Induced Circular Dichroism of a Cationic Porphyrin Bound to α-Helical Poly(L-glutamic acid) and β-Form Poly(*S*-carboxymethyl-L-cysteine) in Aqueous Solutions

Takashi NEZU and Shoichi IKEDA
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464
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Absorption spectra and circular dichroism (CD) of aqueous solutions of a water-soluble porphyrin derivative, porphine-*meso*-tetra(4-*N*-methylpyridinium) tosylate (TMpyP tosylate), have been measured in the presence of poly(L-glutamic acid) or poly(*S*-carboxymethyl-L-cysteine) at different pH. At neutral and alkaline pH where poly(L-glutamic acid) is randomly coiled, essentially no CD is induced in the Soret region over a wide range of [P]/[D] ratios. At acid pH where poly(L-glutamic acid) is helical, a positive CD band at 415 nm and a stronger negative CD band at 442 nm are induced if [P]/[D] is high, but the induced CD is considerably weak as [P]/[D] is lowered, owing to partial disruption of the helical conformation by densely bound TMpyP ions, and it substantially vanishes at [P]/[D] as low as 1.

While random coil poly(*S*-carboxymethyl-L-cysteine) does not induce CD in the Soret region of TMpyP, β-form poly(*S*-carboxymethyl-L-cysteine) induces a weak, positive CD band at 510 nm, accompanied by an extensive weakening of the absorption bands.

Poly(L-glutamic acid) has the α-helical conformation in solutions of pH lower than 6, while it is randomly coiled at higher pH. Symmetric cationic dyes such as Acridine Orange and Pinacyanol are induced strong circular dichroism (CD) at their absorption bands when α-helical poly(L-glutamic acid) is present in their solutions.[1—3]

Porphine-*meso*-tetra(4-*N*-methylpyridinium) tosylate (TMpyP tosylate) is a water-soluble derivative of porphyrin.[4,5] In the previous work[6] it was demonstrated that TMpyP is induced strong CD at the Soret bands when helical poly(L-glutamic acid) is present in its solutions. In the present work the absorption spectra and the extrinsic optical activity of aqueous solutions of TMpyP in the Soret region are investigated in the presence of poly(L-glutamic acid) at different pH and at different molar ratios of glutamic acid residue-to-porphyrin dye, [P]/[D].

Poly(*S*-carboxymethyl-L-cysteine) has the β-conformation in solutions of pH lower than 5, while it is randomly coiled at higher pH. Acridine Orange and its derivatives are induced characteristic CD at their absorption bands when β-form poly(*S*-carboxymethyl-L-cysteine) is in their solutions.[7,8] In the present work the absorption spectra and CD of aqueous solutions of TMpyP mixed with poly(*S*-carboxymethyl-L-cysteine) are measured at different pH. The molar ratio of *S*-carboxymethyl-L-cysteine residue-to-porphyrin dye, [P]/[D], is kept at 100.

Experimental

Materials. Porphine-*meso*-tetra(4-*N*-methylpyridinium) tosylate (TMpyP tosylate) was purchased from Porphyrin Products, Logan, Utah, U.S.A. Sodium poly(L-glutamate) was obtained from Institute for Protein Research Foundation, Minoh, Osaka, Japan. Poly(*S*-carboxymethyl-L-cysteine) was prepared in our laboratory and had the form of its sodium salt.

A stock solution of 1×10^{-3} M TMpyP was prepared by dissolving purple crystals of TMpyP in water (1 M=1 mol dm^{-3}). A stock solution of 2×10^{-3} M poly(L-glutamic acid) was prepared by dissolving sodium poly(L-glutamate) in distilled water. A stock solution of poly(*S*-carboxymethyl-L-cysteine) was prepared in a similar way.

For measurements of absorption spectra and CD, the stock solution of TMpyP was added to the polymer solution to a desired mixing ratio, and then the pH of the solution was adjusted by adding a 0.1 or 1 M HCl or NaOH solution. The solution of 1.0×10^{-5} M TMpyP had a yellow color at alkaline and neutral pH, both in the presence and absence of poly(L-glutamic acid) or poly(*S*-carboxymethyl-L-cysteine).

The concentration of TMpyP, [D], was kept at 1.0×10^{-5} M or 1.0×10^{-6} M throughout the present measurements.

Apparatus. The pH of solutions was measured by a Horiba N-8F Ion Meter before each spectral measurement. Absorption spectra were recorded on a Shimadzu UV-2200 (or sometimes UV-200S) Spectrophotometer, using quartz cells of 0.5 and 1cm path over the wavelength region, mostly, between 350 and 500 nm at room temperature around 25°C. CD was recorded on a JASCO J-40A Circular Dichrometer, using the same cells with a jacket thermostatted to 25°C, over the same wavelength region. Far ultraviolet CD was measured in a similar way, using a 0.5 cm cell, in order to examine the conformation of poly(L-glutamic acid) and poly(*S*-carboxymethyl-L-cysteine).

In the visible and ultraviolet region, the molar extinction coefficient and the molar ellipticity of aqueous solutions of TMpyP in the presence of poly(L-glutamic acid) or poly(*S*-carboxymethyl-L-cysteine) are given on the basis of molar concentration of total dye and are expressed by ε_D (dm^3 mol^{-1} cm^{-1}) and $[\theta_D]$ (deg cm^2 dmol^{-1}), respectively. The far ultraviolet CD is examined by the molar ellipticity, $[\theta_P]$, on the basis of residue mole of poly(L-glutamic acid) or poly(*S*-carboxymethyl-L-cysteine). The wavelength is expressed by λ (nm).

Results

Absorption Spectra of Free (Unbound) TMpyP. The absorption spectra of aqueous solution of TMpyP give a strong Soret band with the molar extinction coefficient, $\varepsilon=233000$ at 421 nm, together with a weak band, $\varepsilon=35000$ at 380 nm, and several weaker bands, $\varepsilon \lesssim 10^4$ at longer wavelengths. In the region of pH from 2.5 to 10.5, the absorption spectra of TMpyP remain nearly identical.

The Soret or B transition consists of two components, whose polarizations are mutually perpendicular to each other. The B_y component at a shorter wavelength has a polarization perpendicular to the line connecting H atoms of opposing pyrrole groups, i. e., to the H–H axis, and the B_x component at a longer wavelength is polarized parallel to the H–H axis.[9,10] The observed Soret band of free TMpyP can be decomposed into two components, of which one having $\varepsilon=35000$ at 403 nm is assigned to the B_y component and the other having $\varepsilon=210000$ at 422 nm is to the B_x component.

The weak shoulder at 380 nm is associated with the N transition, and four weaker bands at 518, 554, 584, and 641 nm may be assigned to the vibrational branches of the Q transition.[10,11]

The wavelength and molar extinction coefficient of the near ultraviolet absorption bands of free TMpyP are tabulated in Table 1.

In the following, however, we will use values of the spectral parameters which are directly observed from spectra without spectral decomposition.

Spectra of TMpyP in the Presence of Poly(L-glutamic acid). Figure 1 shows absorption spectra of the solutions of TMpyP mixed with poly(L-glutamic acid) to [P]/[D] 100. At neutral or alkaline pH where poly(L-glutamic acid) is fully ionized and randomly coiled, the absorption spectra have a main band with $\varepsilon_D=217000$ at 425 nm. With lowering pH, the absorption band shifts toward red and weakens further. At pH 4.4 where poly(L-glutamic acid) is α-helical, the absorption band has $\varepsilon_D=170000$ at 430 nm.

Figure 2 shows CD of the solutions of TMpyP mixed with poly(L-glutamic acid) at [P]/[D] 100. At alkaline and neutral pH essentially no CD band is induced in the Soret region. (It is suspected that very weak CD bands are induced in this region, but their magnitudes are quite small as compared with those at lower pH.) With lowering pH below 6, appreciable CD emerges in the Soret region, and at pH 5.3 where poly(L-glutamic acid) is in the interrupted helix, positive bands are induced at 401 and 438 nm and a negative band is at 426 nm. At pH below 5 the signs of two CD bands at longer wavelengths are apparently reversed, and a positive band is at a shorter wavelength and a stronger negative band is at a longer wavelength. Two CD bands having $[\theta_D]=84000$ at 414 nm and $[\theta_D]=-277000$ at 442 nm are induced at pH 4.4.

Figure 3 shows changes in molar ellipticities of the

Table 1. Absorption Spectra of Aqueous Solution of TMpyP at pH 2.5—10.5 (TMpyP in the Free Base Form)

Assignment	λ / nm	ε / $dm^3\ mol^{-1}\ cm^{-1}$
N	380	33000
B_y	403	35000
B_x	422	210000
Q_y (1←0)	518	14900
Q_y (0←0)	554	5400
Q_x (1←0)	584	6200
Q_x (0←0)	641	1500

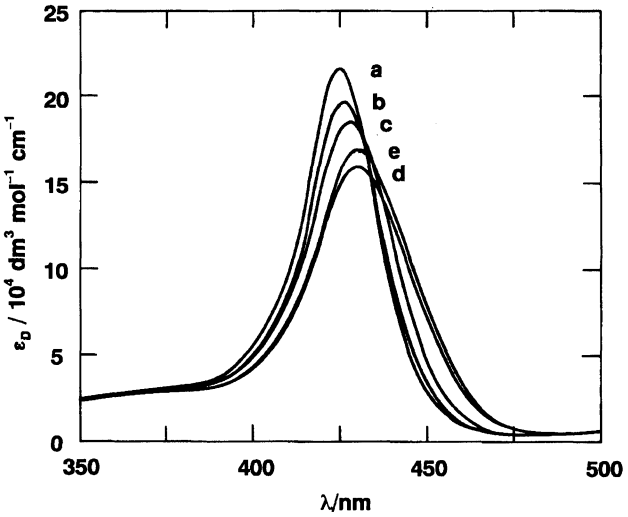

Fig. 1. Absorption spectra of aqueous solutions of TMpyP in the presence of poly(L-glutamic acid) at [P]/[D] 100. $[D]=1.00\times10^{-5}$ M. a, pH 7.82—6.27; b, pH 5.39; c, pH 5.09; d, pH 4.76; e, pH 4.41.

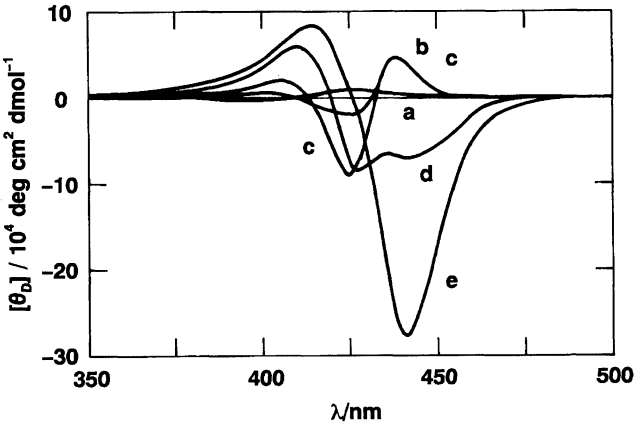

Fig. 2. CD of aqueous solutions of TMpyP in the presence of poly(L-glutamic acid) at [P]/[D] 100. [D]= 1.00×10^{-5} M. a, pH 7.82—6.16; b, pH 5.33; c, pH 5.01; d, pH 4.71; e, pH 4.39.

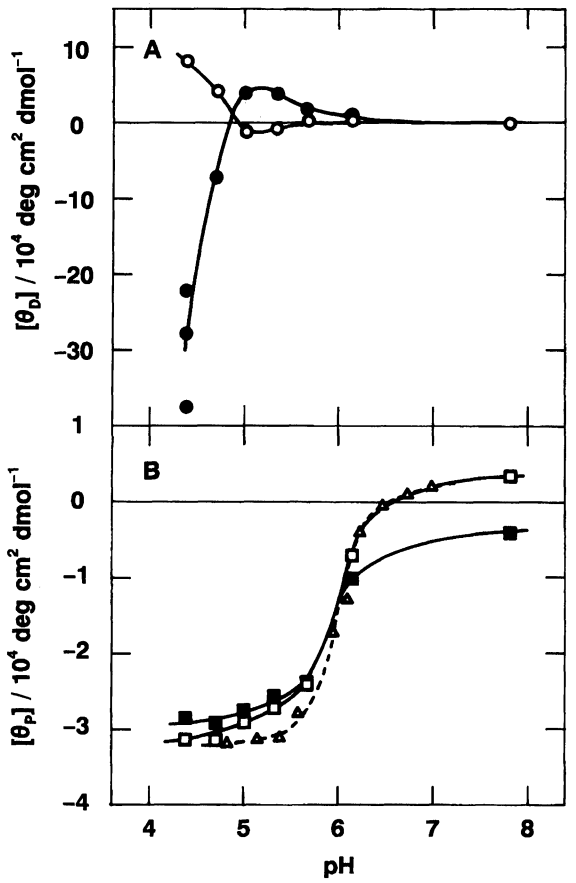

Fig. 3. Molar ellipticities of CD bands at the Soret (A) and the far ultraviolet (B) transitions plotted against pH. [P]/[D] 100, [D]=1.00×10^{-5} M. A: ○, 414 nm; ●, 442 nm. B: ■, 208 nm; □, 222nm. ---△---, 222 nm, in the absence of TMpyP.

CD bands for the near and far ultraviolet region with pH. Molar ellipticities of the Soret transition sharply changes with lowering pH below 5. Poly(L-glutamic acid) is subject to the helix-coil transition at pH 6, as exhibited in the residue molar ellipticities, irrespective of whether TMpyP is present or not. Accordingly, the Soret transition of TMpyP is induced strong CD only when the α-helix is completely formed.

Since poly(L-glutamic acid) has the α-helical conformation at acid pH, the induced CD must be caused by strong interaction, i. e., binding, of TMpyP with the α-helix of poly(L-glutamic acid). The α-helix would exert dissymmetric fields on the chromophore of bound TMpyP ions. With lowering pH further, the CD becomes weaker, owing to precipitation of poly(L-glutamic acid) or its complex with TMpyP and a decrease in number of bound TMpyP ions.

Values of parameters for the Soret band of TMpyP bound to poly(L-glutamic acid) are given in Table 2. Assuming that the absorption band of the B_y component is weaker and hidden in that of the B_x component, the weaker positive CD band at a shorter wavelength is assigned to the B_y transition, while the main absorption band and the stronger negative CD band at a longer wavelength are assigned to the B_x transition.

Values of the dissymmetry factor, $[\theta_D]/\varepsilon_D$, of the two components of the Soret transition of TMpyP are also given in Table 2 in the presence of helical poly(L-glutamic acid).

The red-shift of the Soret band from 421 to 425 nm at neutral pH reflects electrostatic interaction or binding of TMpyP ions with random coil poly(L-glutamic acid), and bound TMpyP ions are subject to a weak dissymmetric effect of the chiral random coil. However, at pH 7.3 essentially no CD is induced in the region of [P]/[D] ratios from 1 to 100. As shown in Fig. 4, the absorption band is weaker and located at 421 nm at lower [P]/[D], but it is at 425 nm at higher [P]/[D]. The far ultraviolet CD assures the random coil form of poly(L-glutamic acid) at pH 7.3 in the presence of TMpyP. The hypochromism at 421 nm and the red-shift to 425 nm of the Soret band suggest two kinds of modes of binding of TMpyP ions to poly(L-glutamic acid).

The absorption spectra are scarcely dependent on [P]/[D] ratio higher than 20, indicating that all TMpyP ions are bound to poly(L-glutamic acid). No or very weak CD is induced for TMpyP ions bound to poly(L-glutamic acid) at neutral and alkaline pH even at very low [P]/[D] ratios. This is different from strong induced CD observed for other dyes such as Acridine Orange bound to poly(L-glutamic acid),[2,3,12—14] for which strong CD is induced at neutral and alkaline pH only at very low [P]/[D].

Figure 5 shows the absorption spectra of TMpyP in the presence of poly(L-glutamic acid) to different [P]/[D] at pH 4.4. As [P]/[D] is increased from 1 to 10, the molar extinction coefficient of the Soret band decreases sharply, followed by its slight red-shift from 421 to 425 nm. The sharp decrease in molar extinction coefficient and the following red-shift reflect two different modes of binding of TMpyP to partially ionized poly(L-glutamic acid), as are observed at neutral and alkaline pH. The absorption band shifts further to red with a further increase in [P]/[D]. The absorption band at 430 nm remains nearly unaltered at [P]/[D] 50 and 100. This red-shift occurs by the perturbation due to the static field of the α-helix of poly(L-glutamic acid).

The induced CD changes with [P]/[D] in a somewhat complicate way at pH 4.4, as shown in Fig. 6. At lower [P]/[D] a negative CD band around 420 nm and a stronger positive band at 440 nm are induced. At [P]/[D] higher than 20 the signs of these CD bands are reversed; a positive band at 414 nm and a stronger negative band at 442 nm are manifest. There would be, at least, two different modes of interacton between TMpyP and poly(L-glutamic acid) at pH 4.4, depending on [P]/[D].

At lower [P]/[D] poly(L-glutamic acid) is only partially helical or in the interrupted helix, even at pH 4.4. This must be caused by some conformational effects of

Table 2. Absorption Spectra and CD of TMpyP in the Presence of Poly(L-glutamic acid) and Poly(*S*-carboxymethyl-L-cysteine)

A. Poly(L-glutamic acid), [P]/[D]=100

	(a) Random coil, pH 7.8		(b) α-Helix, pH 4.4				
	λ / nm	ε_D / dm^3 mol^{-1} cm^{-1}	λ / nm	ε_D / dm^3 mol^{-1} cm^{-1}	λ / nm	$[\theta_D]$ / deg cm^2 dmol^{-1}	$[\theta_D]/\varepsilon_D$
B_y	408[sh a)]	87500	410[sh a)]	72000	414	84200	1.17
B_x	425	217000	430	172000	442	−277000	−1.61

B. Poly(*S*-carboxymethyl-L-cysteine), [P]/[D] = 100

	(a) Random coil, pH 7.4		(b) β-Form, pH 4.5				
	λ / nm	ε_D / dm^3 mol^{-1} cm^{-1}	λ / nm	ε_D / dm^3 mol^{-1} cm^{-1}	λ / nm	$[\theta_D]$ / deg cm^2 dmol^{-1}	$[\theta_D]/\varepsilon_D$
B_y	408[sh a)]	77000	442	70000	450	6000	0.09
B_x	428	202000	475[sh a)]	48000	484	13500	0.28
Q	520	14000	524	47000	540	18000	0.38

a) sh: shoulder.

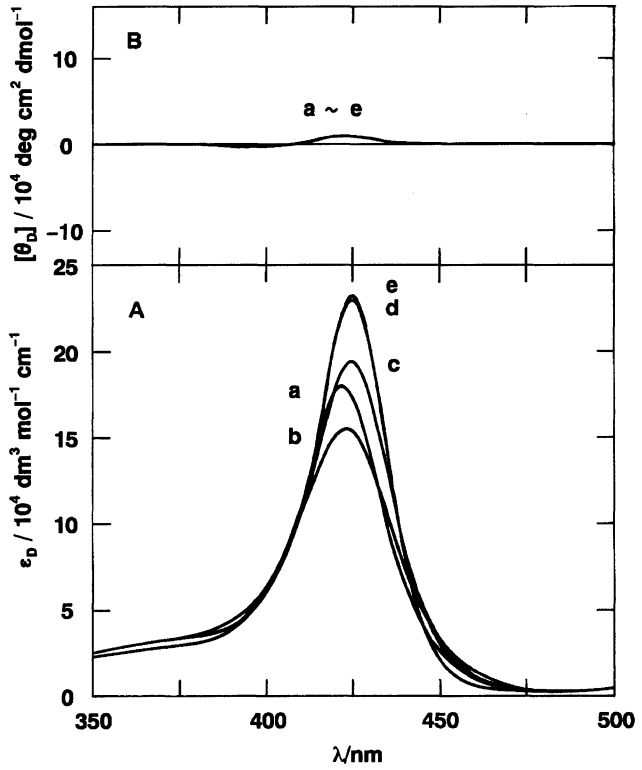

Fig. 4. Absorption spectra and CD of TMpyP in the presence of poly(L-glutamic acid) at pH 7.3, [D]=1.00×10^{-5} M. A, absorption spectra; B, CD. [P]/[D]: a, 1.0; b, 5.0; c, 10.0; d, 50.0; e, 100.

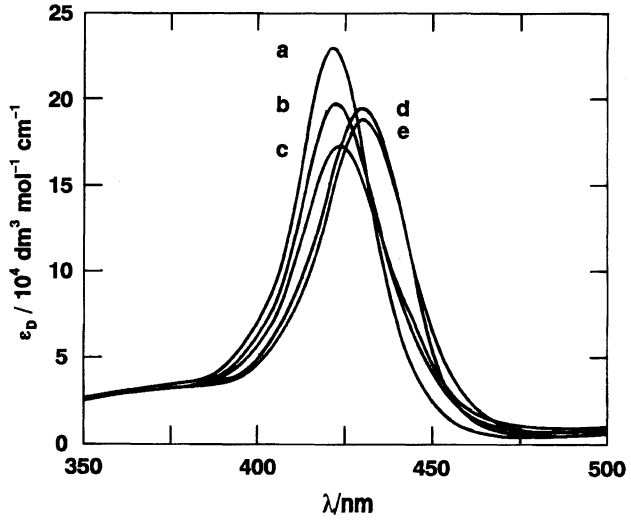

Fig. 5. Absorption spectra of TMpyP mixed with poly(L-glutamic acid) at pH 4.4, [D]=1.00×10^{-5} M. [P]/[D]: a, 1.0; b, 5.0; c, 10.0; d, 50.0; e, 100.

binding of a greater number of TMpyP ions. The CD has a feature similar to that at pH 5.3 and [P]/[D] 100. The far ultraviolet CD supports lowered helical contents of poly(L-glutamic acid) at lower [P]/[D]; $[\theta_P]$ is lowered in magnitude at 222 nm. However, the stronger increase in $[\theta_P]$ at 208 nm with lowering [P]/[D] is associated with aggregate formation of partially helical poly(L-glutamic acid), as was observed in solutions of poly(L-glutamic acid) at neutral pH in the presence of some cationic surfactants or some divalent metal ions.[15—17] That is, at [P]/[D] around 20 poly(L-glutamic acid) is in the aggregated states of interrupted helices that are caused by the cross-linking through bound TMpyP ions. At [P]/[D] 1 no CD is induced in the Soret region.

At higher [P]/[D] the conformational effects of binding of TMpyP weaken owing to the decreased number of bound TMpyP ions. The perfect helix of poly(L-glutamic acid) is stable at [P]/[D] from 50 to 100 for [D]=1.0×10^{-5} M; the dissymmetric perturbation due to α-helical poly(L-glutamic acid) becomes more dominant on the Soret transition of bound TMpyP ions, and the strong induced CD is not very much dependent on [P]/[D]. This also suggests that all TMpyP ions in solution are bound to helical poly(L-glutamic acid).

Even if [D] is kept lower, i. e., at 1.0×10^{-6} M rather

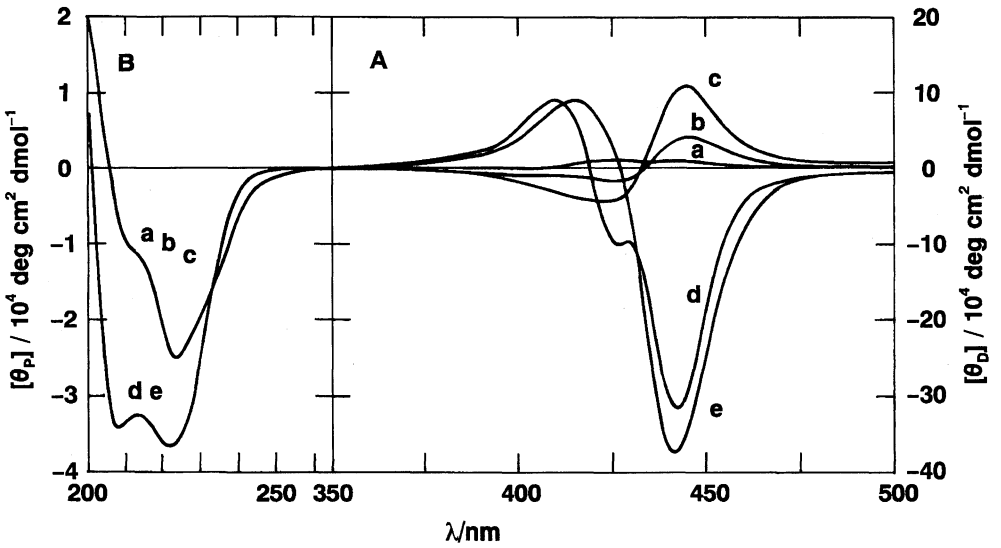

Fig. 6. CD of TMpyP mixed with poly(L-glutamic acid) at pH 4.4, [D]=1.00×10^{-5} M. [P]/[D]: a, 1.0; b, 5.0; c, 10.0; d, 50.0; e, 100. A, [θ_D]; B, [θ_P].

than at 1.0×10^{-5} M, both absorption spectra and CD change with [P]/[D] similarly, but, owing to the less number of bound TMpyP ions, the perfect helix is formed at a lower [P]/[D] ratio and is stable at [P]/[D] beyond 10 up to 1,000.

Table 3 gives the wavelength of the Soret band of TMpyP bound to poly(L-glutamic acid), in relation to the mode of binding and polypeptide conformation, as deduced below.

Spectra of TMpyP in the Presence of Poly(*S*-carboxymethyl-L-cysteine). Figure 7 shows absorption spectra and CD of TMpyP mixed with poly(*S*-carboxymethyl-L-cysteine) in solutions of [P]/[D] 100. At pH 7.4 where poly(*S*-carboxymethyl-L-cysteine) is randomly coiled, the Soret band shifts to 428 nm and is weakly hypochromic with ε_D=201000 but remains optically inactive, without having any CD. Lowering pH below 5.5, the absorption band is strongly red-shifted, broadens and becomes very weak, and the bands at longer wavelengths become stronger.

At pH 4.5 where poly(*S*-carboxymethyl-L-cysteine) is in the β-conformation, the Soret band is subject to a strong red-shift and a large intensity decrease as well as an extensive broadening; ε_D=70000 at 442 nm and ε_D=48000 at 475 nm. Nevertheless, only weak CD is observable in the Soret region as well as in the longer wavelength region; ε_D=47000 at 524 nm and [θ_D]=18000 at 510 nm.

Table 3. The Location of the Soret Band of TMpyP Bound to Poly(L-glutamic acid)

Condition	[P]/[D]	λ nm	Binding mode of TMpyP	Polypeptide conformation
Unbound	0	421		
pH 7.3	1—5	421	Monovalent	Random coil
	10—100	425	Divalent	Random coil
pH 4.4	1—10	421	Monovalent	Interrupted helix
	50—100	430	Divalent	Helix

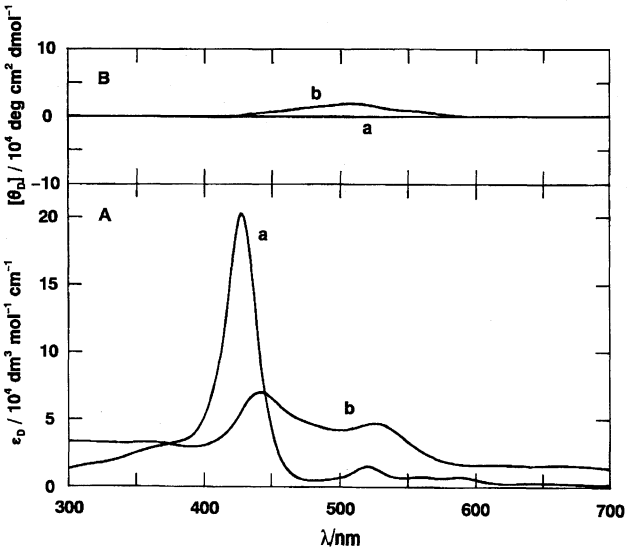

Fig. 7. Absorption spectra and CD of aqueous solutions of TMpyP in the presence of poly(*S*-carboxymethyl-L-cysteine) at [P]/[D] 100. [D]=1.00×10^{-5} M. a, pH 7.41; b, pH 4.49. A, absorption spectra; B, CD.

Tentative assignment of absorption and CD bands of TMpyP in the presence of β-form poly(*S*-carboxymethyl-L-cysteine) is also given in Table 2. The slight decrease in molar extinction coefficient at neutral pH is caused by the binding of TMpyP ions to random coil poly(*S*-carboxymethyl-L-cysteine). The red-shift and hypochromism of the absorption band on lowering pH must be caused by the conformational transition of poly(*S*-carboxymethyl-L-cysteine) from the random coil to the β-form. The two absorption bands at 442 and

475 nm could be assigned to the B_y and B_x components, respectively, but the positive CD band at 510 nm would be associated with the weakly allowed Q transition.[10,11]

Discussion

The Interaction between TMpyP and Poly(L-glutamic acid). A TMpyP ion is a tetravalent cation, and it can interact with ionized poly(L-glutamic acid) strongly. The electrostatic binding assisted by hydrophobic interaction would occur. As a result, a TMpyP ion can bind to a carboxylate group of poly(L-glutamic acid) through one of its *N*-methylpyridinium groups. Such monovalent binding of a TMpyP ion to poly(L-glutamic acid) does not only neutralize a negative charge but also conversely increases three positive charges locally. A TMpyP ion can also bind divalently to two carboxylate groups of poly(L-glutamic acid), through its neighboring or diagonal *N*-methylpyridinium groups. If a sufficient number of ionized carboxyl groups are available and two of them are separated by a distance equal to the neighboring or diagonal *N*-methylpyridinium groups, the divalent binding of TMpyP would occur.

When randomly coiled poly(L-glutamic acid) is added to TMpyP in solution, the Soret band at 421 nm largely weakens and then slightly shifts to red, i. e., to 425 nm. The weakening of the Soret band at 421 nm can be assigned to the monovalent binding of TMpyP, as a less number of carboxylate groups are available for TMpyP at lower [P]/[D]. The band at 421 nm is associated with both free (unbound) and monovalently bound TMpyP ions, so that it is weaker as [P]/[D] increases from 1 to 5. The red-shift of the Soret band can be attributed to the divalent binding of TMpyP ions, as a greater number of carboxylate groups are present at higher [P]/[D]. With a sufficient number of binding sites, i. e., at [P]/[D] 50 and 100, all TMpyP ions are bound divalently and the molar extinction coefficient remains independent of [P]/[D]. The chiral effect of a residue or two to which TMpyP ion binds should be operative for the Soret transition, but its dissymmetry factor, $[\theta_D]/\varepsilon_D$, is so low that essentially no CD is observable.

At acid pH where poly(L-glutamic acid) is α-helical, the Soret band of TMpyP further shifts to red, i. e., to 430 nm and gives strong CD if [P]/[D] is high. A TMpyP ion interacts with helical poly(L-glutamic acid) at acid pH through ionized side chain carboxyl groups. If poly(L-glutamic acid) is present to a sufficiently large amount, that is, at higher [P]/[D], TMpyP ions can bind sparsely and divalently without interacting mutually, so that the Soret transition is mainly subject to the dissymmetric effect of the α-helix of poly(L-glutamic acid). Then a single divalently bound TMpyP ion on an α-helix of poly(L-glutamic acid) will be responsible for the induction of strong CD bands. Bound TMpyP ions would be separated from one another, so that they cannot couple together electronically.

As the dissymmetry factors, $[\theta_D]/\varepsilon_D$, of the induced CD bands are not very low and their location is relatively far apart from that of the absorption bands, it might be possible that the positive and negative CD bands are both associated with the B_x components and only very weak CD is induced for the weak B_y components; in such a case two TMpyP ions must be consecutively bound on an α-helix. However, such a mode of binding would not occur so often for TMpyP, because of its low tendency for aggregation.

As the amount of poly(L-glutamic acid) is decreased, that is, with lowering [P]/[D], more TMpyP ions are bound to an α-helix, so that the monovalent binding would be more feasible than the divalent binding. The monovalent binding increases the number of positive charges on the helix locally, and it tends to disrupt the helix and also to cross-link interrupted helices of poly(L-glutamic acid).

It is known that TMpyP gives induced CD in the Soret region when polydeoxynucleotide, i. e., DNA, poly $(dG-dC)_2$ or poly $(dA-dT)_2$, is present in solution.[18] Furthermore, poly $(dG-dC)_2$ undergoes the conformational change from the Z-form to the B-form in the presence of TMpyP in 60% ethanol 1 mM sodium phosphate solutions, when the nucleotide-to-porphyrin molar ratio is decreased.[19] This phenomena must involve the initial process of unwinding or disruption of the Z-form and might be related to the helix-breaking power of TMpyP such as seen in the present system.

Interaction between TMpyP and Poly(*S*-carboxymethyl-L-cysteine). When random coil poly(*S*-carboxymethyl-L-cysteine) is mixed with TMpyP in solution, the Soret band shifts toward red but no CD is induced. When β-form poly(*S*-carboxymethyl-L-cysteine) is present in solution, the Soret band of TMpyP is subject to stronger red-shift and extensive weakening, but the band at 524 nm becomes stronger. These observations indicate the binding of TMpyP ions to β-form poly(*S*-carboxymethyl-L-cysteine). The location of absorption bands at 475 and 428 nm as well as of CD bands at 510 and 484 nm indicates that the CD is mainly induced at the Q transition of bound TMpyP ions and partly at their Soret transition. However, their dissymmetry factors, $[\theta_D]/\varepsilon_D$, are of small magnitudes.

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