Induction of Circular Dichroism on the Soret Bands of a Symmetric Water-Soluble Porphyrin by Poly(L-lysine) in Three Conformations

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Absorption spectra and circular dichroism (CD) of aqueous solutions of porphine-*meso*-tetra(4-benzenesulfonic acid) (TPPS) in the presence of poly(L-lysine) have been measured at different pH and at different mixing ratios, and also after heated at alkaline pH higher than 10.5. Even at pH 3.0—8.0, where poly(L-lysine) is in the random coil conformation, strong CD is induced in the Soret region, giving many CD bands, and their magnitudes are not very dependent on the mixing ratio, [P]/[D], higher than 5. TPPS bound to random coil poly(L-lysine) is mostly in the dimeric form having a blue-shifted absorption band at 399 nm. The pH-induced helix-coil transition of poly(L-lysine) part occurs, accompanied by the conversion of bound TPPS from the dimeric form to the monomeric form. At pH 11 where poly(L-lysine) is in the perfect α-helix, strong CD is induced in the Soret region, consisting of a pair of positive and negative bands, and their magnitudes increase with decreasing [P]/[D] ratio and have a maximum at an intermediate [P]/[D] ratio. TPPS bound to helical poly(L-lysine) is monomeric, having an absorption band at 418nm. The induction of strong CD on the Soret region of TPPS when bound to either coiled or helical poly(L-lysine) can be attributed to dissymmetric electronic coupling of two or more TPPS ions bound to poly(L-lysine). Only weak CD is induced on the Soret transition of TPPS bound to β-form poly(L-lysine) that has been formed by heating aqueous solution of helical poly(L-lysine) at alkaline pH.

In the previous work[1] we have demonstrated that three kinds of water-soluble porphyrin derivatives can bind electrostatically with α-helical polypeptides in aqueous solution and induce a similar type of circular dichroism (CD) in the Soret region. Among them porphine-*meso*-tetra(4-*N*-methylpyridinium) (TMpyP) ion binds to α-helical poly(L-glutamic acid) and shows a positive and a negative CD bands in the Soret region,[2] while porphine-*meso*-tetra(4-benzenesulfonate) (TPPS) ion[3] binds to α-helical poly(L-lysine) and shows two overlapping strong pairs of positive and negative CD bands in the Soret region. It was imagined that the principal mode of binding would be different between these two systems.

Poly(L-lysine) is randomly coiled in aqueous solutions at pH lower than 9, while it is α-helical at pH higher than 10.[4,5] At alkaline pH the conformation of poly(L-lysine) converts from the α-helix to the β-form when heated at temperatures above 45°C,[6—8] and the β-form of poly(L-lysine) is stable for several days even after brought to room temperature.[8,9]

In order to investigate the mode of interaction and mechanism of induction of CD of TPPS with poly(L-lysine) in more detail, we examine the absorption spectra and CD of aqueous solutions of TPPS in the presence of poly(L-lysine) at different pH and at different mixing ratios, [P]/[D], and also after heat treatment at high pH.

Experimental

Materials. Porphine-*meso*-tetra(4-benzenesulfonic acid) dihydrochloride was purchased from Porphyrin Products, Inc., Logan, Utah, U.S.A. and used without further purification. Poly(L-lysine) hydrochloride was obtained from Protein Research Foundation, Minoh, Osaka, Japan.

A stock solution of 1×10^{-3} M TPPS was prepared by dissolving the dark green crystals in distilled water (1 M=1 mol dm^{-3}). Poly(L-lysine) hydrochloride was dissolved in water to prepare its stock solution of residue molar concentration, 2×10^{-3} M. To the polymer solution the porphyrin dye solution was added in such a way as to make the polymer-to-dye molar ratio, [P]/[D], to a desired value, where [P] is the residue molar concentration of poly(L-lysine) and [D] is the molar concentration of TPPS, and then the pH of the solution was varied from 3 to 11, by adding 0.1 or 1 M HCl or NaOH. The value of mixing ratio, [P]/[D], was varied from 1 to 1000 at pH 7.0 and 10.8, keeping [D]=1.0×10^{-5} or 1.0×10^{-6} M. At very low [P]/[D] ratios for solutions of higher [D], the solutions were slightly turbid. All the solutions were kept for an hour before spectral measurements.

In order to examine the effect of heat treatment, solutions were prepared by two methods. First, to the solution of 2×10^{-4} M poly(L-lysine) an equal volume of a solution of 4×10^{-6} M TPPS was added, and after brought to pH 11.1, the mixture was heated at 50°C for an hour and cooled down to room temperature. Secondly, a solution of 2×10^{-4} M poly(L-lysine) at pH 11 was heated at 50°C for an hour and cooled down to room temperature, and then an equal volume of a solution of 4×10^{-6} M TPPS at pH 11 was added to it. Both solutions were slightly turbid but the former solution became more turbid with time.

Measurements. Absorption spectra of solutions were measured on a Shimadzu UV-2200 (or sometimes UV-200S) Spectrophotometer over 500—350 nm at room temperature around 25°C, using quartz cells of 0.5 or 1 cm path. CD of solutions was measured on a JASCO J-40A Circular Dichrometer over the wavelength region, mostly from 500 to 350 nm, using quartz cells of 0.1, 0.5, and 1 cm path at 25 °C. Through the jacket of cells, water of constant temperature was circulated. Far ultraviolet CD was measured similarly, by using a 0.5 or 1 cm quartz cell.

The pH of solutions was measured by a Horiba N-8F Ion Meter before and after each spectroscopic measurement.

Results

The molar extinction coefficient, ε_D ($dm^3\,mol^{-1}\,cm^{-1}$), and the molar ellipticity, $[\theta_D]$ ($deg\,cm^2\,dmol^{-1}$), of solutions of TPPS in the presence of poly(L-lysine) are given on the basis of total molar concentration of added TPPS. In the far ultraviolet region the residue molar ellipticity, $[\theta_P]$, is given for solutions of TPPS in the presence of poly(L-lysine), on the basis of total mole of L-lysine residue. The wavelength is expressed by λ (nm).

Absorption Spectra of Free (Unbound) TPPS. The aqueous solution of 1×10^{-5} M TPPS has a red color at pH higher than 5, while it has a green color at pH below 5. Here we will be concerned with aqueous solutions of TPPS in the presence of poly(L-lysine), mainly at pH higher than 6, which are yellow-colored. The absorption spectra of aqueous solutions of TPPS have a strong Soret band at 413 nm, toghether with a weak shoulder at 393 nm, when the pH is higher than 6, and they are scarcely dependent on pH over the range of pH from 6 to 11. The main band has a molar extinction coefficient ε=484000 at 413 nm.

The Soret band, or the B band, of porphyrin belongs to the second longest wavelength transition of porphine and consists of two components having polarizations perpendicular to each other.[10,11] The B_x component has a polarization parallel to the line connecting two opposing pyrrole groups, i. e., to the H–H axis, and is at a longer wavelength, while the B_y component has a polarization perpendicular to the H–H axis and is at a shorter wavelength.

The B_y and B_x bands of TPPS in the free base form locate at 393 and 413 nm, respectively, and the B_x band has a much stronger molar extinction coefficient than the B_y band.

Because of its strong hypochromism such as noted below, it is likely that only the B_x component of the Soret band will be observable for aqueous solutions of TPPS in the presence of poly(L-lysine).

Dependence of Spectral Properties of the TPPS–Poly(L-lysine) System on pH. Figure 1 shows absorption spectra of the solutions of TPPS in the presence of poly(L-lysine) to [P]/[D] 80 at different pH. At pH 3.0—8.0 two Soret bands locate at 399 and 415 nm, indicating that TPPS ions bound to random coil poly(L-lysine) are in two forms. The shoulder band at 415 nm may be attributed to TPPS ions bound simply electrostatically. The binding of TPPS ion would be mainly electrostatic through two sulfonate groups with two ε-ammonium groups of poly(L-lysine); that is, a TPPS ion can bind to poly(L-lysine) divalently. The main band at 399 nm must be assigned to a strongly perturbed form, or a dimeric form, of bound TPPS ions. (A band at 490 nm appearing at pH 3.0 may be assigned to the diacid form of bound TPPS,[3] but we will not be concerned with it here.)

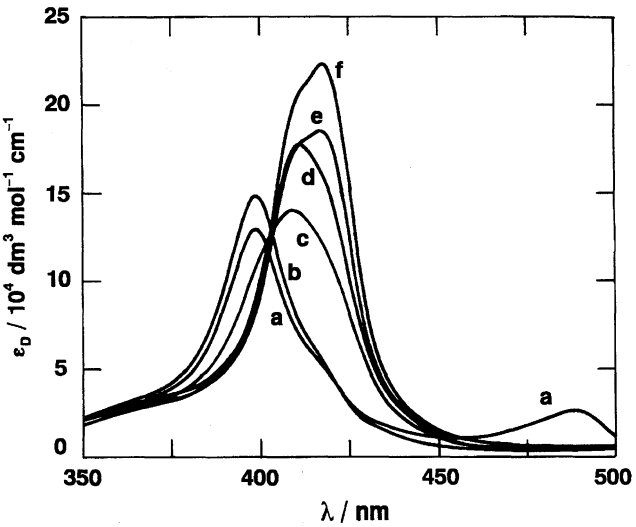

Fig. 1. Absorption spectra of aqueous solutions of TPPS in the presence of poly(L-lysine) at different pH. [P]/[D] 80, [D]=1.25×10^{-5} M: a, pH 3.01; b, pH 4.86; c, pH 8.66; d, pH 9.08; e, pH 9.88. [P]/]D] 100, [D]=1.00×10^{-5} M: f, pH 10.57.

It has been observed that dimerization of dye molecules often causes a substantial blue-shift of its absorption band,[12—15] and its linear array induces further blue-shift. It is likely that the binding of TPPS ions to fully charged poly(L-lysine) induces their dimerization and their electronic coupling. TPPS is known to have some tendency for dimerization in water.[16] Furthermore, it has also been noted that some dye tends to dimerize when bound to polyelectrolyte.[17—20]

Beyond pH 8.2 the band at 412 nm becomes the main band in place of that at 399 nm. At alkaline pH higher than 8.5 the band at 412 nm is manifest, with a shoulder at 418 nm, while at pH higher than 9.8 the band at 418 nm is the main band, with a shoulder at 412 nm. Poly(L-lysine) undergoes the transition from the random coil to the α-helix at pH between 8.5 and 10, as will be seen below, and the bands at 412 and 418 nm can then be ascribed to the monomeric form of TPPS bound to the interrupted and perfect helix, respectively, of poly(L-lysine).

Table 1 lists location and intensities of the Soret bands of TPPS in the presence of random coil and helical poly(L-lysine), which are characteristic of bound species of TPPS. It is remarkable to observe that in the presence of poly(L-lysine), either helical or randomly coiled, the molar extinction coefficient of TPPS reduces to a half or a third, as compared with that of free TPPS, and the band width broadens. All these bands are assigned to the B_x component.

Figure 2 shows induced CD of the solutions of TPPS in the presence of poly(L-lysine) to [P]/[D] 80 at different pH. It exhibits somewhat complicated changes

Table 1. Absorption Spectra and CD of TPPS in the Presence of Poly(L-lysine)

(A) Random coil at neutral pH, 7.0 $[D]=1.0\times10^{-6}$ M

[P]/[D]	λ / nm	ε_D / $dm^3\,mol^{-1}\,cm^{-1}$	λ / nm	$[\theta_D]$ / deg $cm^2\,dmol^{-1}$	$[\theta_D]/\varepsilon_D$	Bound form of TPPS
1—2	413	255000[b]				(Unbound free base form)
	(399)		396	75000[b]		Dimeric form
			409	−125000[b]		
			434	−55000[b]		
5	399	124000	397	1045000	8.43	Dimeric form
			410	−1065000	−8.59	
			434[sh a]	−220000	−1.77	
10—1000	399	164000[c]	392	30000[c]	0.18	Dimeric form
			402	−225000[c]	−1.37	
			425	−20000[c](+)	−0.12	
	415[sh a]	113000[c]	414	−25000[c](+)	−0.22	Monomeric form
			434	−110000[c]	−0.97	
			445[sh a]	−80000[c]	−0.71	

(B) α-Helix at alkaline pH, 10.8 $[D]=1.0\times10^{-6}$ M

[P]/[D]	λ / nm	ε_D / $dm^3\,mol^{-1}\,cm^{-1}$	λ / nm	$[\theta_D]$ / deg $cm^2\,dmol^{-1}$	$[\theta_D]/\varepsilon_D$	Bound form of TPPS
1	413					(Unbound free base form)
5—10	413	289000[d]	411	225000[d]	0.79	Monomeric form
			430	−300000[d]	−1.04	(On interrupted helix)
	417[sh a]	199000[d]				
50—1000	412[sh a]	176000[e]				
	418	239000[e]	416	820000[e]	3.43[e]	Monomeric form
			426	−990000[e]	−4.14[e]	

(C) β-Form at alkaline pH, 11.1 $[D]=2.0\times10^{-6}$ M

[P]/[D]	λ / nm	ε_D / $dm^3\,mol^{-1}\,cm^{-1}$	λ / nm	$[\theta_D]$ / deg $cm^2\,dmol^{-1}$	$[\theta_D]/\varepsilon_D$	Bound form of TPPS
50	414	230000	400	30000	0.13	Monomeric form
			410	−40000	−0.17	
			425	−60000	−0.26	

a) [sh]shoulder, b) [P]/[D]=1, c) [P]/[D]=1000, d) [P]/[D]=10, e) [P]/[D]=50.

depending on pH, since even random coil poly(L-lysine) induces CD on the Soret transition of TPPS. Relevant data of induced CD are summarized in Table 1.

At lower pH from 3.0 to 8.0, two groups of CD bands, i. e., a weak positive band at 392 nm and a negative band at 402 nm, together with a positive band at 417 nm and a negative band at 434 nm, are induced. The former pair and the shoulder band at 425 nm are associated with the absorption band at 399 nm which can be assigned to the dimeric TPPS ions. And the latter pair and the band at 445 nm may be attributed to the absorption band at 415 nm, which is assignable to the monomeric TPPS ions. The splitting of an absorption band into three CD bands would be caused by electronic coupling of many bound TPPS ions, either in the dimeric or monomeric form. (A negative CD band, which is broad but shallow, is manifest at pH 3.0 in the longer wavelength region, i. e., up to 480 nm. This CD band must be associated with the absorption band at 490 nm, which can be attributed to the diacid form of bound TPPS.[3]) All these solutions exhibit far ultraviolet CD characteristic of the random coil conformation of poly(L-lysine).

The induced CD is subject to a large change across pH 9. At pH 9.1 it has a positive band at 405 nm and a negative band at 420 nm. The far ultraviolet CD shows that the content of α-helix of poly(L-lysine) increases with increasing pH in this region.

At pH higher than 10 where poly(L-lysine) is perfectly helical, a pair of positive and negative bands at 416 and 426 nm is induced. The pair is associated with the absorption band at 418 nm, which is assigned to the B_x component, and the splitting of the Soret transition into two suggests that, at least, two TPPS ions are bound consecutively to different sites located on an α-helix and they are electronically coupled together. Then the Soret band splits into two, and the CD bands associated with the split components should have opposite signs.[21,22]

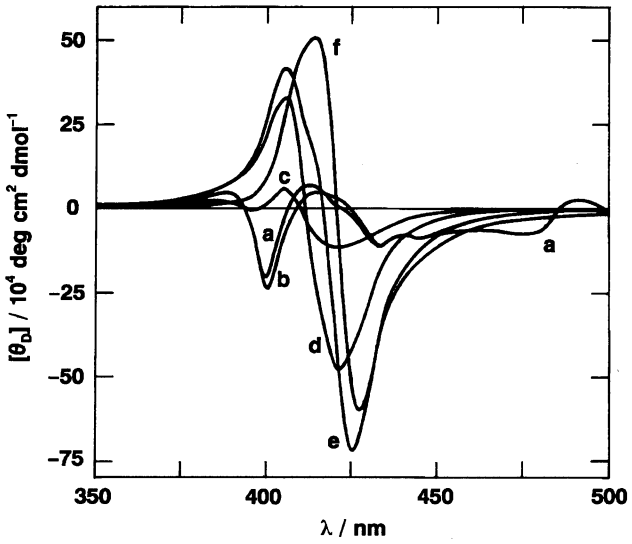

Fig. 2. CD of aqueous solutions of TPPS in the presence of poly(L-lysine) at different pH. [P]/[D]=80, [D]=1.25×10^{-5} M: a, pH 3.01; b, pH 4.86; c, pH 8.66; d, pH 9.08; e, pH 9.88. [P]/[D] 100, [D]=1.00×10^{-5} M: f, pH 10.57.

Figure 3 shows changes in molar ellipticities of the CD bands for the near and far ultraviolet region with pH. Molar ellipticities of the Soret transition tend to be constant at pH below 8.5 and above pH 9.5, respectively. Their sharp changes across pH 9.2 must be connected with the conformational change of poly(L-lysine), to which TPPS binds. The coil-to-helix transition of poly(L-lysine) part converts bound TPPS ions from the dimeric to the monomeric form. The residue molar ellipticities of the far ultraviolet transition clearly show the occurrence of the pH-induced helix-coil transition of poly(L-lysine) part across pH 9.5. It has been observed that the helix-coil transition of poly(L-lysine) is induced at pH 10.0 in the absence and presence of added salt.[4,23] Accordingly, the transition pH shifts toward a lower pH, i. e., to pH 9.5, in the presence of TPPS to [P]/[D] 80; the α-helix of poly(L-lysine) is stabilized by the binding of TPPS.

At the most alkaline pH the molar ellipticities of the Soret transition sharply increase, together with the residue ellipticities of the peptide transition. Since the residue ellipticity at 208 nm serves as a measure of aggregation state of the α-helix,[2] the increase in the molar ellipticities of the Soret transition can be ascribed to the aggregation of α-helical poly(L-lysine), possibly caused by the cross-linking with TPPS ions.

Dependence of Spectral Properties of the TPPS–poly(L-lysine) System on [P]/[D]. Figures 4 and 5 illustrate absorption spectra and CD, respectively, of solutions of TPPS mixed with poly(L-lysine) of pH 7.0 at different [P]/[D] ratios. In Table 1 assignment of the absorption bands and the associated CD bands, together with values of relevant spectral parameters, is given for the TPPS–poly(L-lysine) systems at pH 7.0.

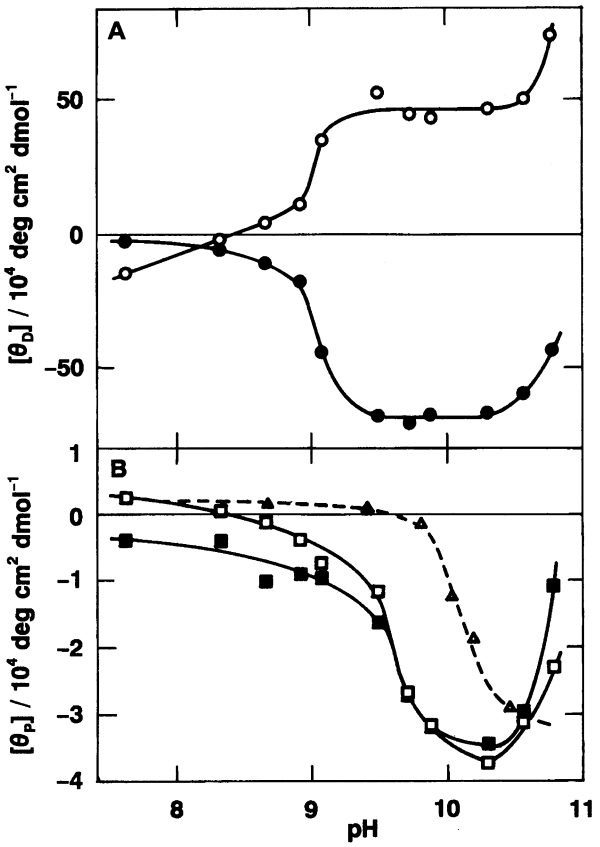

Fig. 3. Molar ellipticities of CD bands at the Soret (A) and the far ultraviolet (B) transitions plotted against pH. [P]/[D] 80, [D]=1.25×10^{-5} M. A: ○, 405 or 416 nm; ●, 425 or 426 nm. B: ■, 208 nm; □, 222nm. For data above pH 10.5, [P]/[D] 100, [D]=1.00×10^{-5} M. --- Δ---, 222 nm, in the absence of TPPS, from Ref. 23.

The Soret band at 413 nm is subject to hypochromism in the presence of poly(L-lysine) to [P]/[D] 1, and the Soret band shifts to 399 nm when [P]/[D] is 5. The hypochromism indicates that some TPPS ions are bound to poly(L-lysine) simply electrostatically. The blue-shift of the Soret band and the induction of associated CD bands suggest dimerization of bound TPPS ions and their rigid binding to poly(L-lysine).

At [P]/[D] 5 the induced CD is the strongest; the pair of positive and negative CD bands would be generated from the most effective coupling of dimeric TPPS ions.

At [P]/[D] higher than 5, the absorption band at 399 nm is nearly independent of [P]/[D], but, with increasing [P]/[D] ratio, the shoulder at 415 nm becomes stronger. The induced CD is complex but is nearly independent of [P]/[D]. The scarce dependence of the main absorption band and the induced CD on [P]/[D] suggests that almost all TPPS ions are bound to random coil poly(L-lysine) and they are mostly in the dimeric

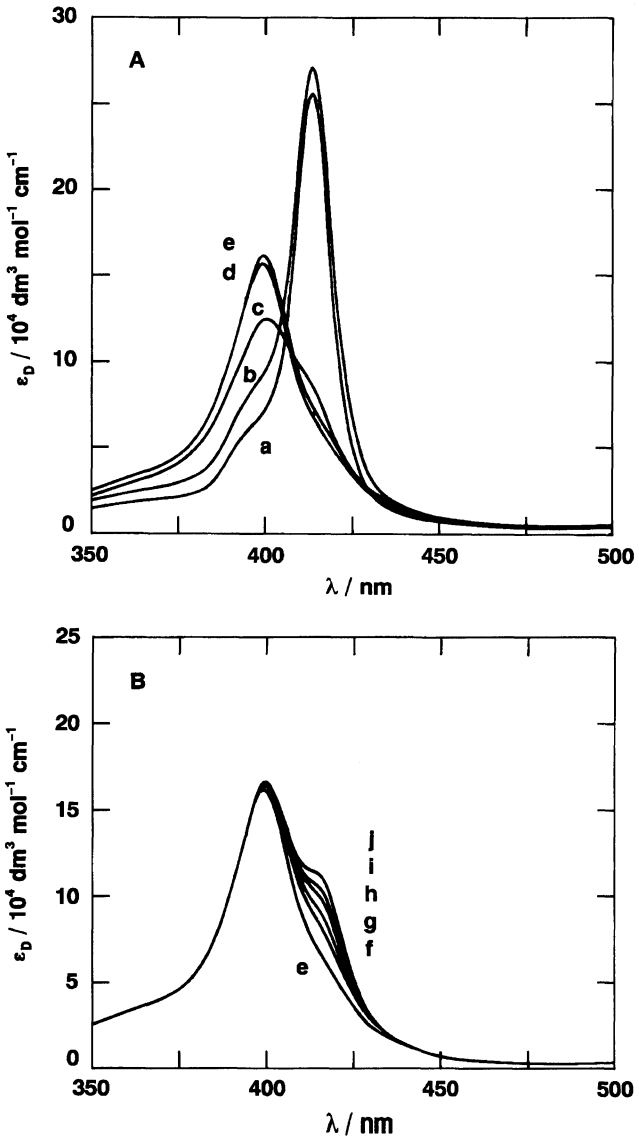

Fig. 4. Absorption spectra of aqueous solutions of TPPS in the presence of random coil poly(L-lysine) at different mixing ratios. pH 7.0, [D]=1.00×10^{-6} M. A, [P]/[D]: a, 1.0; b, 2.0; c, 5.1; d, 10; e, 20. B, [P]/[D]: e, 20; f, 50; g, 100; h, 200; i, 501; j, 1000.

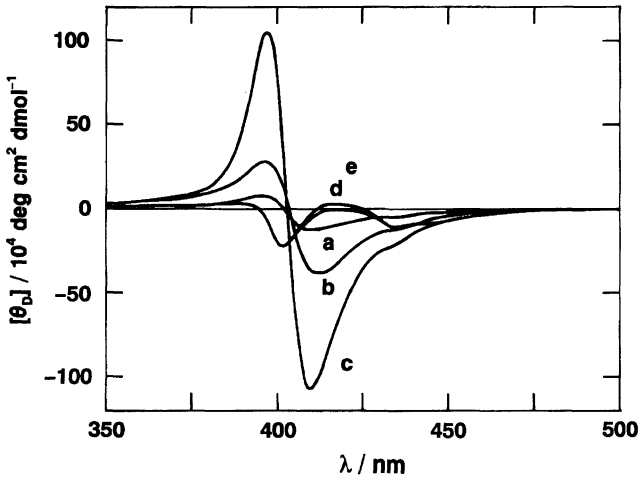

Fig. 5. CD of aqueous solutions of TPPS in the presence of random coil poly(L-lysine) at different mixing ratios. pH 7.0, [D]=1.00×10^{-6} M. [P]/[D]: a, 1.0; b, 2.0; c, 5.1; d, 10; e, 20—1000.

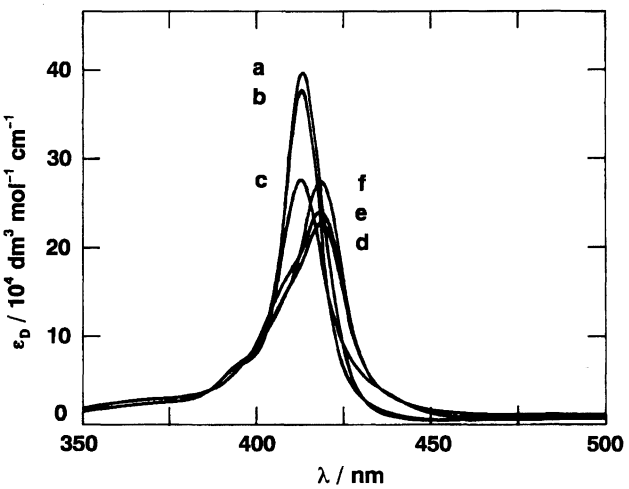

Fig. 6. Absorption spectra of aqueous solutions of TPPS in the presence of helical poly(L-lysine) at different mixing ratios. pH 10.8, [D]=1.00×10^{-6} M. [P]/[D]: a, 1.0; b, 5.0; c, 10.0; d, 50.0; e, 95.9; f, 501.

form. The shoulder band at 415 nm becomes gradually stronger with increasing [P]/[D], which is in accord with the assignment to the monomeric form. The dimeric and monomeric TPPS ions are closely bound to poly(L-lysine) so that they may be electronically coupled together. Then to these arrays several CD bands would be assignable, as given in Table 1.

At alkaline pH higher than 10, two strong absorption bands appear at 412 and 418 nm, and they change their intensities with [P]/[D] ratio, in such a way as shown in Fig. 6. The induction of CD in the Soret region is shown in Fig. 7. Values of the spectral parameters are tabulated in Table 1.

In the absence of poly(L-lysine), the absorption band at 413 nm is assignable to unbound TPPS ions. With increasing [P]/[D] strong hypochromism occurs for the band at 413 nm, and between [P]/[D] 10 and 50 the main band shifts to 418 nm. The simple binding of TPPS ions causes hypochromism on the absorption band, and the mutual electronic coupling of bound TPPS ions shifts its location to red and induces a positive CD band at 416 nm and a negative band at 426 nm. At [P]/[D] higher than 10, the absorption band is stronger at 418 nm, with a shoulder at 412 nm, and corresponding CD bands are induced. The sudden red-shift and the strongest hypochromism as well as the strongest induction of CD occur at the intermediate mixing ratio, [P]/[D] 50, where the electronic coupling of consecutively bound TPPS ions would be the most effective, owing to an optimum number of divalently bound TPPS ions for the dissymmetric arrangement, arising from a

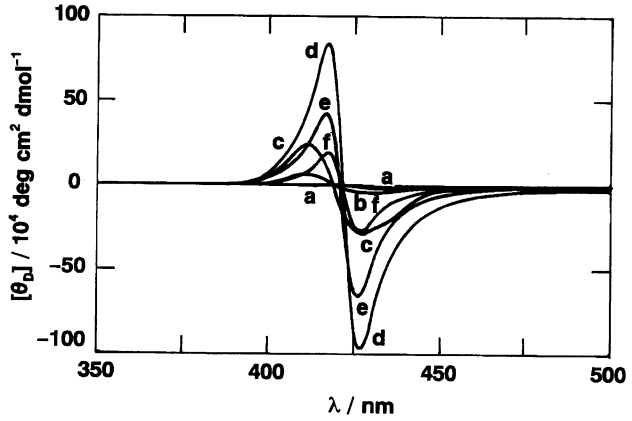

Fig. 7. CD of aqueous solutions of TPPS in the presence of helical poly(L-lysine) at different mixing ratios. pH 10.8, [D] 1.00×10^{-6} M. [P]/[D]: a, 1.0; b, 5.0; c, 10.0; d, 50.0; e, 95.9; f, 501.

decreased number of ionized groups.

Discussion

The Interaction between TPPS and Randomly Coiled Poly(L-lysine). Usually random coil polypeptides cannot induce CD on the absorption bands of a symmetrical dye such as Acridine Orange, when the mixing ratio, [P]/[D], is as high as 100; only at [P]/[D] less than 10 some induced CD is observable for Acridine Orange in the presence of random coil poly(L-glutamic acid)[24—28] or random coil poly(*S*-carboxymethyl-L-cysteine).[29,30]

Nevertheless, aqueous solutions of TPPS in the presence of random coil poly(L-lysine) show strong CD in the Soret region, even if [P]/[D] is as high as 1000. It should be noted, however, that induced CD is observed for Methyl Orange in the presence of random coil poly(L-lysine) even at high [P]/[D] ratios.[18]

The mode of interaction of TPPS with random coil poly(L-lysine) must be different from that of TMpyP with random coil poly(L-glutamic acid) or poly(*S*-carboxymethyl-L-cysteine). TMpyP ions are bound sparsely on these anionic polypeptides, so that they are not electronically coupled together; then essentially no CD is induced for the TMpyP-polypeptide systems.[2] Accordingly, the binding and formation of many dimeric TPPS ions on poly(L-lysine) and their mutual electronic coupling would be the origin for inducing CD on the Soret transition.

The Interaction between TPPS and α-Helical Poly(L-lysine). The induced CD observed for TPPS in the presence of α-helical poly(L-lysine) exhibits a single pair of positive and negative CD bands. Previously[1] we noted two overlapping pairs of such CD bands for the TPPS-helical poly(L-lysine) system at pH 10.3, but we have now found that a pair of CD bands arises from the binding of TPPS ions to the interrupted helix at pH 10. The absorption band at 418 nm is characteristic of TPPS ions bound to the perfect helix of poly(L-lysine).

The dissymmetry factors, $[\theta_D]/\varepsilon_D$, of the CD bands for helical poly(L-lysine) have relatively large magnitudes, as shown in Table 1, so that the CD would be induced by the Moffitt-Tinoco mechanism arising from the dissymmetric electronic coupling of Soret transition of two TPPS ions consecutively bound to the α-helix of poly(L-lysine).[21,31—33]

As shown in Fig. 3, the binding of TPPS serves not only to stabilize the α-helix of poly(L-lysine), but the Soret transition of bound TPPS is also subject to the dissymmetric field of poly(L-lysine), even if it is not perfectly helical but is only partially helical. These observations are in contrast to those for TMpyP bound to helical poly(L-glutamic acid).[2] Such differences would arise from the difference in binding affinity of porphyrin with polypeptide, mainly coming from the higher hydrophobicity of TPPS[16] and poly(L-lysine).[9]

The Mode of Binding of TPPS to β-Form Poly(L-lysine). When aqueous solution of TPPS mixed with helical poly(L-lysine) is heated at pH 11 to 50°C for an hour and then cooled to room temperature, the magnitude of the induced CD characteristic of the TPPS-helical poly(L-lysine) complex decreases as the content of helix decreases. TPPS ions bound divalently to poly(L-lysine) confer the increased stability on

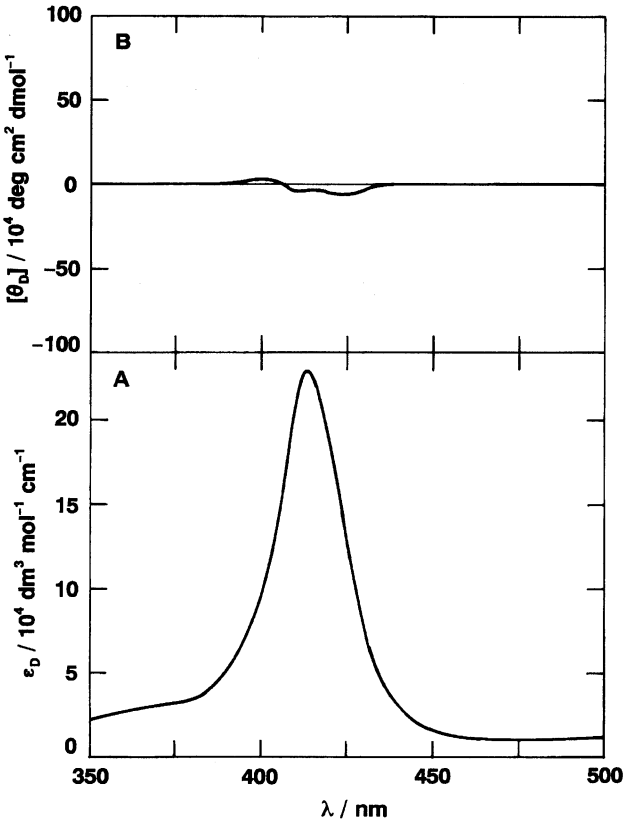

Fig. 8. Absorption Spectra (A) and CD (B) of TPPS in the presence of β-form poly(L-lysine) at pH 11.1 and [P]/[D] 100 with [D]=2.00×10^{-6} M.

the α-helix, so that the α-helix is prevented from being converted to the β-form.

If aqueous solution of TPPS at pH 11 is added to the solution of poly(L-lysine) of pH 11 that has been heated at 50°C for an hour and cooled down to the room temperature in advance, the mixture gives only weak induced CD in the Soret region, as shown in Fig. 8.

Both solutions show the absorption band at 414 nm, blue-shifted relative to the band at 418 nm, which is attained when TPPS is bound to α-helical poly(L-lysine) at alkaline pH. However, the former still exhibits the far ultraviolet CD of the α-helix, and only the latter gives the ultraviolet CD characteristic of the β-form, having $[\theta_p]=-15000$ at 218 nm.[6,7] The spectral data for the Soret transition of the latter are given in Table 1.

Previously[2] we observed that the Soret transition of TMpyP ion is strongly perturbed by β-form poly(*S*-carboxymethyl-L-cysteine). The absorption band shifts largely to red and a broad positive CD band is induced.

Both β-form poly(L-lysine) and β-form poly(*S*-carboxymethyl-L-cysteine) induce weak CD on the Soret transition of bound porphyrin, with some bands differing in their signs. Only detailed modes of their interaction of TMpyP and TPPS with the β-forms would be different, mainly arising from geometrical difference between the two porphyrins or from different electronic structure between the side chains of two polypeptides.

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