

Unique property of cyanine dyes on charged poly(L-lysine)

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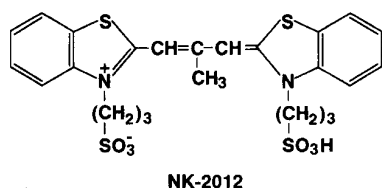
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Charged poly(L-lysine) forms a random coil in water (pH 7) and an α -helix in methanol. Cyanine (NK-2012) dyes combine with these charged poly(L-lysine)s to produce S-chiral and R-chiral dimers in water and methanol, respectively. The dimers on the α -helix show a circular dichroism strength (with a single Davydov splitting) 10 times greater than on the random coil (with several circular dichroism bands). These results indicate that the binding state of NK-2012 dyes is remarkably dependent on the secondary structure of poly(L-lysine) and the α -helix provides a single binding mode for the dyes.

(Keywords: poly(L-lysine); cyanine dye; dimer formation; circular dichroism; α -helix)

Introduction

Poly(α -amino acid) belongs to the simplest class of synthetic polymers that can produce secondary structures spontaneously. Therefore, it has been widely utilized as a protein model and functional polymer. For example, some ionic poly(α -amino acid)s can provide specific microenvironments. A typical example is that methyl orange dyes combine with poly(L-lysine·HBr) (PLL·HBr) in an aqueous solution to form dimers with strong exciton coupling in the circular dichroism (CD) spectra^{1,2}. This phenomenon is explained in terms of a chiral dimer formation of dyes on residual ammonium groups of PLL·HBr whose main chain is in a random coil¹. On the other hand, it is known that PLL undergoes a random coil-to- α -helix transition in the main chain, depending on the pH³ and the concentrations of perchlorate ion³, poly(acrylic acid)^{4,5} or methanol^{6,7} in aqueous solutions. However, there is no study on the dispersion state of dyes bound to α -helical PLL·HBr. This communication discusses the fact that binding states of cyanine (NK-2012) dyes, which attract wide attention because of their photochemical and photophysical properties, are remarkably dependent on the secondary structures of PLL.



Results and discussion

PLL·HBr (degree of polymerization, 600) was dissolved in water (pH 7). This solution showed a CD spectrum with 3000 deg cm² dmol⁻¹ at 222 nm. This shows that the PLL forms a random coil in water.

Cyanine dye NK-2012 (Nippon Kanko Shikiso Laboratories) in water provided a visible spectrum with λ_{\max} values of 505 and 541 nm. A remarkable λ_{\max} shift to 463 nm was induced in the presence of PLL·HBr at pH 7. Figure 1a includes a visible spectrum of the mixture of NK-2012 and PLL·HBr. When the concentration of the residual ammonium groups of PLL·HBr was higher than that of the sulphonium groups of NK-2012, the absorption due to the monomeric NK-2012 almost disappeared. A blue-shift of λ_{\max} of NK-2012 dyes is known to occur on cationic lipid bilayer aggregates in water⁸. In these cases, the NK-2012 dyes form head-to-head stacked dimers on the bilayer membranes⁸. Therefore, the blue-shift in the presence of PLL·HBr also indicates the formation of head-to-head stacked dimers on PLL. In addition, the CD spectrum showed strong exciton coupling (the broken line in Figure 2). The CD pattern showed a typical Davydov splitting around the absorption band of the dimers and was assigned to S-chirality according to the exciton chirality method^{9,10}. In addition, no exciton coupling was observed in the absence of PLL·HBr. These results indicate that the NK-2012 dimers are in S-chiral conformation through interaction with PLL·HBr.

On the other hand, it was observed that PLL·HBr formed a right-handed α -helix ($[\theta]_{222} = -34000$ deg cm² dmol⁻¹) when PLL·HBr was dissolved in methanol. The induction of α -helix is attributed to lowering of electrostatic repulsion among the residual groups. The λ_{\max} of NK-2012 dyes in methanol is located at 543 nm. No exciton coupling was observed in the absence of PLL·HBr. A remarkable λ_{\max} shift (to 455 nm) of NK-2012 dyes in the methanol solution was observed in the presence of PLL·HBr (Figure 1b). This is due to the formation of head-to-head stacked dimers. This λ_{\max} shift includes the induction of large CD to the dimeric NK-2012 dyes (the solid line in Figure 2). In addition, the CD pattern below 300 nm showed that the PLL·HBr maintained α -helical conformation in the presence of NK-2012 dyes. These results are also explained by the chiral dimer formation on α -helical PLL·HBr. However, the CD pattern in a methanol solution shows that the

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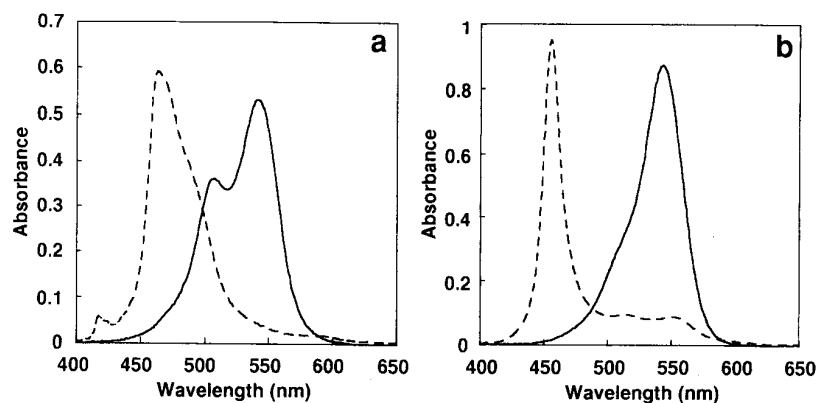


Figure 1 Visible spectra of NK-2012 in water at pH 7 (a) and in methanol (b): —, without PLL·HBr; ----, with PLL·HBr (4.1×10^{-5} unit mol^{-1}). $[\text{NK-2012}] = 8.2 \times 10^{-6}$ mol l^{-1}

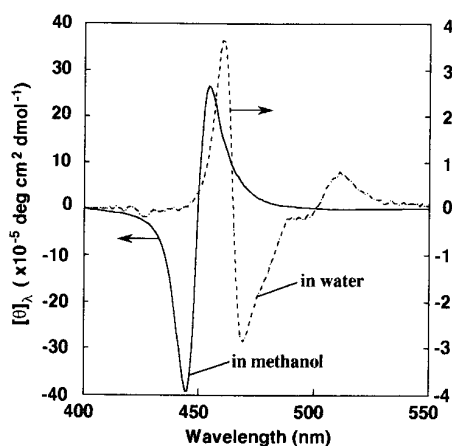


Figure 2 CD spectra of NK-2012-PLL·HBr complexes in water at pH 7 (----) and methanol (—). $[\text{PLL}\cdot\text{HBr}] = 4.1 \times 10^{-5}$ unit mol^{-1} . $[\text{NK-2012}] = 8.2 \times 10^{-6}$ mol l^{-1}

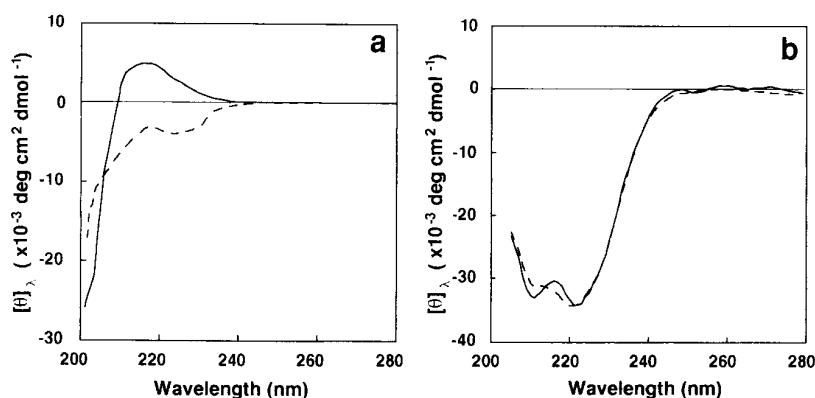


Figure 3 CD spectra of PLL·HBr in water at pH 7 (a) and in methanol (b): —, without NK-2012; ----, with NK-2012 (8.2×10^{-6} mol l^{-1}). $[\text{PLL}\cdot\text{HBr}] = 4.1 \times 10^{-5}$ unit mol^{-1}

dimers are in R-chiral conformation, in contrast to the S-chiral dimers in water. This indicates that PLL·HBr can provide two chiral microenvironments, depending on the type of secondary structures.

The induced CD strength in methanol is about 10 times greater than the strength in water (Figure 2). In addition, the CD spectrum in water includes R-chiral exciton

coupling at wavelengths between 480 and 530 nm (Figure 2). The visible spectrum also shows a shoulder at wavelengths (around 500 nm) corresponding to this CD band (Figure 1a). These results indicate that PLL·HBr 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 Davydov splitting in the CD spectrum and a symmetrical peak in the visible spectrum. This shows that α -helical PLL·HBr lying in a more ordered structure must provide homogeneous binding sites against the dyes.

Conclusions

We have found for the first time that NK-2012 dyes form chiral dimers on charged PLL and the chirality is remarkably dependent on the secondary structure of PLL. If the dimers are formed assuming that two sulphonium groups of an NK-2012 molecule interact with two residual ammonium groups of PLL through ionic interaction, the structure of the dimer must depend remarkably on the conformation of the PLL main chain. Figure 3 shows the CD spectra of PLL·HBr in neutral water and methanol. The addition of NK-2012 dyes to the aqueous solution caused a significant spectral change around 220 nm. This change indicates partial induction of right-handed α -helical conformation. On the contrary, almost no change of the CD pattern was observed in the methanol: the right-handed α -helical conformation is preferentially produced despite the addition. Therefore,

PLL in methanol can provide more homogeneous binding sites for NK-2012 dyes than PLL in water.

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