-one complex from NK-2012 and 1,6-diaminohexane (a) and a dimeric NK-2012 bound to 1- and 5- positioned ammonium groups on a right-handed $\alpha$-helical dodecamer from L-lysines (b).

In conclusion, we have found that NK-2012 dyes on α-helical PLL in methanol not only provided strong exciton coupling in the CD spectra, but the strength was also remarkably influenced by additional ions such as diamines. The sensitivity towards ionic interaction and binding seems to be closely related to the three-dimensional configuration of the helix. In addition, these results were observed in a methanol solution, providing an advantage over an aqueous system, which has limited solubility for hydrophobic compounds.

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