Letter

Geometrically selective recognition using α-helical poly(L-lysine)

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Abstract

Poly(L-lysine hydrobromide) in α-helical conformation showed selective binding for geometrical isomers of dicarboxylic acids. This selectivity was spectrophotometrically detected by monitoring the dissociation of poly(L-lysine)-cyanine dye complexes. The computer calculation indicated that the distance between residual ammonium groups in the α-helix was more closed to that between two carboxylic groups in the *trans* isomers than in the *cis* isomers.

Keywords: Poly(L-lysine); Selective recognition; α-Helices

1. Introduction

Secondary structural polymers are very attractive as protein models and functional polymers. Especially, poly(α-amino acid)s belong to the simplest class of synthetic polymers which can produce secondary structures spontaneously and provide specific microenvironments for ionic molecular substances. A typical example is that some achiral dyes combine to poly(L-lysine hydrobromide) (PLL·HBr) in aqueous solution. Interestingly, the bound dyes form dimers with strong exciton coupling in the circular dichloism (CD) spectra [1–3]. These phenomena are explained in terms of a chiral dimer formation of dyes on residual ammonium groups of PLL·HBr. These observations may increase the applicability of poly(α-amino acid)s as biomimetic sensing materials. However, unfortunately, the main chain in these dye-binding works was in a random coil and thus the binding sites were too heterogeneous.

In this communication, we describe that the α-helical conformation of PLL·HBr is very useful for that of selective binding, especially for geometrical molecular recognition, because in that conformation the molecule is rather rigid and the residual ammonium groups assume identical positions as binding sites.

2. Experimental details

Poly(L-lysine hydrobromide) was prepared by polymerization of N^ϵ-benzyloxycarbonyl L-lysine initiated with triethylamine followed by debenzyloxycarbonylation using acetic acid saturated with hydrobromide. The degree of polymerization (n) was determined by viscosity of solutions using the following equation: $\log n = 0.79 \log \eta_{sp} + 2.46$ [4]. Cyanine dye, NK-2012 (Nippon Kanko Shikiso Laboratories, Japan), was used without further purification. The chemical structures are given in Fig. 1.

The conformation was estimated using a JASCO spectorphotometer J-500C. The visible spectra of NK-2012 was measured using a Shimadzu UV-Visible spectrophotometer 160A.

The computer calculation of the conformation of PLL·HBr and dicarboxylic acids were carried out using
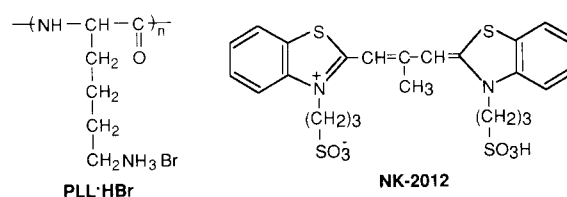

Fig. 1. Chemical structures of poly(L-lysine hydrobromide) and cyanine dye NK-2012.

the PEPCON program [5–7] and the MOPAC 6.00 (Teijin MATERIA) with the PM3 option respectively.

3. Results and discussion

PLL·HBr (n ≃ 600) in water provided a CD spectrum with $[\theta]_{222} = 2 \times 10^3$ deg cm dmol^{-1} which was assigned to a random coil. However, when PLL·HBr was dissolved in methanol, the CD spectrum ($[\theta]_{222} = -34 \times 10^3$ deg cm dmol^{-1}) showed formation of right-handed α-helices. This random coil-to-α-helix transition is due to lowering of electrostatic repulsion among residual ammonium groups of PLL·HBr. As described above, α-helical conformation of PLL·HBr is useful for selective binding study, because this conformation is more rigid than that of random coils and provide homogeneous binding sites for electrostatic interaction on the residual ammonium groups. However, it is always difficult to detect specific interaction, when the interaction is not accompanied by spectrophotometrically detectable response. Therefore, in order to detect selective binding behavior on α-helical PLL, we adopted the induced chirality method [8]. This technique is based on the fact that dyes bound to chiral macromolecules produce chiral dimers (or aggregates) with remarkable λ_{max}-shift in UV-visible spectra and strong exciton coupling in CD spectra, and that the dissociation of dye-macromolecule complexes due to substrates can be detected by monitoring the spectral change due to dye release. In this study, selective binding ability of PLL for geometrical isomerism was examined according to this method. A cyanine dye NK-2012 provided a visible spectrum with λ_{max} of 543 nm in methanol. When 5 times equimolar (in the residual ammonium group) of PLL·HBr was added to the NK-2012 methanol solution, a remarkable λ_{max} shift from 543 nm to 455 nm was observed (Fig. 2). In addition, CD spectra provided significant information for this λ_{max} shift: (1) extremely strong exciton coupling ($[\theta]_{453} = -3.7 \times 10^6$ and $[\theta]_{462} = 2.2 \times 10^6$ deg cm dmol^{-1}) was induced by addition of PLL·HBr although no exciton coupling was observed in its absence; (2) CD spectra showed almost no change in the conformation of the main chain of PLL·HBr. These results indicate that NK-2012 dyes bound to right-handed α-helical PLL·HBr to produce H-like (head-to-head) aggregates [9,10] with R-chirality. In this study, the aggregated dyes on PLL·HBr are abbreviated by A_1. It was observed that the aggregation of bound NK-2012 dyes came loose by addition of fumaric acid $(3.8 \times 10^{-5}$ mol l^{-1}). Fig. 2(a) shows that the NK-2012 aggregates (A_1) dissociate to monomeric species (M) with fumaric acid. This A_1-to-M transition was accompanied by a significant decrease in CD strength. The value of $[\theta]_{453}$ in NK-2012 dye-PLL complexes decreased remarkably from -3.7×10^6 to
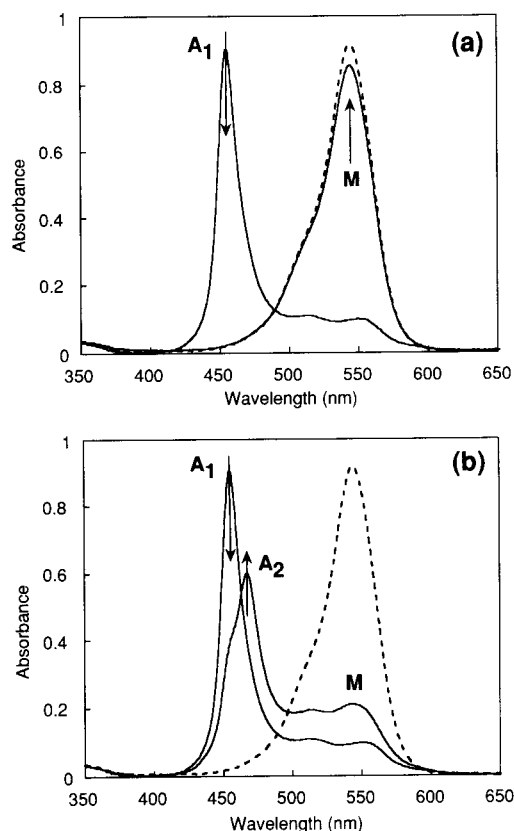

Fig. 2. Visible spectra of NK-2012 dye $(8.2 \times 10^{-6}$ mol l$^{-1})$ in the presence of PLL·HBr $(4.1 \times 10^{-5}$ mol l$^{-1})$ in methanol. (a) When fumaric acid $(3.8 \times 10^{-5}$ mol l$^{-1})$ was added to the NK-2012-PLL complexes $(\lambda_{max} = 455$ nm), the aggregated species (A_1) disappeared to produce monomeric species (M). (b) When maleic acid $(3.8 \times 10^{-5}$ mol l$^{-1})$ was added to the NK-2012-PLL complexes, the aggregated species with λ_{max} of 468 nm (A_2) appeared with decrease of A_1 species. The broken lines present NK-2012 dye in the absence of PLL·HBr in methanol.

-7.0×10^4 deg cm dmol^{-1} by addition of 3.8×10^{-5} mol l^{-1} of fumaric acid ([PLL] = 4.1×10^{-5} mol l^{-1}, [NK-2012] = 8.2×10^{-6} mol l^{-1}). These results indicate that fumaric acid interacts with PLL·HBr to induce the dissociation of dye aggregation (A_1), and thus this interaction can be readily followed by monitoring visible and CD spectra. Interestingly, a new peak $(\lambda_{max}, 468$ nm) was observed in visible spectra (Fig. 2(b)), when maleic acid $(3.8 \times 10^{-5}$ mol l$^{-1})$, a geometric isomer of fumaric acid, was added to the NK-2012-PLL·HBr complexes. This λ_{max} shift was accompanied by the appearance of a new CD band. Addition of 4.7×10^{-5} mol l^{-1} of maleic acid induced a new CD band ($[\theta]_{466} = -12.5 \times 10^5$ and $[\theta]_{478} = 8.8 \times 10^5$ deg cm dmol^{-1}) with remarkable decrease of CD strength around the absorption band of A_2 species (468 nm). These results indicate that maleic acid also interacts with PLL·HBr but does not dissociate dye aggregation in this condition. NK-2012 dyes still maintain R-chiral aggregation on PLL·HBr, whose stacking state is influenced by maleic acid. In this study, the aggregated
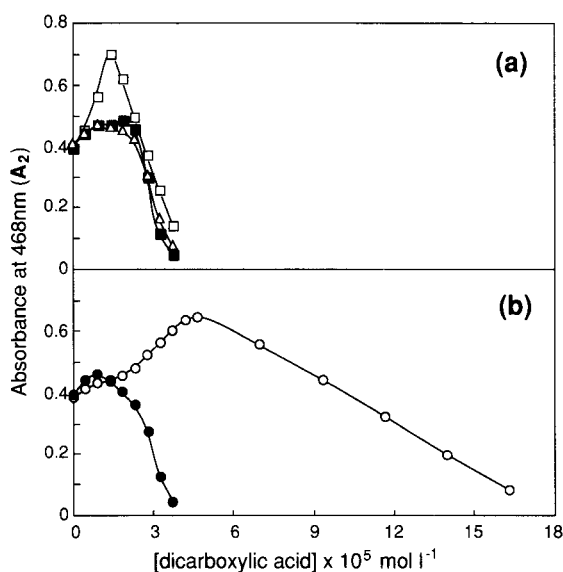
Fig. 3. Relationships between the concentration of dicarboxylic acids and the absorbance at 468 nm of NK-2012-PLL mixtures in methanol. (NK-2012) = 8.2×10^{-6} mol l^{-1}; (PLL·HBr) = 4.1×10^{-5} mol l^{-1}. (a) O, maleic acid (cis-isomer); ●, fumaric acid (trans-isomer). (b) □, phtalic acid (o-isomer); ■, isophtalic acid (m-isomer); ▲, terephtalic acid (p-isomer).

dyes are abbreviated by A_2. In order to understand the different spectral changes between fumaric and maleic acids, the dissociation mode in NK-2012 dye-PLL complexes was examined using position isomers of benzene dicarboxylic acids. Fig. 3 shows the concentration dependencies of the absorbance (Abs_{468}) at 468 nm (including the absorption of aggregates A_2) on dicarboxylic acids. Abs_{455} (belonging to A_1 species) decreased remarkably by addition of all isomers (3.8×10^{-5} mol l^{-1}). However, the o-isomer showed critical increase of Abs_{468} as shown in Fig. 3(a). This indicates that the dye-PLL complexes undergo the A_1-to-M transition by addition of benzene dicarboxylic acids, but the o-isomer includes A_1-to-A_2 transition in this process. A similar increase of Abs_{468} was clearly observed for maleic acid (Fig. 3(b)). These results show that the formation of A_2 species is specifically observed for the cis- or o-isomers. It was confirmed that addition of fumaric or maleic acids (3.8×10^{-5} mol l^{-1}) to the dye-PLL complexes showed almost no change in their CD spectra in the absorption bands related to the conformation of PLL. This indicates that right-handed α-helical conformation in PLL is not disturbed by dicarboxylic acids. However, we previously estimated that NK-2012 dyes bound to 1- and 5-positioned residual ammonium groups in α-helical PLL [11]. This assumption is based on calculations with MOPAC 6.00 program using the PM3

option [12] and PEPCON program [5–7]. According to these calculations, the center-to-center distance between the closest two nitrogen atoms of α-helical PLL is 8.4. Similar calculation for maleic acid (cis-isomer) provided 2.8 and 4.1 for the center-to-center distances of carboxylate oxygen atoms which were in symmetric and asymmetric conformations respectively. These distances seem to be too short to bond to 1- and 5-positioned ammonium groups of α-helical PLL. Moreover, the trans-isomer (fumaric acid) showed 5.2 and 6.0 for those conformations. These values also are smaller than the 8.4, but closer than those of the cis-isomer. Therefore, we estimate that the trans-isomer can combine more tightly to PLL to dissociate A_1 aggregates than the cis-isomer, and that the A_2 species is produced as an intermediate through loose binding of the cis-isomer. In conclusion, we have found for the first time that poly(L-lysine) interacts with dicarboxylic acids recognizing geometrical conformations of their isomers. We emphasize that this finding is gained by using homogeneous binding sites derived from the α-helical conformation.

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