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Fluoroscent properties of 22,24-diprotonated 5,10,15,20-tetrakis (4-sulfonatophenyl)porphyrin interacting with a β-sheet structure of a zwitterionic polypeptide

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Summary

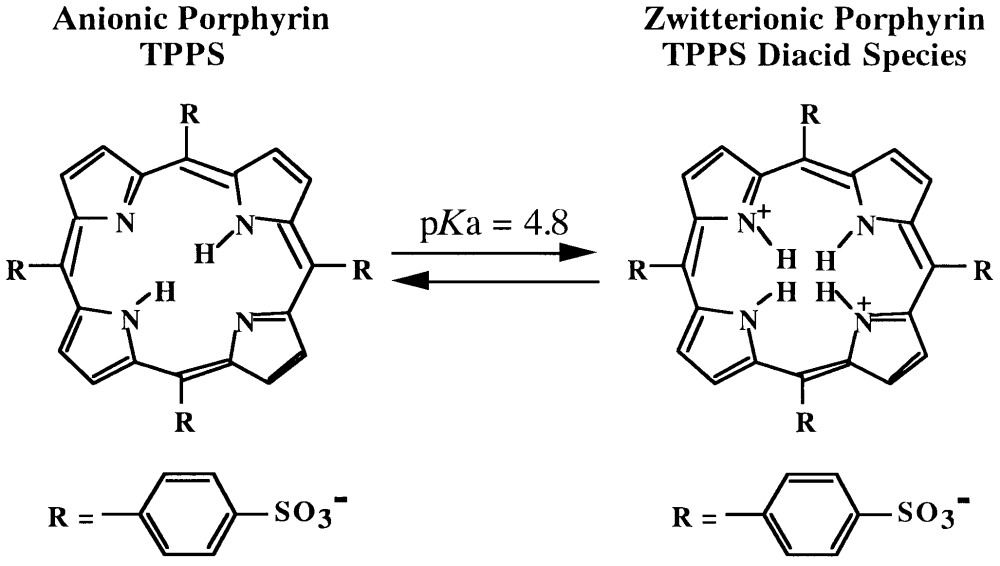
The fluorescent properties of 22,24-Diprotonated 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin interacting with the β-sheet structure of a zwitterionic Poly(Glu-Val-Lys-Val) were investigated under various conditions. The TPPS diacid species formed four types of aggregated species by the addition of the polypeptide and/or NaCl. The fluorescent bands were observed for the monomeric and their three aggregated species. The fluorescent intensity changes and maximum wavelength shifts indicated that the monomeric and their aggregated species bound to the polypeptide by electrostatic interaction. The poly(Glu-Val-Lys-Val)/TPPS diacid species system may be provided a good model for studying the porphyrin assembly binding to specific protein domains with definite orientations and alignments.

Introduction

The study of porphyrins and metalloporphyrins has been an active field of research because of their involvement in many reactions of chemical and biological interest [1, 2]. The porphyrins are assembled by binding to specific protein domains with definite orientations and alignments, so that the sophisticated properties such as the photoinitiated electron transfers and catalytic chemical reactions can be acquired. The study of interactions between porphyrin derivatives and synthetic peptides is an approach to the determination of spatial arrangement and binding sites of the porphyrin derivatives bound to numerous macromolecules of biological interest [3-7].

The aggregation of water-soluble porphyrins in aqueous solutions is well-known by adding salts or surfactants [8-10]. We investigated the interactions of poly(Glu-Val-Lys-Val) with a water-soluble porphyrin derivative, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS). TPPS interacted with the β-sheet structure of zwitterionic poly(Glu-Val-Lys-Val) when TPPS existed as 22,24-diprotonated TPPS (TPPS diacid species), whereas an interaction of free base TPPS with the polypeptide was not observed [3, 7]. The monomeric TPPS diacid species aggregated to four types of aggregated species by the interaction with the polypeptide under various [P]/[D] ratios ([P] is the residue molar concentration of the polypeptide and [D] is the molar concentration of TPPS molecule), NaCl concentration, or pH [7].

In an acidic aqueous solution, TPPS forms a diacid species containing four hydrogens at the center of the molecule because p*K*a of TPPS is approximately 4.8 as shown in scheme 1 [12]. 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. Therefore, we found that the positive charges might play a key role in the complex formation [3].



Scheme 1 Equilibrium between anionic and zwitterionic porphyrins

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 1M 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 the present study, we characterized fluorescent properties of the TPPS diacid species bound to poly(Glu-Val-Lys-Val) since it has been not known the properties of the aggregated TPPS diacid species. The fluorescent spectra of the aqueous solutions of porphyrin-polypeptide complexes were measured at different pH and different [P]/[D] ratios, and different NaCl concentrations. Poly(Glu-Val-Lys-Val) is expected to adopt a β-sheet conformation under all experimental conditions.

Experimental

Materials.

Poly(Glu-Val-Lys-Val) was synthesized according to a method described in the previous paper; the molecular weight was determined by size-exclusion chromatography to be approximately 14000 with 2.69 of molecular weight distribution [11]. 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 x 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 x 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 0.1M HCl for absorption and CD (circular dichroism) 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 x 10^{-5} M. pH and the sodium chloride concentration was varied from 2.0 to 4.0 and from 0 to 1000 mM, respectively. The spectra of all mixture solution were measured within an hour after preparation.

Measurements

Fluorescent spectra of the solutions were measured on a Jasco FP-777 spectrofluorometer. The measurements were carried out at 20 °C under aerobic conditions unless otherwise noted. The spectroscopic measurements were done 30 min after each sample was prepared. pH was measured with a Horiba pH meter F-16 before fluorescence measurement.

Results and Discussion

Complex formation of the TPPS diacid species with poly(Glu-Val-Lys-Val) was studied by absorption and CD spectroscopies [3, 7]. The monomeric TPPS diacid species could form at least four types of aggregates by the addition of the polypeptide and/or NaCl, as shown in Figure 1 and Table 1 [7]. Figure 2 shows the absorption spectra of the monomeric diacid TPPS and Aggregates (I)-(IV). In the absence of poly(Glu-Val-Lys-Val) and NaCl, the absorption spectrum of 6.67 x 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. TPPS diacid species forms Aggregates (I) and (II) at [P]/[D] below 5 and above 10 by the addition of the polypeptide, respectively. However, some amounts of the monomeric TPPS diacid species remain, even in the presence of the polypeptide. Binding of a diacid monomer and Aggregate (I) to the polypeptide is more effective with decreasing pH because the positive charge in porphyrin center increases and negatively charged glutamic acid residues by protonation of the γ-carboxylic groups decreases with decreasing pH. This would be caused by an increase in electrostatic attraction between the positive charge and glutamic acid residues as well as by a decrease in electrostatic repulsion between the sulfonate groups of TPPS molecules and glutamic acid residues. The amount of Aggregate (I) increases with decreasing pH because electrostatic repulsion between the sulfonate groups decreases by the positive charges.

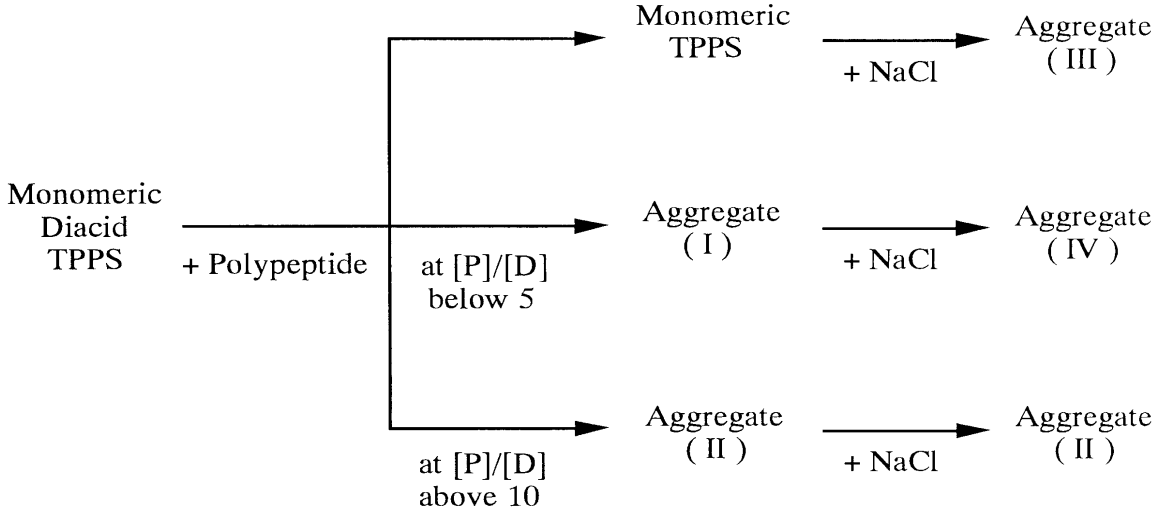


Figure 1 Schematic representation of TPPS aggregate formation

After adding the polypeptide, the monomeric TPPS diacid species forms Aggregate (III), which has an absorption band at 424 nm in the presence of NaCl. Aggregate (I) changes to Aggregate (IV) upon addition of NaCl. However, Aggregate

(II) remains unchanged even at 1000 mM of added NaCl. Aggregate (I) gradually changes to Aggregate (IV) as the NaCl concentration increases.

It is likely that Aggregates (I) and (IV) result from J-aggregates since absorption spectra for Aggregates (I) and (IV) from Figure 2 are consistent with those of J-aggregates previously described [13, 14]. On the other hand, information about the type of aggregation for Aggregates (II) and (III) were not obtained.

Table 1 Absorption and induced CD bands for monomeric diacid TPPS and four types of aggregates

	Monomeric Diacid TPPS	Aggregate [a]			
		(I)	(II)	(III)	(IV)
Absorption Band	434 nm 593 nm 644 nm	489 nm 701 nm	406 nm	424 nm	489 nm 701 nm
Induce CD Band (sign)		472 nm (+) 489 nm (–) 705 nm (–)	406 nm (+) 425 nm (–)	416 nm (+) 434 nm (–)	472 nm (–) 489 nm (+) 705 nm (+)

a) From Figure 1

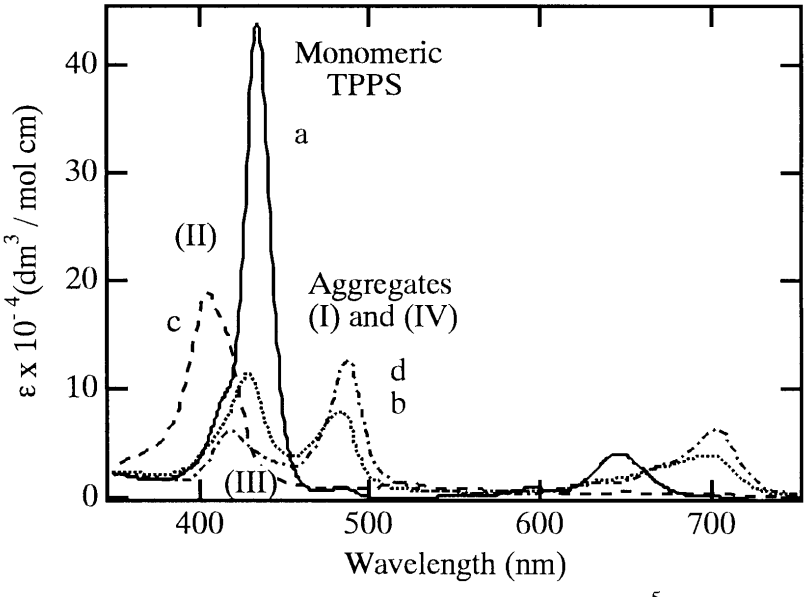


Figure 2 Absorption spectra of 6.67×10^{-5} M TPPS in aqueous solution at pH 4.0 in the absence or presence of poly(Glu-Val-Lys-Val) and NaCl.
a, in the absence of poly(Glu-Val-Lys-Val); b, [P]/[D]=2.0; c, [P]/[D]=20; d, [P]/[D]=2.0 and 1000 mM NaCl.

Figure 3 illustrates the fluorescence spectra of solutions of TPPS in the presence of poly(Glu-Val-Lys-Val) in a β-sheet structure at pH 4.0 and different [P]/[D] values. In the absence of poly(Glu-Val-Lys-Val), broad fluorescent bands appeared at 456 and 665 nm which corresponded to the monomeric TPPS diacid species. The intensity of the fluorescent band at 665 nm is much higher than that of the band at 456 nm. At pH 7.0 in the absence of the polypeptide, free base TPPS has the 0-0 and 0-1 fluorescent bands centered at 645 and 700 nm, respectively [15]. The two defined bands changed the one broad band at 665 nm by changing from free base TPPS to the diacid species.

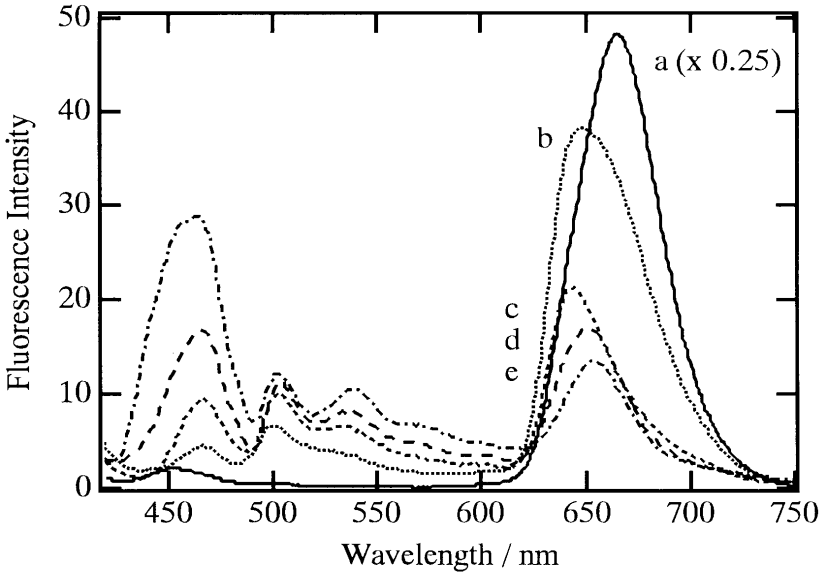


Figure 3 Fluorescence spectra of 6.67 x 10^{-5} M TPPS in aqueous solution at pH 4.0 in the presence of poly(Glu-Val-Lys-Val) at different [P]/[D] ratios with excitation at 400 nm.
[P]/[D] ratio: a, 0; b, 2.0; c, 5.0; d, 10.0; e, 20.0.

The addition of the polypeptide resulted in a disappearance of the small band at 456 nm. The band at 665 nm shifted to shorter wavelength and decreased in intensity with increasing [P]/[D] values. These results indicated that the content of the monomeric diacid species decreased with increasing [P]/[D] values due to the formation of the aggregated species as well as TPPS diacid species was fixed in the polypeptide binding site as the monomeric species by electrostatic attraction.

New bands around at 503 and 539 nm were observed and increased in intensity with a longer wavelength shift with increasing [P]/[D] values. These bands would be assignable to Aggregate (I) with the absorption band at 489 nm. The increase in fluorescent intensity would be cause by an increase in the content of Aggregate (I) up to [P]/[D] = 5, but from [P]/[D] = 10 to 20, strong binding of Aggregate (I) to the polypeptide by electrostatic interaction in spite of a decrease in the content of Aggregate (I).

In addition, another new fluorescent band at 467 nm was observed. This band would be assigned to Aggregate (II) with the absorption band at 406 nm. The band intensity increased with increasing [P]/[D] ratio due to increase in the content of Aggregate (II).

The fluorescence spectra of solutions of TPPS in the presence of poly(Glu-Val-Lys-Val) at different pH at [P]/[D] = 2 is shown in Figure 4. The fluorescent band around 650 nm decreased in intensity due to the reduce in the content of the monomeric

TPPS diacid species with decreasing pH. On the other hand, the two fluorescent bands at 501 and 533 nm at pH 4.0 changed to one broad band at 510 nm assigned to Aggregate (I) by pH change to lower pH such as 2.0 and 3.5. The intensity of the band around 510 nm much increased with decreasing pH because of an increase in the content of the aggregated (I) as well as the stronger interaction of Aggregate (I) with the polypeptide.

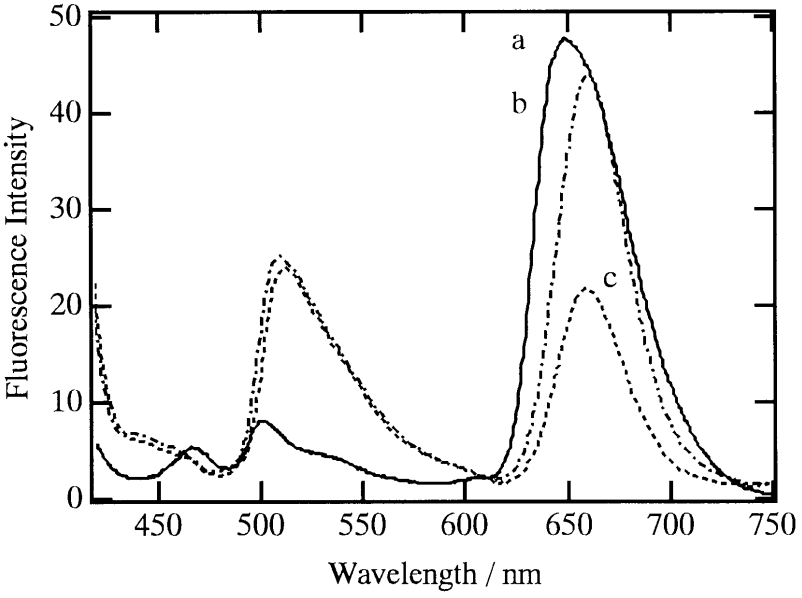


Figure 4 Fluorescence spectra of 6.67 x 10^{-5} M TPPS in aqueous solution in the presence of poly(Glu-Val-Lys-Val) at [P]/[D]= 2 at different pH with excitation at 400 nm. pH value: a, 4.0; b, 3.5; c, 2.0.

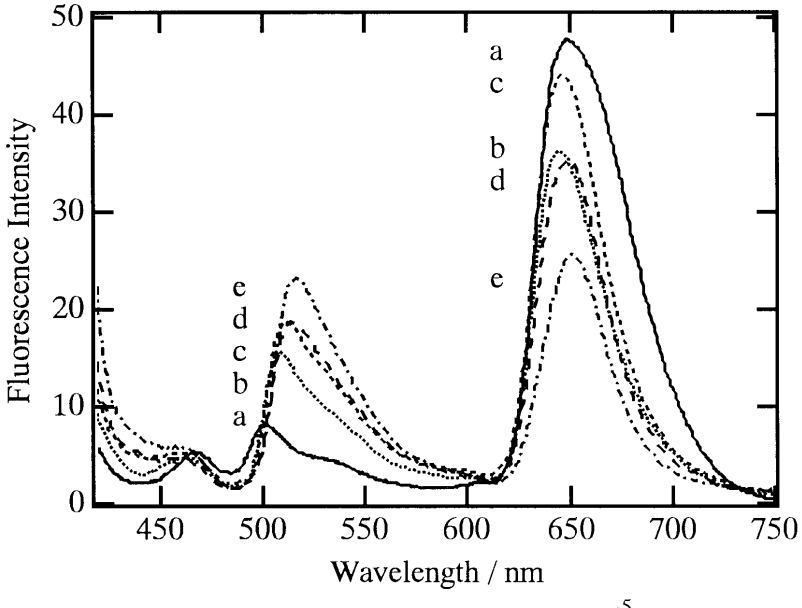


Figure 5 Fluorescence spectra of 6.67 x 10^{-5} M TPPS in aqueous solution at pH 4.0 and [P]/[D] = 2 in the presence of poly(Glu-Val-Lys-Val) at different NaCl concentration with excitation at 400 nm. NaCl concentration: a, 0 mM; b,100 mM; c, 250 mM; d, 500 mM; e, 1000 mM.

The salt effect on the fluorescence spectra of solutions of TPPS-poly(Glu-Val-Lys-Val) complex is shown in Figure 5. An decrease in the intensity of the fluorescent band around 650 nm assigned to the monomeric diacid TPPS was observed with increasing added NaCl concentration. The two fluorescent bands at 501 and 533 nm in the absence of NaCl changed to one broad band at 516 nm by an addition of 500 or 1000 mM of NaCl. The fluorescent band around 516 nm which could be assignable to Aggregate (IV) increased in intensity and a red shift of the fluorescent maxima was also observed with an increase of the added NaCl concentration. These results were caused by a decrease in the monomeric TPPS diacid and an increase in Aggregate (IV) as well as the strong interaction of Aggregate (IV) with the polypeptide.

Figure 6 illustrates the fluorescent spectra of solutions of TPPS-poly(Glu-Val-Lys-Val) mixture at pH 4.0, 1000 mM NaCl, and various [P]/[D] ratios. The fluorescent intensity around 650 nm increased with an increase in [P]/[D] ratios up to 5, while the intensity decreased with increasing [P]/[D] ratios above [P]/[D] = 10. Although the content of the monomeric species reduced with an increase in [P]/[D] ratios up to 5, the fluorescent intensity increased due to the strong binding of the monomer to the polypeptide even at the high salt condition. Above [P]/[D] = 10, a reduce in the fluorescent intensity could be caused by a steep decrease in the monomer content.

The fluorescent bands around 516 nm, would be assignable to Aggregate (IV) owing to high NaCl concentration solution, decreased in intensity with increasing [P]/[D] ratios. In addition, at [P]/[D] = 20, the fluorescent band around 467 nm, could be assigned to Aggregate (II), was observed even at the high salt condition. However, any fluorescent bands which correspond to Aggregate (III) formed by adding the polypeptide and NaCl was not distinguished with the fluorescent bands for the other species.

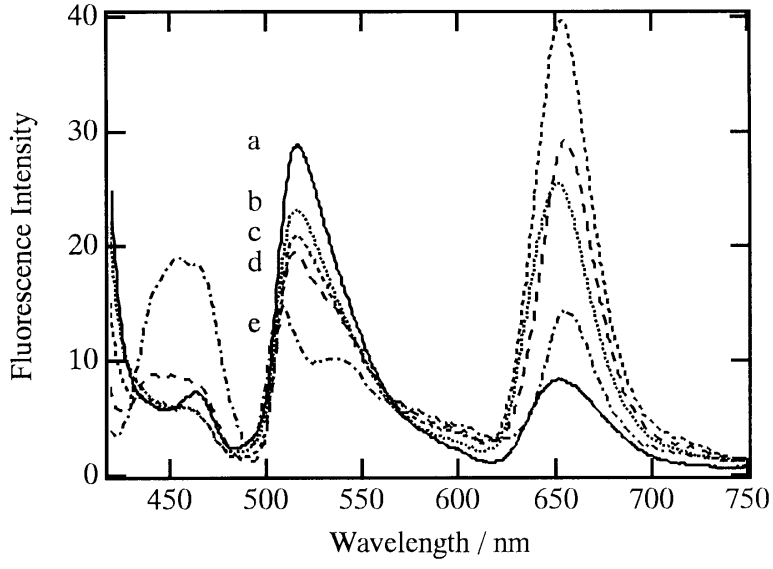


Figure 6 Fluorescence spectra of 6.67 x 10^{-5} M TPPS in aqueous solution at pH 4.0 and 1000 mM NaCl concentration in the presence of poly(Glu-Val-Lys-Val) at different [P]/[D] ratios with excitation at 400 nm.
[P]/[D] ratio: a, 0; b, 2.0; c, 5.0; d, 10.0; e, 20.0.

The monomeric TPPS diacid species could form four types of aggregated species due to the addition of poly(Glu-Val-Lys-Val) and/or NaCl. The fluorescent bands would be assignable to the monomeric and the three aggregated species except Aggregate (III)

were observed. The fluorescent properties for the monomeric diacid TPPS, Aggregates (I), (II), and (IV) were summarized in Table 2. The fluorescent behavior showed that the monomeric species, Aggregates (I), (II) and (IV) interacted with poly(Glu-Val-Lys-Val) by electrostatic interaction.

Table 2 Fluorescent bands for monomeric diacid TPPS and four types of aggregates

Monomeric Diacid TPPS	Aggregate [a]			
	(I)	(II)	(III)	(IV)
456 nm 655 nm	503 nm 539 nm	467 nm	No observation	516 nm

a) From Figure 1

Poly(Glu-Val-Lys-Val) is a zwitterionic polypeptide with a β-sheet structure, which interacts with a zwitterionic porphyrin derivative by two types of electrostatic attractive interactions such as between the positive porphyrin center and the negative glutamic acid residues as well as between the negative sulfonate groups of TPPS and the positive lysine residues. In addition, the polypeptide can induce to arrange the binding molecule dissymmetically. We suggest that poly(Glu-Val-Lys-Val)-TPPS complex may provide a generally useful model for studying the spatial arrangement and binding site of porphyrin derivatives bound to biological macromolecules by absorption, CD, and fluorescent spectra measurements.

Acknowledgments

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