Salt Effect on the Interaction of 22,24-Diprotonated 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin with a β-Sheet Structure of a Zwitterionic Polypeptide

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The effect of sodium chloride on the absorption and CD spectra of the β-sheet structure of a zwitterionic poly(Glu–Val–Lys–Val)/22,24-diprotonated 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin system in aqueous solution was studied under various conditions. The monomeric TPPS was aggregated by the addition of the polypeptide and/or NaCl; at least four kinds of TPPS aggregation were observed. Although the aggregated species with an absorption band at around 489 nm formed an *S*-chiral conformation at [P]/[D] from 1 to 5 below 100 mM NaCl, the *S*-chirality changed to *R*-chirality by increasing to 500 mM NaCl or above. These results may indicate that an increase in the ionic strength induces a chiral conformational transition due to the prevention of electrostatic interactions between TPPS and the polypeptide; also the *S*- and *R*-chiralities could be caused by the secondary structure of the polypeptide and original property of TPPS, respectively. It is presumed that the electrostatic interactions play an important role in binding TPPS with the polypeptide as a major driving force. However, even 1000 mM NaCl could not completely shield the electrostatic interactions.

Porphyrins and metalloporphyrins have been an active field of research for several decades owing to their involvement in many reactions of chemical and biological interest.[1,2] These properties are acquired primarily by binding them to specific proteins. The study of interactions between porphyrin derivatives and synthetic peptides may provide useful information about the mechanisms for sophisticated functions of porphyrin–protein complexes, and for determining the spatial arrangement and binding sites of porphyrin derivatives bound to numerous macromolecules of biological interest.

When some symmetric dye molecules interact with biopolymers, such as polypeptides, polysaccharides, and polynucleic acids adopting specific regular conformations, they exhibit induced optical activity in the region corresponding to their characteristic absorption bands of the bound dye,[3] even though the free symmetric dyes are optically inactive. Many investigators have studied the interaction of the α-helical conformation of poly(L-glutamic) with some cationic dyes or various secondary conformations of poly(L-lysine) with some anionic dyes.[4—6] Recently, the interactions of water-soluble porphyrin derivatives with water-soluble biopolymers have been extensively studied.[7—10] It was reported that an anionic porphyrin, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS), interacted with poly(L-lysine) in three conformation by an electrostatic interaction between the sulfonate groups and the ε-ammonium groups of the lysine moieties;[10] also, a cationic porphyrin, 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin (TMPyP), binded to α-helical poly(L-glutamic acid) by electrostatic interactions between the *N*-methylpyridinium groups and the γ-carboxylate groups.[9]

We investigated the interactions of poly(Gul–Val–Lys–Val) with anionic and cationic water-soluble porphyrin derivatives, because the polypeptide is a zwitterionic compound including both negatively and positively charged amino acid residues, to which may become attached to both anionic and cationic dye chromophores.[7] TPPS interacted with the β-sheet structure of zwitterionic poly(Glu–Val–Lys–Val) when TPPS existed as a diacid species, whereas interactions of TMPyP and free base TPPS with the polypeptide were not observed. The monomeric 22,24-diprotonated TPPS (TPPS diacid species) aggregated by the addition of the polypeptide, and two types of aggregated species with *S*-chiral conformation were observed. During the course of the studies of the interaction between TPPS and the polypeptide under the conditions of various pH or [P]/[D] ratios ([P] is the residue molar concentration of the polypeptide and [D] is the molar concentration of TPPS molecule), we found that positive charges in porphyrin center for the TPPS diacid species might play an important role in complex formation.

It was previously reported that poly(Glu–Val–Lys–Val) can form an unusually stable β-sheet conformation in aqueous solutions in the pH 2.0 to 12.0 region and at pH 7, even in the presence of 1 M NaCl (1 M = 1 mol dm^{-3}) or denaturation agents by an ionic self-complementary interaction between a glutamic acid and a lysine residue in addition to hydrophobic interactions between valine residues.[11]

In this paper we discuss the effect of the NaCl concentration on the interaction of the β-sheet conformation of poly(Glu–Val–Lys–Val) with the TPPS diacid species. The absorption and CD spectra were measured with aqueous so-

lutions of the TPPS diacid mixed with the polypeptide at different NaCl concentrations; also, the effect of complex formation on the spectroscopic properties was examined at different pH or various [P]/[D] ratios in high ionic-strength solutions. Poly(Glu–Val–Lys–Val) is imagined to form a β-sheet structure under the experimental conditions.

Experimental

Materials. Poly(Gul–Val–Lys–Val) was synthesized according to a method described in the previous paper; the molecular weight was determined by viscosity and size-exclusion chromatography to be approximately 14000.[11]

5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin disulfonic acid salt tetrahydrate (TPPS) was purchased from Dojindo Laboratories and used without further purification.

A stock solution of poly(Glu–Val–Lys–Val) was prepared at a residue molar concentration of 4.72×10^{-2} M, based on the total moles of the lysine residue, and dissolved in deionized distilled water. TPPS was dissolved in water to prepare a stock solution of 6.67×10^{-4} M. A TPPS–polypeptide mixture solution was prepared by adding the TPPS solution to the polypeptide solution to make the desired [P]/[D]; the pH of the solution was adjusted to the desired value by 0.1 M HCl for absorption and CD spectra measurements in the presence of sodium chloride. [P]/[D] was varied from 0 to 20, but the final concentration of the porphyrin was fixed at 6.67×10^{-5} M. pH; the sodium chloride concentration was varied from 2.0 to 4.5 and from 0 to 1000 mM, respectively. The spectra of all mixture solutions were measured within an hour after preparation.

Measurements. The absorption spectra of the solution were measured on a Hitachi U-4000 Spectrophotometer over 750—350 nm at room temperature, using a quartz cuvette of 0.1 cm pathlength. The CD spectra of the solutions were obtained in the 750 to 350 nm range at 20 °C under a constant flow of nitrogen on a Jovin Ivon CD6 spectropolarimeter equipped with an interface, and a personal computer using a quartz cuvette of 0.1 cm pathlength. The instruments were calibrated with an aqueous solution of ammonium *d*-camphorsulfate.[12] The absorption and CD spectra were expressed as the molar extinction coefficient [ε], normalized to decimeter cube units per mol centimeter and the molar ellipticity [θ], which was normalized to units of degrees centimeter squared per decimole, based on the molar concentration of the total added porphyrin. The pH was measured with a Horiba pH meter F-16 before a CD measurement.

Results

Effect of the Salt Concentration on the Absorption and CD Spectra. In an acidic aqueous solution, TPPS forms a diacid species (H_2 $TPPS^{2+}$) containing four hydrogens at the center of the molecule. pK_a of TPPS (TPPS + $2H^+ \longleftrightarrow H_2TPPS^{2+}$) is approximately 4.8.[13] Therefore, the diacid species is a zwitterionic compound having both positive charges at the central region, and negatively charged sulfonate groups below approximately pH 4.5.

In the absence of poly(Glu–Val–Lys–Val), the absorption spectrum of 6.67×10^{-5} M TPPS in an aqueous solution at pH 4.0 has bands at 434 (Soret), 593 and 644 nm (Q band), which were assigned to a monomeric TPPS diacid species. New absorption bands at 489 and 701 nm were observed in addition to those of the diacid species by the addition of the polypeptide.[7] It is presumed that these new bands can be assigned to aggregation (a dimer or an aggregate) of the diacid species.[13]

Figures 1 and 2 illustrate the absorption and CD spectra, respectively, of solutions of TPPS in the presence of poly(Glu–Val–Lys–Val) in a β-sheet structure at pH 4.0, [P]/[D] = 2, and various NaCl concentrations. From 0 to 100 mM in NaCl concentration, for the Soret region, two absorption bands were observed at around 434 and 489 nm; for the Q bands, two absorption bands exist at around 647 and 701 nm. The absorption bands assigned to a monomeric TPPS diacid species decrease in magnitude with increasing NaCl concentration, whereas those assigned to an aggregated TPPS species increase slightly in intensity with increasing NaCl concentration. Because the presence of univalent salts, such as KCl or NaCl, enhances the aggregation of TPPS free base species by suppressing any electrostatic repulsive interactions between the TPPS species, it is expected that NaCl will induce TPPS diacid species to aggregate in a similar

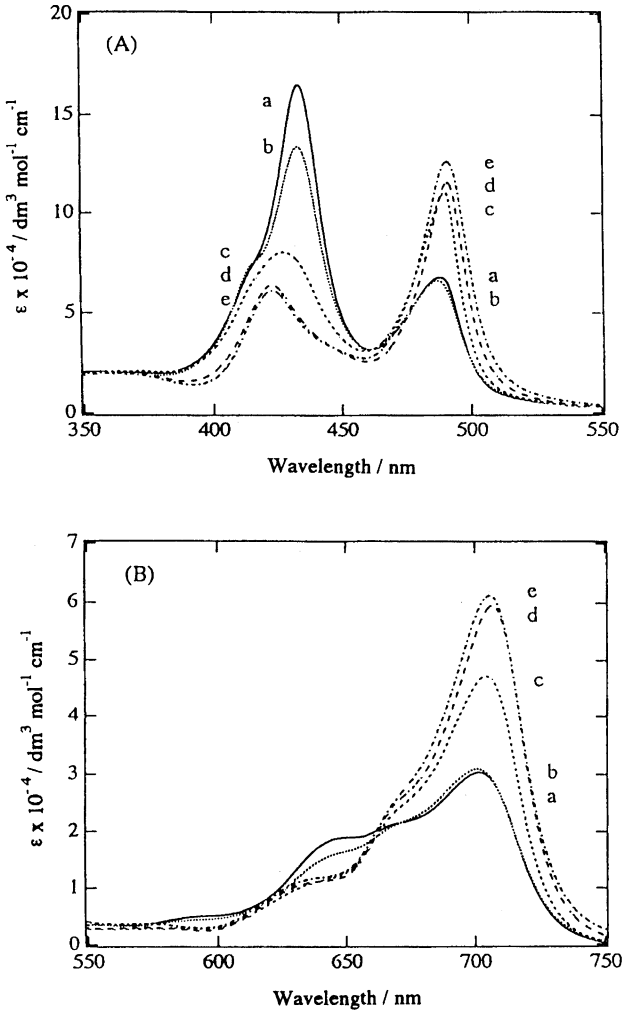

Fig. 1. Absorption spectra of 6.67×10^{-5} M TPPS in aqueous solution at pH 4.0 and [P]/[D] = 2 in the presence of poly(Gul–Val–Lys–Val) at different NaCl concentration. NaCl concentration: a, 0 mM; b, 10 mM; c, 100 mM; d, 500 mM; e, 1000 mM. (A) wavelength, 350—550 nm; (B) wavelength, 550—750 nm.

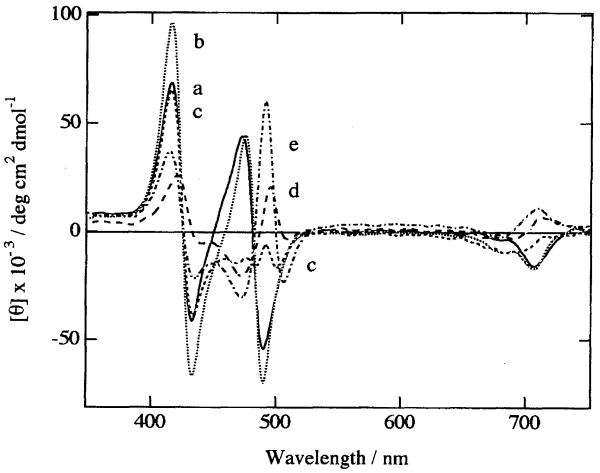

Fig. 2. CD spectra of 6.67×10^{-5} M TPPS in aqueous solution at pH 4.0 and [P]/[D] = 2 in the presence of poly(Glu–Val–Lys–Val) at different NaCl concentration. NaCl concentration: a, 0 mM; b, 100 mM; c, 250 mM; d, 500 mM; e, 1000 mM.

manner.[14]

From 500 to 1000 mM in NaCl concentration, the Soret band at 434 nm shifts to 424 nm, while the other absorption bands in wavelength are independent of the NaCl concentration. The blue-shift of the absorption band, compared to the monomer band of the TPPS diacid species, may be interpreted as the formation of a face-to-face TPPS aggregation which is distinct from aggregation with the absorption bands at 489 and 701 nm. The optical densities at 424 and 647 nm are little dependent on the NaCl concentration, whereas those at 489 and 701 nm increase slightly with the NaCl concentration. These results suggest that an addition of NaCl would slightly promote the formation of aggregated TPPS diacid species.

From 0 to 100 mM in NaCl concentration, five induced CD bands have apparently been observed; at least two dichroic bands are associated with each of TPPS absorption bands around 434 and 489 nm. Both bands consist of a pair of positive and negative dichroic bands, the positive one being at a shorter wavelength and the negative one at a longer wavelength. The induced-pair CD bands at 416 and 433 nm may be attributed to the absorption band at 434 nm, which can be assigned to a monomeric TPPS species. The splitting of the absorption band at 434 nm into two CD bands may suggest that two monomeric TPPS diacid species are bound consecutively to different sites located on the polypeptide, and are electronically coupled together. The induced pair CD bands around 472 and 490 nm are assigned to an aggregation of the TPPS diacid species. The splitting of the absorption band around 489 nm would be due to the exciton coupling of a dimeric or higher aggregates of the TPPS diacid species. The induced negative CD band at 706 nm is associated with the absorption band around 701 nm, which may be assigned to the same aggregated TPPS diacid species as that with absorption band at 489 nm.

At 250 mM of NaCl, the induced CD bands at 416 and 434 nm are obviously observed, whereas those around 476 and 490 nm become complicated and equivocal, and that around 706 nm is broadened. This may suggest that aggregated TPPS diacid species bind more weakly to the polypeptide due to a suppression of the electrostatic interactions by increasing the ionic strength.

From 500 to 1000 mM in NaCl concentration, the induced-pair CD bands associated with the absorption band at around 489 nm consist of a negative one at a shorter wavelength and a positive one at a longer wavelength, and that associated with the absorption band around 706 nm is positive in sign. These CD bands are opposite in sign compared with those from 0 to 100 mM in NaCl concentration. The aggregation is an *S*-chiral conformation from 0 to 100 mM in NaCl concentration, while from 500 to 1000 mM it has *R*-chirality, according to the exciton chirality method.[16,17] These results indicate that the aggregated TPPS species with absorption bands at 489 and 701 nm shows a salt-induced chiral-conformational transition at around 250 mM in NaCl concentration. In addition, the aggregated TPPS could interact with the polypeptide, even at 1000 mM NaCl, by electrostatic interactions between the sulfonate groups and the ε-ammonium groups of the lysine moieties as well as the positively charged TPPS center and γ-carboxylic groups, even though the electrostatic interactions decrease in magnitude with increasing ionic strength.

The ellipticity at 217 nm for the polypeptide is almost independent of the NaCl concentration, and does not have opposite sign upon the addition of NaCl (data not shown); it is thus likely that the absorption and CD bands of TPPS/(the polypeptide system) are not affected by the polypeptide conformation. These results therefore suggest that the chiral-conformational transition upon the addition of NaCl is not caused by a conformational change of the polypeptide.

The pair CD bands around 416 and 434 nm are obviously induced from 500 to 1000 mM in NaCl concentration; since the positive one is at a shorter wavelength, and the negative one is at a longer wavelength, this aggregation adopts a *S*-chiral conformation. Two kinds of aggregated TPPS species are observed above 500 mM NaCl; that with an absorption band at 424 nm has *S*-chirality, and the other with the band at 489 nm has an *R*-chiral conformation.

Figure 3 shows the dependence of the dissymmetric factor ($[\theta]/\varepsilon$) values of the aggregated TPPS species associated with the absorption bands at 489 and 701 nm at [P]/[D]=2 on the NaCl concentration. This aggregation was observed from 0 to 1000 mM in NaCl concentration. The $[\theta]/\varepsilon$ value at 472 nm decreases with the NaCl concentration, whereas the value at 490 and 706 nm increase with the NaCl concentration. These results may indicate that the chiral conformation of the aggregate gradually changes from the *S*-type to the *R*-type with increasing NaCl concentration, because the interaction between the polypeptide and the aggregate becomes weaker with increasing NaCl concentration.

Figure 4 shows the dependence of the $[\theta]/\varepsilon$ values of the aggregated TPPS species associated with the absorption band at 424 nm at [P]/[D] = 2 on the NaCl concentration.

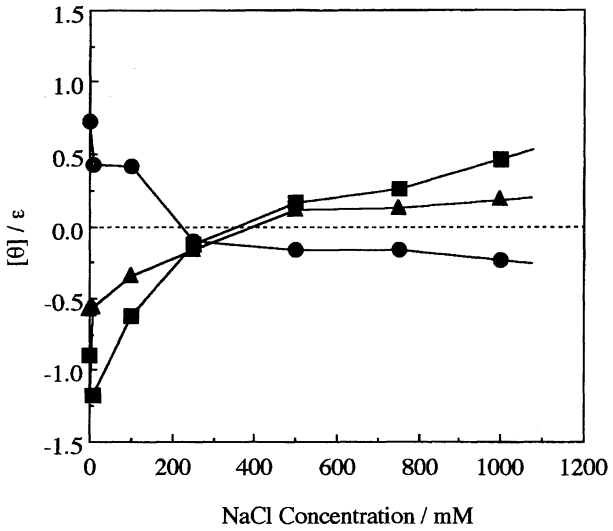

Fig. 3. Dependence of $[\theta]/\varepsilon$ of aggregated TPPS associated with the absorption bands at 489 and 701 nm mixed with poly(Glu–Val–Lys–Val) on NaCl concentration at pH 4.0 and [P]/[D] = 2. (●) 472 nm; (■) 490 nm; (▲) 706 nm.

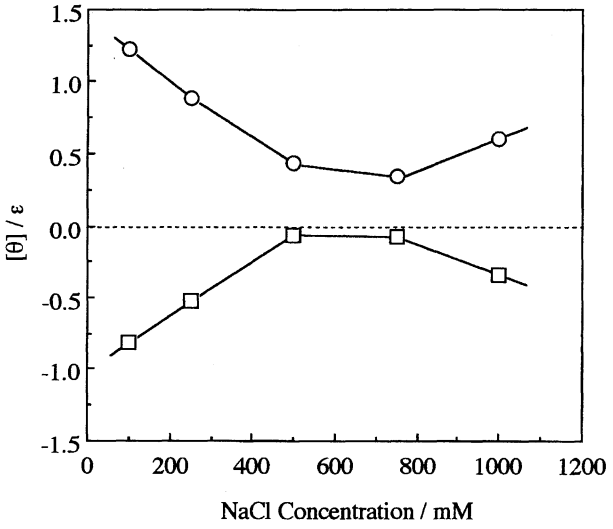

Fig. 4. Dedoes not pendence of $[\theta]/\varepsilon$ of aggregated TPPS associated with the absorption bands at 424 nm mixed poly-(Glu–Cal–Lys–Val) on NaCl concentration at pH 4.0 and [P]/[D] = 2. (○) 416 nm; (□) 433 nm.

This aggregated TPPS species was observed above 100 mM of the NaCl concentration. The absolute values of both $[\theta]/\varepsilon$ decrease with the NaCl concentration from 100 to 750 mM, while they increase with the concentration from 750 to 1000 mM. This decrease may be responsible for decreasing the interaction between the polypeptide and the aggregate with increasing ionic strength, while the increase may be due to an increase in the interaction between the TPPS molecules by suppressing the electrostatic repulsion, although the interaction between the polypeptide and the aggregate decreases with the ionic strength.[14]

Effect of the [P]/[D] Ratio on the Absorption and CD Spectra at a High Salt Concentration. Figures 5 and 6 illustrate the absorption and CD spectra, respectively, of

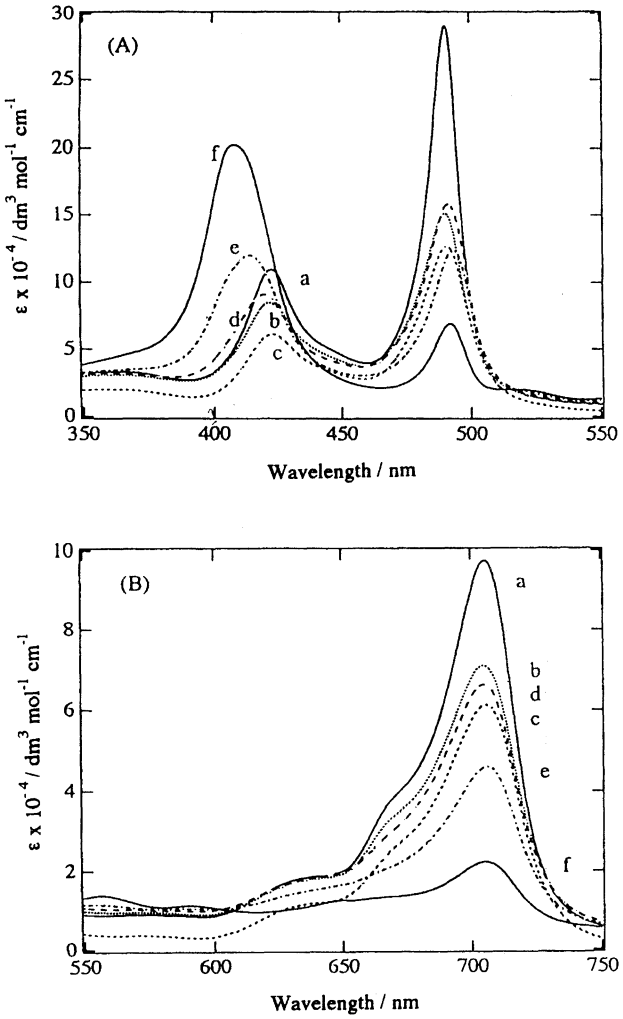

Fig. 5. Absorption spectra of 6.67×10^{-5} M TPPS in aqueous solution at pH 4.0 in the presence of poly(Glu–Val–Lys–Val) and 1000 mM NaCl at different [P]/[D] ratios. [P]/[D] ratio: a, 0; b, 1.0; c, 2.0; d, 5.0; e, 10.0; f, 20.0. (A) wavelength, 350–550 nm; (B) wavelength, 550— 750 nm.

solutions of TPPS mixed with a β-sheet structure of poly-(Glu–Val–Lys–Val) at pH 4.0, 1000 mM NaCl, and various [P]/[D] ratios. In the presence of the polypeptide at [P]/[D] from 1 to 20, the absorption bands around 489 and 701 nm undergo hypochromism with increasing [P]/[D] ratio. The absorption band around 489 nm splits into three complicated CD bands, two negative bands around 470 and 510 nm together with a positive band around 490 nm. An induced positive CD band around 706 nm, associated with the absorption band at 701 nm, was observed. This result may suggest that the aggregation forms a *R*-chiral conformation regardless of the [P]/[D] ratio.

The absorption band around 424 nm also decreases in magnitude as the [P]/[D] ratio increases to 2; above 5 the band shifts to 410 nm and undergoes hyperchromism. The CD spectra show a typical Davydov splitting around the absorption bands from 424 to 410 nm. These induced bands comprise a positive band at a shorter wavelength and a negative band at a longer wavelength. Therefore, the absorption

band at around 410 nm may be assignable to different aggregated TPPS species in *S*-chiral conformation from that corresponding to the band around 424 nm.

Effect of pH on the Absorption and CD Spectra at a High Salt Concentration. Figures 7 and 8 illustrate the absorption and CD spectra, respectively, of solutions of TPPS diacid species mixed with a β-sheet structure of poly(Glu–Val–Lys–Val) at [P]/[D] = 2, 1000 mM NaCl, and various pH. From pH 2.0 to 4.5, all absorption of the spectra have four absorption bands at almost the same wavelength, and no simple relationship between the extinction coefficient and the pH is observed. The induced CD bands are observed at the same wavelength from pH 2.0 to 4.5, and those around 490 and 706 nm increase in magnitude with the pH. A correlation between the pH and the magnitude does not exist for the other bands.

Figure 9 shows the dependence of $[\theta]/\varepsilon$ on the pH for induced CD bands around 472, 490, and 706 nm, which are assigned to the aggregated TPPS species. Two $[\theta]/\varepsilon$ values at 490 and 706 nm increase with increasing pH, whereas that at 472 nm decreases with increasing pH in the pH range of 3.5—4.5. In the absence of NaCl, the absolute values of all $[\theta]/\varepsilon$ decrease with pH, because the TPPS diacid species binds to the polypeptide more effectively as the pH decreases.[7] Because the induced CD bands at the three wavelengths reverse in sign upon an addition of NaCl, an increase in the absolute $[\theta]/\varepsilon$ values associated with increasing pH may indicate that the TPPS species interacts less strongly with the polypeptide as the pH increases. Therefore, the pH dependence of the interaction between TPPS and the polypeptide in the presence of NaCl is similar to that in its absence. On the other hand, for the induced CD bands around 416 and 434 nm, no simple relationship between $[\theta]/\varepsilon$ and pH is observed.

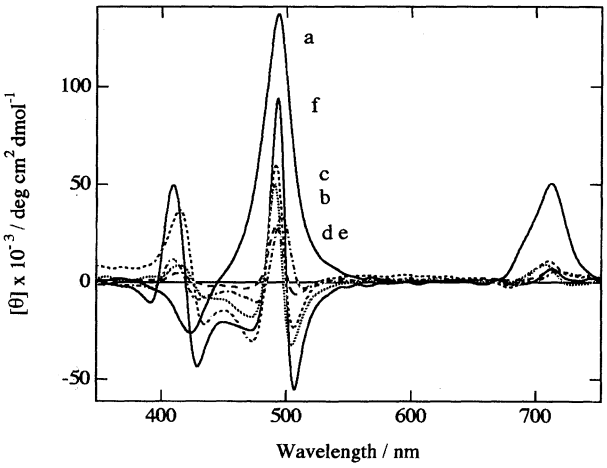

Fig. 6. CD spectra of 6.67×10^{-5} M TPPS in aqueous solution at pH 4.0 in the presence of poly(Glu–Val–Lys–Val) and 1000 mM NaCl at different [P]/[D] ratios. [P]/[D] ratio : a, 0; b, 1.0; c, 2.0; d, 5.0; e, 10.0; f, 20.0.

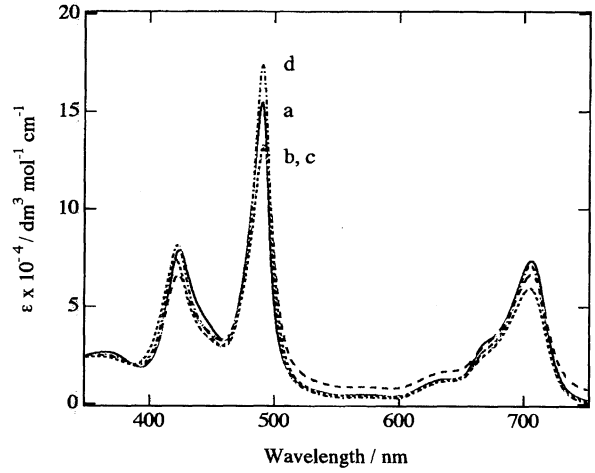

Fig. 7. Absorption spectra of 6.67×10^{-5} M TPPS in aqueous solution in the presence of poly(Glu–Val–Lys–Val) and 1000 mM NaCl at different pH. [P]/[D] = 2 : a, pH 2.0; b, pH 3.5; c, pH 4.0; d, pH 4.5.

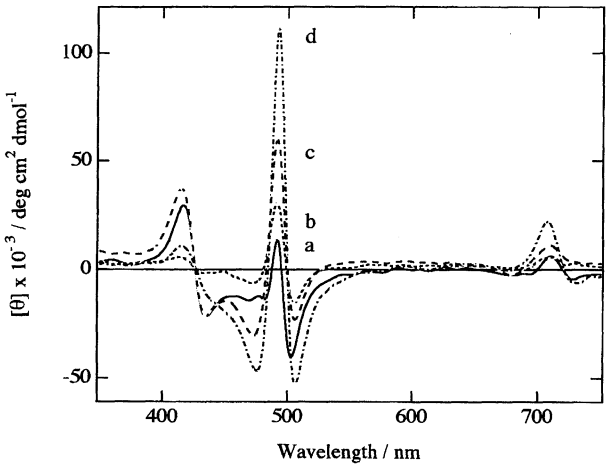

Fig. 8. CD spectra of 6.67×10^{-5} M TPPS in aqueous solution in the pressence of poly(Glu–Val–Lys–Val) and 1000 mM NaCl at different pH. [P]/[D] = 2 : a, pH 2.0; b, pH 3.5; c, pH 4.0; d, pH 4.5.

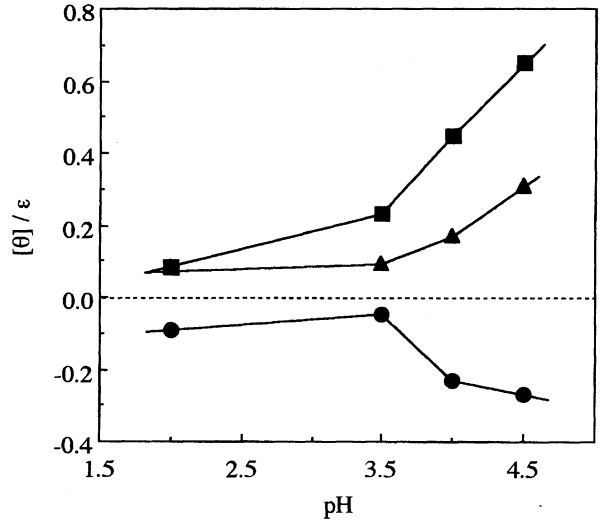

Fig. 9. Dependence of $[\theta]/\varepsilon$ of aggregated TPPS associated with the absorption bands at 489 and 701 nm mixed with poly(Glu–Val–Lys–Val) on pH at [P]/[D] = 2 and 1000 mM NaCl. (●) 472 nm; (■) 490 nm; (▲) 706 nm.

Discussion

A free-base species of TPPS cannot bind to the β-sheet structure of zwitterionic poly(Glu–Val–Lys–Val), while its diacid species can interact electrostatically with the polypeptide because the diacid one has a positive charge at the porphyrin center in addition to anionic sulfonate groups.[7] The monomeric TPPS diacid species could form at least four types of aggregations due to the addition of the polypeptide and/or NaCl, as shown in Fig. 10 and Table 1. In the absence of NaCl, TPPS forms aggregations (I) and (II) at [P]/[D] below 5 and above 10, respectively; however, some amounts of monomeric TPPS remain, even in the presence of the polypeptide. Both aggregations are present in *S*-chiral conformations, although they have absorption bands at different wavelengths.[7]

After adding the polypeptide, the monomeric TPPS forms aggregation (IV), which has an absorption band at 424 nm, existing as an *S*-chiral conformation in the presence of NaCl. Aggregation (I) changes to Aggregation (III) upon the addition of NaCl. Aggregation (III) has absorption bands at the same wavelengths as those of Aggregation (I), but they exist as different chiral conformations than each other. However, Aggregation (II) remains unchanged, even upon adding 1000 mM NaCl (Fig. 5). Therefore, interaction between TPPS and the polypeptide exists even at 1000 mM of NaCl, suggesting that this ionic strength cannot completely shield the electrostatic interactions.

On the other hand, the monomeric diacid TPPS could aggregate through suppressing the electrostatic repulsion between them by the addition of 1000 mM NaCl in the absence of the polypeptide (Fig. 6). These aggregates are optically active; one having an absorption band around 424 nm may adopt the *S*-chiral conformation, while the other, possessing an absorption band around 489 nm, may have the *R*-chirality, even though monomeric TPPS is a symmetric compound. The former aggregation may be identical with Aggregation (IV), whereas the latter may be the same structure as that of Aggregation (III). It is likely that the *R*-chiral conformation for Aggregation (III) is due to the original property of the TPPS, while *S*-chiral conformation for Aggregation (I) is derived from the secondary structure of the polypeptide.

The *S*-chiral conformation for Aggregation (I) gradually changes to the *R*-chiral conformation for Aggregation (III) as the NaCl concentration increases from Fig. 2. These results may indicate that NaCl weakens the electrostatic interactions between the polypeptide and TPPS, and that the bound TPPS gradually dissociates from the polypeptide. It is presumed that the electrostatic interactions play an important role in binding TPPS with the polypeptide as the major driving force.

Figure 11 shows the proposed mechanism for the aggregation formation of the TPPS diacid species. Aggregations (I) and (III) could be a face-to-face aggregated structure (a dimer or a higher aggregate) with close π–π interactions between two porphyrins by the rotation of the phenyl groups of TPPS.[13] Aggregations (I) and (III), formed on the polypeptide surface, can be envisioned as the following two types: One is an aggregation sited parallel to the polypeptide surface; the other is an aggregation sited perpendicular to the polypeptide surface. However, it is not obvious which aggregated structure would be more plausible. Aggregation (III) would be formed by a weaker electrostatic interaction between the aggregation and the polypeptide as well as a suppression of the electrostatic repulsion between the porphyrins upon addition of NaCl. Aggregation (II) would be another type of aggregation with a face-to-face structure; it is likely that the difference between Aggregations (I) or (III) and (II) is due to different aggregation numbers, or different shapes with the same aggregation number. Although aggregation (IV) would be a face-to-face aggregated structure (a dimer or a higher aggregate) by suppressing an electrostatic repulsion with NaCl, it is presumed that this aggregation cannot interact with the polypeptide, which is distinct from the other three aggregations. All aggregation would be face-to-face structures, though the exact structures, the aggregation numbers, and the modes to interact with the polypeptide are

Table 1. Absorption Band and Chiral Conformation for Four Types of Aggregation

	Aggregation [a]			
	(I)	(II)	(III)	(IV)
Absorption band / nm	489 and 701	406	489 and 701	424
Chiral conformation	*S*-chirality	*S*-chirality	*R*-chirality	*S*-chirality

a) From Fig. 10.

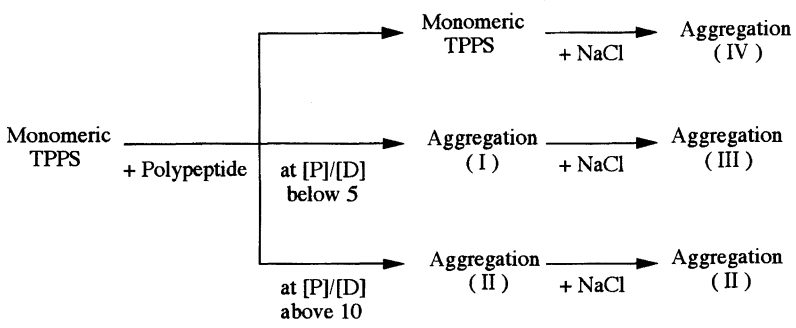

Fig. 10. Schematic representation of TPPS aggregation formaton.

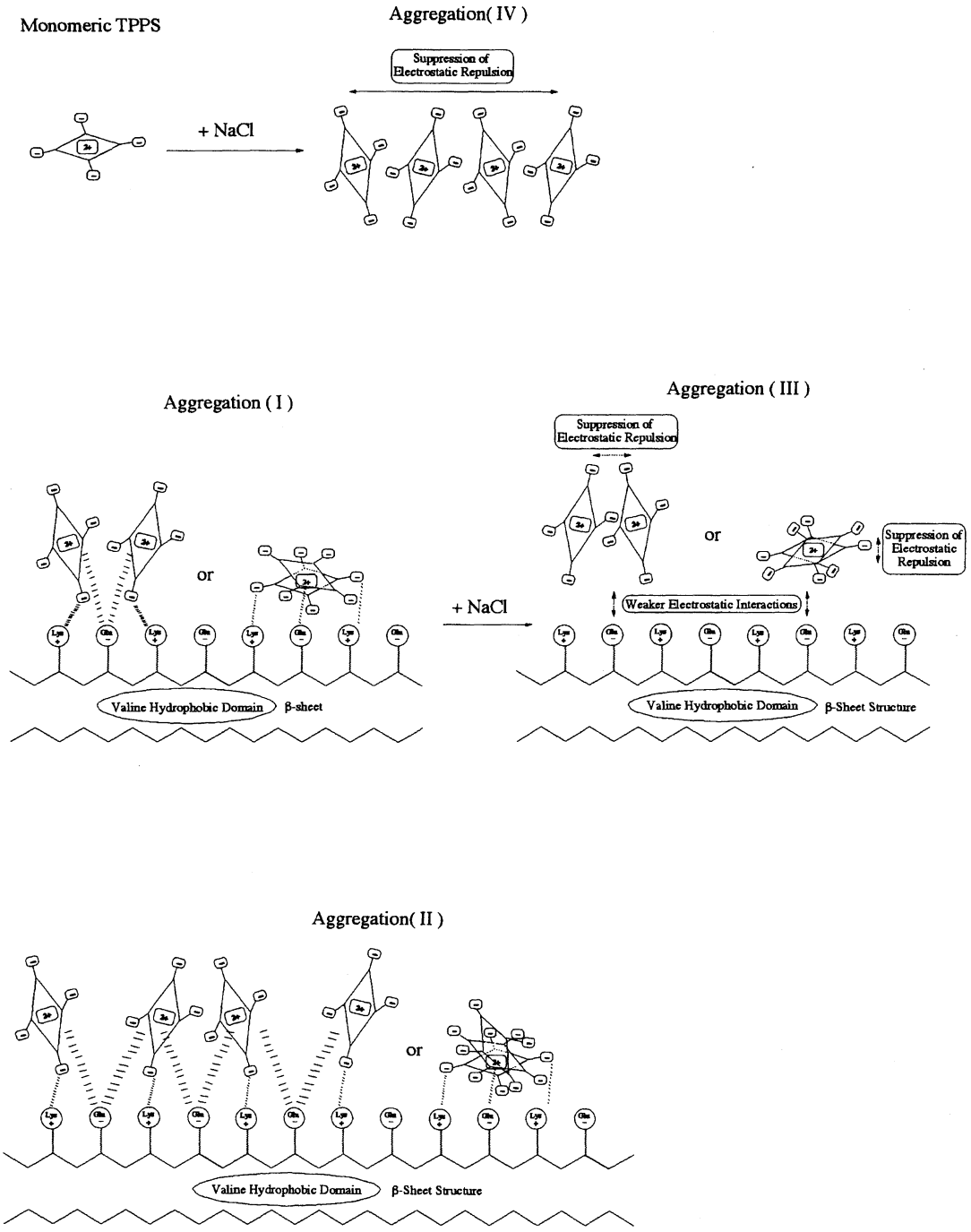

Fig. 11. Proposed mechanism for aggregation formation of TPPS diacid species by addition of poly(Glu–Val–Lys–Val) and NaCl.

not apparent.

The results of this paper indicate that zwitterionic poly-(Glu–Val–Lys–Val) interacts with a zwitterionic porphyrin derivative by predominantly electrostatic interactions. This peptides/TPPS system could be a useful model for investigating the electrostatic interactions between biological macromolecules and charged porphyrin derivatives.

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