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Aggregation behavior of dabsylated poly(ε-L-lysine) in aqueous DMSO solution

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Summary

Side chain amino groups of microbial Poly(ε-L-lysine) (ε-PL) were chemically modified with an amino labeling reagent, 4-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride) to give dabsylated ε-PL (Dabs-ε-PL). Aggregation behavior of Dabs-ε-PL in aqueous DMSO solution was studied through UV-Vis and CD spectra. In DMSO solution, Dabs-ε-PL shows a symmetric visible absorption at 450 nm, corresponding to the azobenzene chromophore in the monomeric state. Upon addition of water to the DMSO solution, the λ_{max} is shifted to around 400 nm. CD spectra were detected in the region of the blue shifted band: a pair of CD signals of opposite sign appears with a crossover point related to the shifted λ_{max}. Similar trends were also observed for UV-Vis and CD spectra of dabsylated oligo(ε-L-lysine)mers. These spectroscopic results indicate that side chain azo chromophores of Dabs-ε-PL form H-aggregates and are disposed according to a chiral arrangement with right handedness in aqueous DMSO solution.

Introduction

Poly(ε-L-lysine) (ε-PL) is one of a few naturally occurring poly(amino acid)s [1]. Microbial production of ε-PL was first discovered by Shima and Sakai from a culture broth of *Streptomyces albulus*. They have reported the physicochemical and biological properties in some detail [2-5]. Since ε-PL has antibacterial activities, it has been used as a preservative for various food products [6]. The structure of ε-PL is characterized by amide linkages formed between the α-carboxyl and ε-amino group of L-lysine. Thus, ε-PL is regarded as an α-amino substituted derivative of nylon 6. However, ε-PL is water-soluble and biodegradable in contrast with nylon 6. So far, we have focused our research attention on the structural and the conformational aspects of ε-PL both in aqueous solution and in the solid state [7-10].

Polymers containing azobenzene chromophores have drawn much attention in the field of material science. Since ε-PL carries free amino groups at the α-position of the amide carbonyl group, chemical modification may be attained using an amino labeling reagent. It is also known that chemical modification of water-soluble polymers with hydrophobic dye groups leads to amphipathic polymers and aggregates are formed by the self-association of dye moieties of the molecules[11]. Since aggregated dyes exhibit distinct optical properties which their monomer does not have, the aggregation behavior is usually studied through UV-Vis and CD spectroscopies. In this work, as a step for functionalization of ε-PL, we have prepared dabsylated ε-PL using the reaction between ε-PL and 4-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride, Dabs-Cl). Aggregation behavior of Dabs-ε-PL was studied in aqueous DMSO solution by UV-Vis and CD spectra. The spectral data were compared with those of dabsylated derivatives of monomer model, α-Dabs-ε-Boc-L-lysine, and dabsylated monodispersed oligo(ε-L-lysine)mers.

Experimental

Materials

ε-PL in the free base form was obtained from Chisso Corporation (Tokyo, Japan). Its number-averaged molecular weight (Mn) of 4,090 corresponds to the degree of polymerization (DP) of 32 based on the unit molecular weight of 128. Monodispersed oligo(ε-L-lysine)mers (4- and 8-mers) were taken from the same batches of preparations as those used in the previous work[9]. ε-Boc-L-lysine, dabsyl chloride, and other reagents were purchased from Tokyo Kasei Kogyo, Japan.

Dabsylation of ε-PL was carried out according to a conventional Schotten-Baumann reaction described in previous work (scheme 1) [12]. Typically, 61 mg ε-PL (0.15 mmol) was added to a 100 ml MeOH solution of 162 mg Dabs-Cl (0.5 mmol) containing 208 μl triethylamine (1.5 mmol). The mixture was kept at room temperature for 20 h. The resulting precipitates were collected by centrifugation. Reddish-orange product was washed with distilled water several times. Dabs-4-, 8-mers and α-Dabs-ε-Boc-L-lysine were also prepared in the same manner.

Spectral measurements

^{1}H NMR spectra were recorded on a JEOL JNM-LA400 instrument at room temperature at 400 MHz. Samples were dissolved in DMSO-d_6 and tetramethylsilane was used as an internal standard.

IR spectra were recorded on a Perkin Elmer 1650 FT-IR spectrometer by averaging 64 scans with a resolution of 4 cm^{-1}. Samples were prepared as KBr pellets.

FT-Raman spectra were obtained on a Perkin-Elmer 2000R spectrometer equipped with a quartz beam splitter and InGaAs detector. The 1064 nm line of a Spectron Laser System SL300 Nd: YAG laser was used as the exciting source with an output power of about 200 mW at the sample position. All spectra were accumulated for 60 scans with a resolution of 4 cm^{-1}. Solid samples were sealed in glass capillary tubes.

UV-Vis spectra were measured with a Shimadzu UV-2500 PC spectrophotometer. Quartz cells of 1 cm path-length were used. Samples were dissolved in DMSO/water solution and adjusted to an appropriate concentration. Solution temperature was controlled by a circulating water bath.

CD spectra were recorded on a JASCO J-820 spectropolarimeter using 1 cm path-length cells. Sample temperature in the cuvette was regulated by a circulating water bath and was kept at 25 °C. After subtraction of a solvent spectrum, data were expressed in terms of mean residue ellipticity.

Results and Discussion

Characterization of Dabsylated ε-PL

Dabsylation process of amino groups is well characterized by vibrational spectra. Dabs-ε-PL, Dabs-8-mer and Dabs-4-mer exhibit IR and Raman spectra similar to each other. Figure 1 shows IR and Raman spectra of Dabs-ε-PL as a typical example. The IR spectrum of Dabs-ε-PL consists of the spectral features due to the ε-PL and the azo dye moieties. IR bands at 1652 and 1556 cm^{-1} are assigned to the Amide I and the Amide II band of the ε-PL framework. These bands are shifted to the higher frequencies by 13 and 16 cm^{-1}, respectively, compared to those of ε-PL. The bands at 1603 and 1522 cm^{-1} are due to the benzene ring stretching of the N-dimethylaminoazobenzene moiety. Two bands at 1364 and 1136 cm^{-1} are assigned to the asymmetric and the symmetric SO_2 stretching modes of the sulfonamide group. In contrast to the IR spectra, the Raman features are dominated exclusively by the aminoazobenzene moiety. This is because the azobenzene group has high polarizability and therefore, its vibrations are highly Raman active. Raman band assignments were

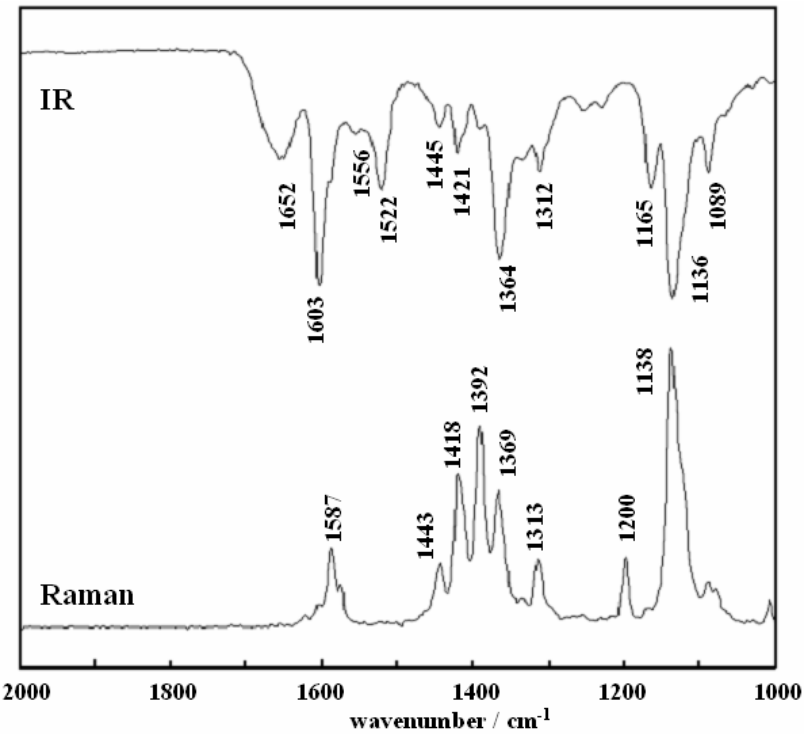
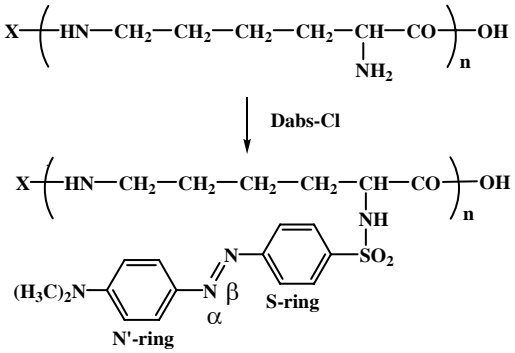


Figure 1. FT-IR and Raman spectra of Dabs-ε-PL.

carried out based on our works of resonance Raman spectra in aqueous solution[13]. The Raman spectrum exhibits intense bands at 1138 (νC-N_α), 1200 (ring, 9a), 1392 (S-ring, 19b), 1418 (νN=N), 1443 (N'-ring, 19b) and 1587 cm^{-1} (bring, 8a) characteristic of the azobenzene chromophore.

Relative IR intensity between the amide I band at 1652 cm^{-1} and the benzene ring stretching band at 1603 cm^{-1} gives rough estimation of dabsylation degree. Degree of dabsylation is more accurately evaluated by measuring the ^{1}H NMR spectra in DMSO-d_6 solution. In the ^{1}H NMR spectra of dabsylated compounds, the molar ratio of [lysyl residue]/[dye] was determined using integral values obtained from the -CH_2- protons of lysine unit and $(CH_3)_2$N- protons of the azobenzene unit. The apparent degree of modification was shown in Table 1.



Scheme 1. Preparation of dabsylated ε-PL and related compounds

Compds.	DP, n=	X=	Degree of dabsylation (%)
Dabs-ε-PL	25-32	H	65
Dabs-8mer	8	H	60
Dabs-4mer	4	H	67
α-Dabs-ε-Boc-L-lysine	1	Boc	100

Table 1. The degree of dabsylation estimated by ^{1}H NMR

UV-Vis absorption spectra of Dabs-ε-PL

4-Dimethylamino azobenzene dyes generally show a characteristic absorption in the visible region. As shown in Fig. 2, α-Dabs-ε-Boc-L-lysine has a symmetric absorption band at 450 nm (ε 27,800) in pure DMSO solution. The λ_{max} and the ε_{max} are practically constant on the change of concentration (10^{-4}-10^{-6} mol dm^{-3}). Thus, α-Dabs-ε-Boc-L-lysine exists as a monomer in this concentration range and the absorption is related to the π-π* of the azobenzene chromophore. The λ_{max} shifts systematically to a longer wavelength from 450 to 470 nm with increasing water content of the solutions from 0% to 90%. This kind of red shift is characteristic of azobenzene containing electron-donating and electron accepting ("push-pull") groups and can be rationalized by a simple solvatochromic effect on the above-mentioned electronic transition[11].

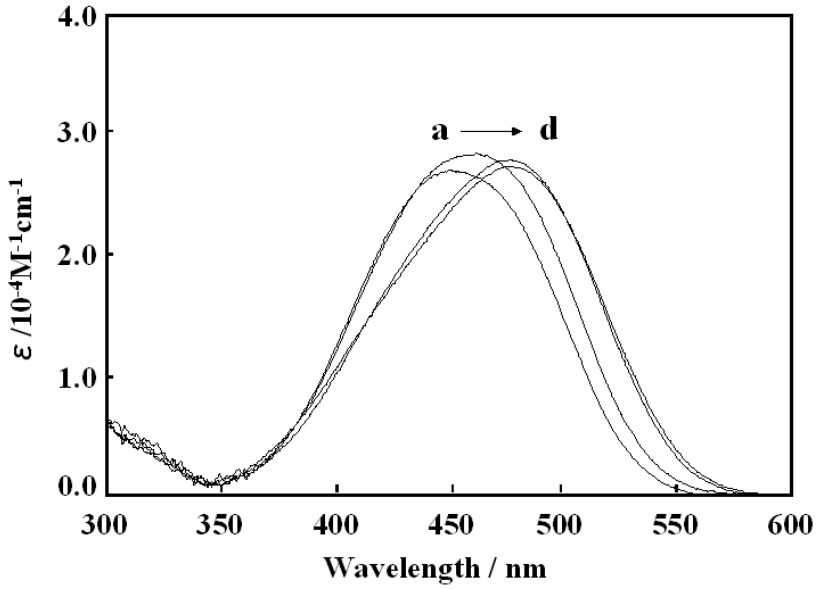


Figure 2. UV-Vis spectra of α-Dabs-ε-Boc-L-lysine (5 x 10^{-6} mol dm^{-3}) in DMSO-H_2O solution. H_2O content: (a) 0%; (b) 20%; (c) 70%; (d) 90%.

UV-Vis spectra of Dabs-ε-PL exhibit different responses to the change of solvent from that of α-Dabs-ε-Boc-L-lysine. As shown in Figure 3, Dabs-ε-PL exhibits a symmetric absorption at 450 nm in pure DMSO solution, which is similar to the

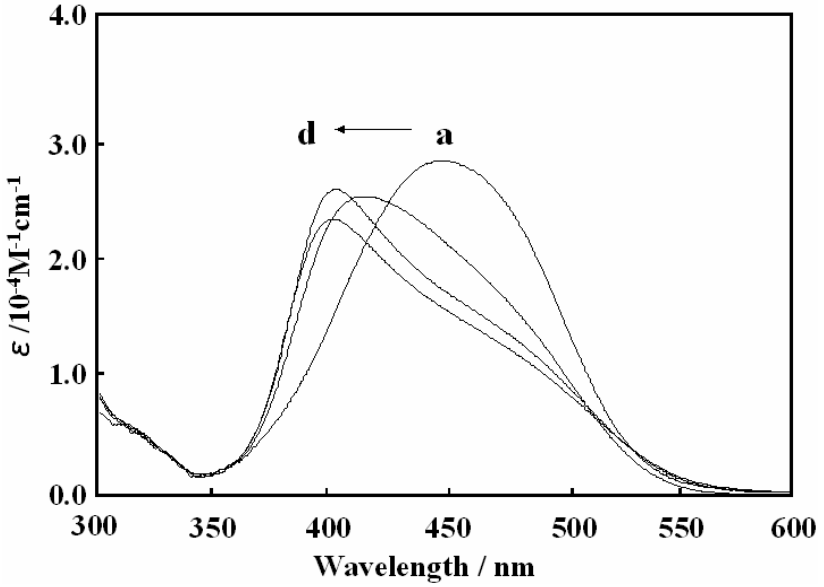


Figure 3. UV-Vis spectra of Dabs-ε-PL (2.0 x 10^{-5} mol dm^{-3}) in DMSO-H_2O solution. H_2O content: (a) 0%; (b) 20%; (c) 70%; (d) 90%.

α-Dabs-ε-Boc-L-lysine. This absorption is ascribed to the electronic transition of the side chain azo chromophore in the monomer state. When water is added to the DMSO solution, a blue shift of the λ_{max} and band shape distortion are observed. Hypsochromicity appears to increase with an increase in the water content. For instance, the λ_{max} is observed at 400 nm in 90% H_2O solution. This kind of hypsochromic effect has been observed for polymeric derivatives bearing azo aromatic chromophores in the side chain and explained as a result of aggregate formation[14,15]. Together with hypsochromicity, a decrease in the molar extinction coefficient (hypochromicity) is also observed upon addition of water. The occurrence of hypsochromism and hypochromicity is related to the parallel intramolecular stacking (H-aggregates) of adjacent dipolar chromophores and interpreted in terms of the molecular exciton theory[16-18]. Thus, two absorption maxima are expected to arise from the azo chromophores in the monomeric and the H-aggregated states. As shown in Fig. 4, the absorption spectra were resolved into two Gaussian components using

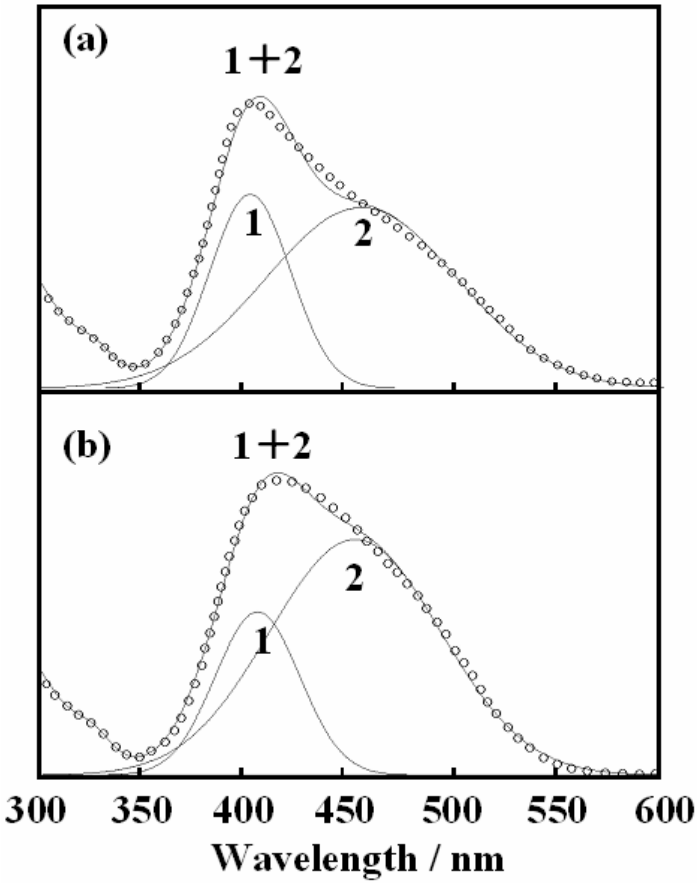


Figure 4. Curve fitting analysis of the absorption spectra of Dabs-ε-PL in DMSO-H_2O solution. H_2O content: (a) 90% (b) 20%. Curves 1 and 2 are the fitting functions related to the H-aggregated and the free un-aggregated azobenzene moieties, respectively. The experimental spectra and the resulting fitting curves are denoted by open circles and full lines.

a curve fitting PC-software (Peak Fit). The absorptions around 450 and 400 nm are assigned to the electronic transitions due to the monomeric and H-aggregated azo chromophores, respectively. It is noted that the absorption maximum of the monomeric component shifts to a longer wavelength with an increase in the water content, whereas that of the H-aggregated component shifts to a shorter wavelength. The molecular exciton theory predicts that an increase in the number of aggregation accompanies a larger hypsochromic shift. Thus the more H_2O content in solution facilitates the H-aggregation of azobenzene chromophores through hydrophobic interaction between these moieties.

Dabs-ε-PL in DMSO-H_2O solution shows little concentration dependence. For example, UV-Vis spectral patterns of the 20% H_2O solutions were practically constant at dye concentrations (1.0×10^{-4} - 5.0×10^{-6} mol dm^{-3}). This finding suggests that H-aggregation occurs between azobenzene chromophores within a Dabs-ε-PL chain.

Aggregation behavior of dabsylated ε-L-lysine oligomers

Dabs-ε-PL has inherently molecular weight distribution owing to the polydispersity of ε-PL. In order to estimate the effect of polydispersity and the chain length dependence on aggregation, we have also measured the UV-Vis spectra of Dabs-8-mer and Dabs-4-mer at the same residual dye concentration. Dabs-8-mer and Dabs-4-mer show spectral changes similar to the Dabs-ε-PL case, upon addition of water to the DMSO solution. However, as shown in Fig. 5, greater amount of water needs to be added to produce the absorption shift in Dabs-8-mer. Moreover, intensity of the shifted band becomes weaker compared with that of Dabs-ε-PL. These propensities are stronger for the Dabs-4-mer case. These findings support the idea that the aggregation occurs

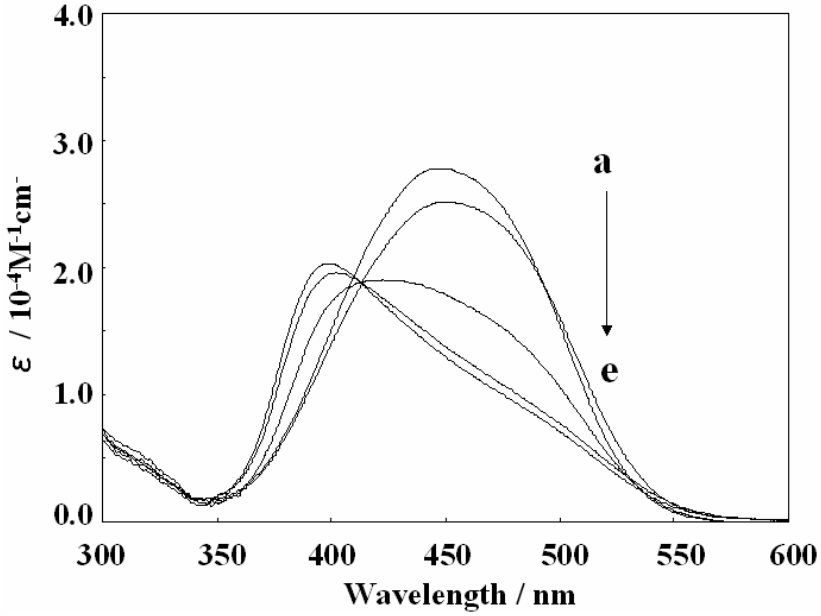


Figure 5. UV-Vis spectra of Dabs-8-mer (1.3×10^{-5} mol dm^{-3}) in DMSO-H_2O solution. H_2O content: (a) 0%; (b) 20%; (c) 30%; (d) 70%; (e) 90%.

through the hydrophobic interaction of the intra-chain azobenzene chromophores of dabsylated derivatives.

CD spectra

When the blue-shifted band is observed, the Dabs-ε-PL solution exhibits an induced CD (ICD) spectrum. Figure 6 shows CD spectra of Dabs-ε-PL measured in the same sample condition as the UV-Vis spectra in Fig. 3. Dabs-ε-PL in DMSO-H_2O solution exhibits two CD bands which are nearly equal in magnitude but have opposite signs. In the spectra, the positive band is at a longer wavelength and the crossover point roughly related to the λ_{max} of the shifted band. In contrast, no CD signals are detected for the 100% DMSO solution where the side-chain azobenzene moieties of Dabs-ε-PL are fully solvated and in the monomeric state. A pair of CD bands of opposite signs can be assigned to a split CD Cotton effect (π-π* electronic transition) induced by electronic interactions between neighboring azobenzene chromophores. This is generally associated with the existence of a chiral arrangement with one prevailing handedness. According to the chiral exciton coupling, this indicates a right-handed screw of two coupled neighboring chromophores[19]. It should be noted that the ICD effect is maximized in 20% H_2O solution; the maximum positive CD Cotton effect (440 nm, Δε +19) and negative Cotton effect (390 nm, Δε -26) with a crossover point at 410 nm are observed for this solution. This finding indicates that this solvent composition facilitates more azo chromophores in H-aggregation to take the chiral arrangement.

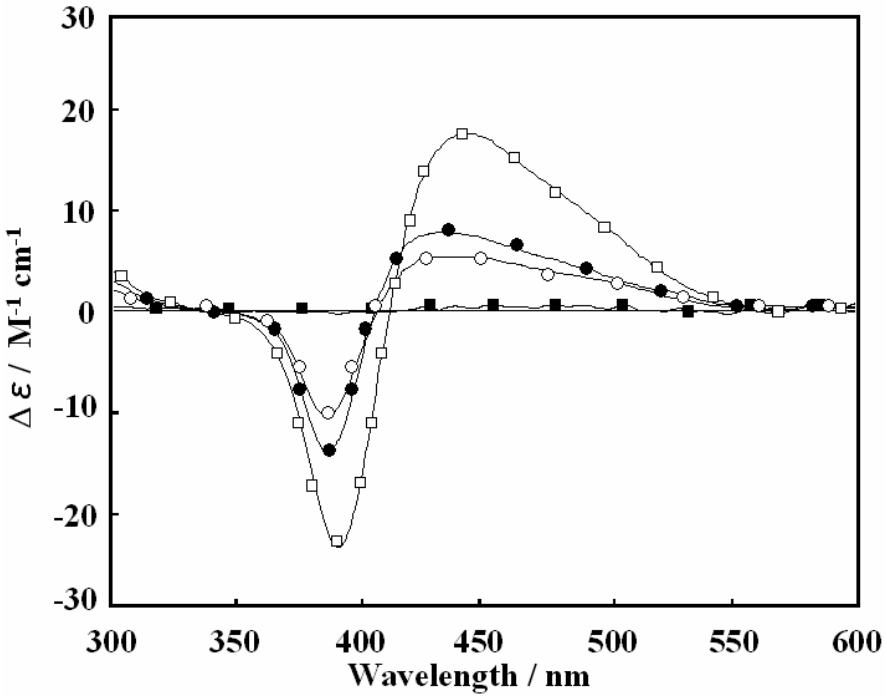


Figure 6. CD spectra of Dabs-ε-PL (2.0 x 10^{-5} mol dm^{-3}) in DMSO-H_2O solution. H_2O content: (a) −■−0%; (b) −□−20%; (c) −●−70%; (d) —○—90% .

Conclusion

The UV-Vis spectra point out that the side chain azo chromophores are non-covalently bound and forms H-type stacked aggregates in DMSO-H_2O solution. Changes in the UV-Vis absorption are accompanied by the appearance of a bisignate CD band in the region of the blue-shifted absorption. A first positive Cotton effect and a second negative one are observed at longer and shorter wavelength, respectively, than that of a crossover point. These spectral data lead to the aggregation model of Dabs-ε-PL in DMSO-H_2O solution, where the side chain azo chromophores are disposed in a chiral arrangement in the H-aggregated state (Figure 7).

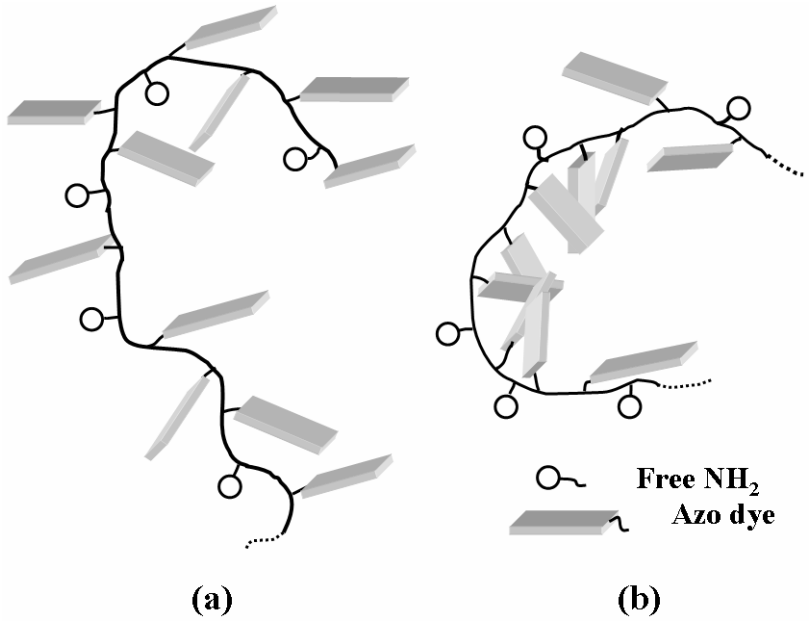


Figure 7. Aggregation model of Dabs-ε-PL (a) un-aggregated Dabs-ε-PL in DMSO, (b) aggregated Dabs-ε-PL in aqueous DMSO solution.

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