Polymer Bulletin 45, 479–485 (2001)

Polymer Bulletin

Interaction of porphyrin derivatives with a β-sheet structure of a zwitterionic polypeptide in aqueous solution

Yasumasa Fukushima

Department of Applied Chemistry, Faculty of Engineering, Toyo University, 2100 Kujirai, Kawagoe, Saitama 350-8585, Japan

Received: 13 September 2000/Revised version: 27 November 2000/Accepted: 27 November 2000

Summary

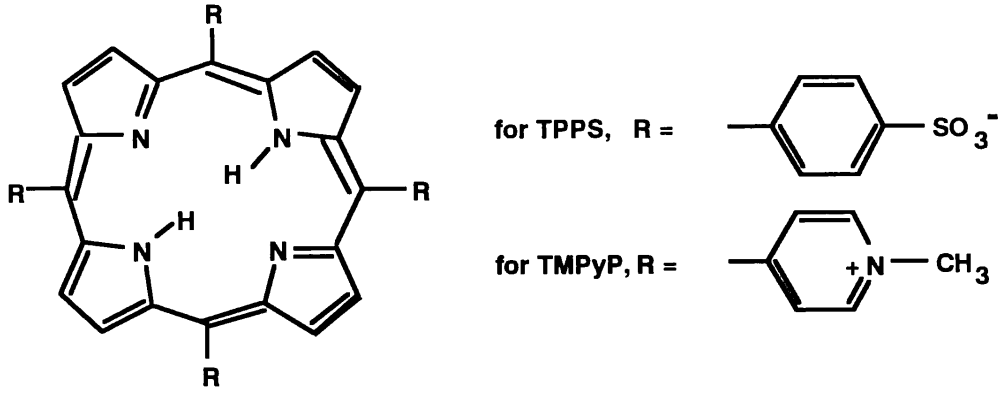
Complex formation of porphyrin derivatives with zwitterionic poly(Glu-Leu-Lys-Leu) was investigated as a protein-porphyrin complex model. The β-sheet structure of poly(Glu-Leu-Lys-Leu) interacted with a 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (TPPS) diacid form which has positive charge in the porphyrin center. On the other hand, no interaction of 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin (TMPyP) or a TPPS free form with the polypeptide was observed. The positive charge in the porphyrin center for the diacid species may play an important role in the complex formation. The TPPS diacid species was aggregated by the addition of the polypeptide and/or NaCl; at least three kinds of TPPS aggregates were observed.

Introduction

Porphyrins and metalloporphyrins have been an active field of research because of their involvement in many reactions of chemical and biological interest [1, 2]. The porphyrins bind 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 [3-5]. It was reported that a cationic porphyrin, 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin (TMPyP), binded to α-helical poly(L-glutamic acid) [4], also, an anionic porphyrin, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS), interacted with poly(L-lysine) in three conformation [5].

Poly(Glu-Leu-Lys-Leu) can form an unusually stable β-sheet conformation in aqueous solutions in the pH 2.0 to 12.0 region by ionic self-complementary interaction between glutamic acid and lysine residues in addition to hydrophobic interactions between leucine residues as reported previously [6]. The polypeptide is a zwitterionic compound including both negatively charged and positively charged amino acid residues, to which may become attached both anionic and cationic dye chromophores.

In this paper I discuss the interaction of TPPS or TMPyP with poly(Glu-Leu-Lys-Leu). The interaction between a porphyrin and a polypeptide may induce a change in absorption spectra and optical activity. Therefore, the absorption and CD spectra were measured with the aqueous solutions of the porphyrin with the polypeptide at different mixing ratios and different NaCl concentrations for investigation of complex formation.



Scheme 1 Structures of TPPS and TMPyP

Experimental

Materials

Poly(Glu-Leu-Lys-Leu) was synthesized according to the method in the previous paper; the molecular weight was determined by viscosity and size-exclusion chromatography to be approximately 14000 [6]. TPPS and TMPyP were purchased from Dojindo Laboratories and used without further purification.

A polypeptide-porphyrin mixture solution was prepared by adding porphyrin solution to polypeptide solution to make the desired polypeptide-porphyrin molar ratio, [P]/[D], where [P] is the residue molar concentration of the polypeptide and [D] is the molar concentration of TPPS or TMPyP.

Measurements

Absorption and CD spectra of the solution were measured on a Hitachi U-4000 Spectrophotometer and a Jovin Ivon CD6 spectropolarimeter, respectively. 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 total added porphyrin. pH was measured with a Horiba pH meter F-16.

Results and discussion

Interaction of TPPS with poly(Glu-Leu-Lys-Leu)

In an acidic aqueous solution, TPPS forms a diacid species (H_2TPPS^{2+}) containing four hydrogens at the center of the molecule. pK_a of TPPS ($TPPS + 2H^+ \rightleftarrows H_2TPPS^{2+}$) is approximately 4.8 [7]. Absorption spectrum of TPPS in neutral aqueous solution has bands at 416 (Soret), 517, 553, 582, and 634 nm (Q band) as well as a weak shoulder at 393 nm which are assigned to a free base species, while at pH 4.0, the absorption spectra has bands at 434 (Soret) and 644 nm (Q band) which are assigned to a monomeric TPPS diacid species [8]. The diacid species, forms in aqueous solution below pH 4.0, is a zwitterionic compound having both positive charges in the central region and negatively charged sulfonate groups.

First I discuss the interaction of the TPPS diacid species with the polypeptide. Figures 1 and 2 show the absorption and induced CD spectra, respectively, of 6.67×10^{-5} M TPPS mixed with poly(Glu-Leu-Lys-Leu) in aqueous solutions at pH 4.0 at different [P]/[D] ratios. In the presence of poly(Glu-Leu-Lys-Leu) to [P]/[D] = 5, absorption bands at 434 and 644 nm undergo hypochromism, isosbestic points are observed at 454 and 657 nm, and new bands at 488 and 702 nm appear and increase in magnitude with increasing [P]/[D] ratios. It is presumed that these new bands are assigned to an aggregate (a dimer or an aggregate) of the diacid species. This aggregated species is named Aggregate (I). It is presumed that Aggregate (I) forms the face-to-face structure with π-π interactions [7]. The induced CD bands are not observed in the absence of the polypeptide, whereas five induced CD bands appear by addition of the polypeptide. Two dichroic bands are associated with each TPPS absorption band at 434 and 488 nm. Both bands are a pair of positive and negative dichroic bands, the positive one is at a shorter wavelength and the negative one is at a longer wavelength. The induced pair CD bands at 417 and 434 nm may be attributed to the absorption band at 434 nm, is assignable to the monomeric TPPS diacid species. The splitting of the absorption band at 434 nm suggests that two monomeric TPPS diacid species bind consecutively to different sites on the polypeptide and are electronically coupled together [9, 10]. The induced CD bands at 472 and 489 nm are assigned to Aggregate (I). The splitting of the absorption band at 488 nm into two CD bands would be caused by exciton coupling of dimeric or aggregated TPPS. The induced CD band around 704 nm may be associated with the absorption band at 702 nm, which is assigned to Aggregate (I). These results indicate that positive charges in porphyrin center for the TPPS diacid species may play an important role in TPPS-poly(Glu-Leu-Lys-Leu) complex and the addition of the polypeptide promotes the aggregation of the TPPS diacid species.

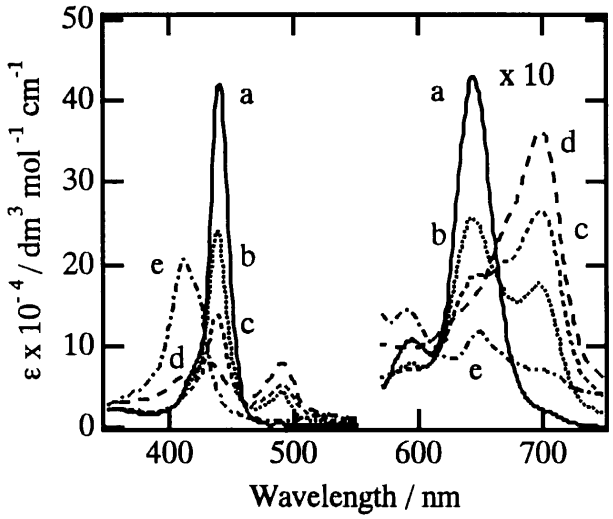


Figure 1 Absorption spectra of 6.67 x 10^{-5} M TPPS in aqueous solution at pH 4.0 in the presence of poly(Glu-Leu-Lys-Leu) at different [P]/[D] ratios. [P]/[D] ratio : a, 0; b, 1.0; c, 2.0; d, 5.0; e, 20.0.

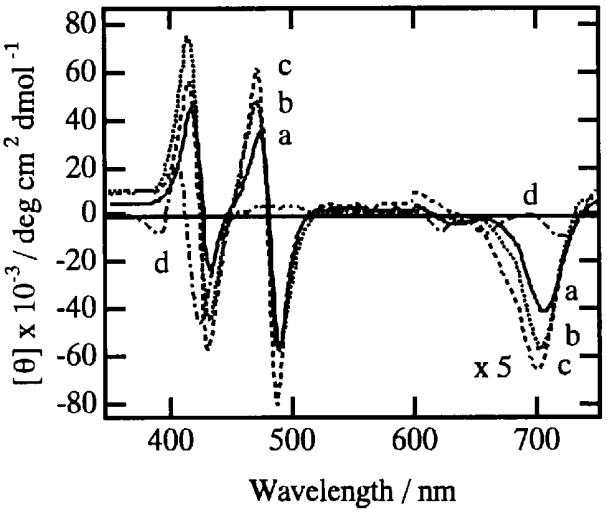


Figure 2 CD spectra of 6.67 x 10-5 M TPPS in aqueous solution at pH 4.0 in the presence of poly(Glu-Leu-Lys-Leu) at different [P]/[D] ratios. [P]/[D] ratio : a, 1.0; b, 2.0; c, 5.0; d, 20.0.

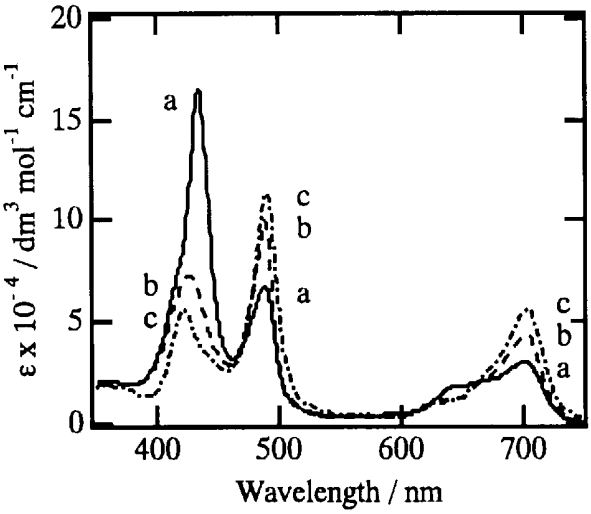


Figure 3 Absorption spectra of 6.67 x 10^{-5} M TPPS in aqueous solution at pH 4.0 and [P]/[D] = 2.0 in the presence of poly(Glu-Leu-Lys-Leu) at different NaCl concentration. NaCl concentration : a, 0 mM; b, 250 mM; c, 1000 mM.

At [P]/[D] ratios = 20, the Soret band at 434 nm shifts to 408 nm, and the bands at 488 and 701 nm disappear. Induced CD bands associated with the absorption band at 408 nm show exciton coupling and are composed of a positive band at a shorter wavelength and a negative band at a longer wavelength. However, induced CD bands around 489 and 704 nm are not observed due to the disappearance of the corresponding absorption bands. The absorption band at 408 nm is subject to blue-shift compared to the monomeric bands of TPPS free base and diacid species, and exciton coupling is

observed. Consequently, the band may be assigned to a different type of TPPS aggregated species (a dimer or an aggregate) from Aggregate (I). This aggregated species is named Aggregate (II).

At [P]/[D] ratios = 20, the Soret band at 434 nm shifts to 408 nm, and the bands at 488 and 701 nm disappear. Induced CD bands associated with the absorption band at 408 nm show exciton coupling and are composed of a positive band at a shorter wavelength and a negative band at a longer wavelength. However, induced CD bands around 489 and 704 nm are not observed due to the disappearance of the corresponding absorption bands. The absorption band at 408 nm is subject to blue-shift compared to the monomeric bands of TPPS free base and diacid species, and exciton coupling is observed. Consequently, the band may be assigned to a different type of TPPS aggregated species (a dimer or an aggregate) from Aggregate (I). This aggregated species is named Aggregate (II).

Next I discuss the effect of NaCl concentration on the interaction of the polypeptide and the TPPS diacid. The absorption bands at 434 and 644 nm decrease in magnitude and the bands at 488 and 702 nm increase in magnitude by increasing NaCl concentration at pH 4.0 and [P]/[D] = 2 as shown in Figure 3. At 250 mM of NaCl, the induced CD bands at 417 and 434 nm are clearly observed, whereas those around 472 and 489 nm become complicated and equivocal, and that around 704 nm is broadened from Figure 4. This may suggest that aggregated TPPS diacid species bind more weakly to the polypeptide due to decrease in the electrostatic interactions by increasing ionic strength [11].

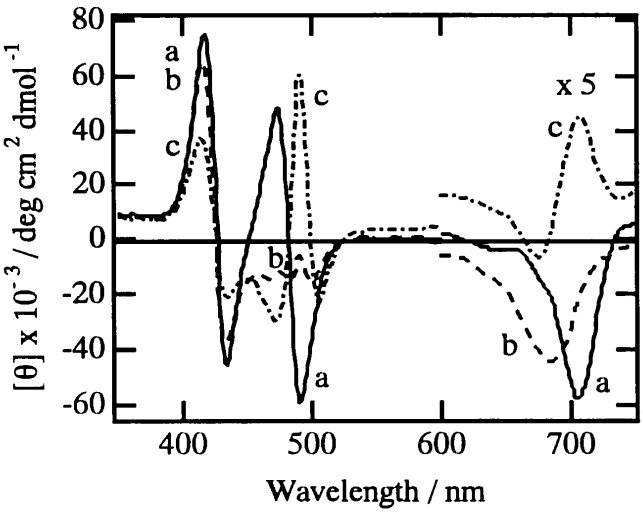


Figure 4 CD spectra of 6.67 x 10^{-5} M TPPS in aqueous solution at pH 4.0 and [P]/[D] = 2.0 in the presence of poly(Glu-Leu-Lys-Leu) at different NaCl concentration. NaCl concentration : a, 0 mM; b, 250 mM; c, 1000 mM.

At 1000 mM of NaCl, the induced-pair CD bands associated with the absorption bands at around 488 and 702 nm are opposite in sign compared with those in absence of NaCl. The new aggregated species is named Aggregate (III). Aggregate (III) has the absorption and induced CD bands at the same wavelength as those of Aggregate (I),

however, induced CD bands are opposite in sign each other. These results may indicate that Aggregate (I) change to Aggregate (III) due to variations of the binding site or mode with the polypeptide around 250 mM NaCl. The spectroscopic properties for Aggregates (I), (II), and (III) are summarized in Table 1.

Table 1 Absorption and Induced CD Bands for Monomeric Diacid TPPS and Three Types of Aggregates

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

Since the ellipticity at 217 nm for the polypeptide is almost independent of the [P]/[D] ratio and NaCl concentration, it is thus likely that the absorption and CD spectra of the TPPS/polypeptide system are not affected by the β-sheet content of the polypeptide.

On the other hand, TPPS free base species would not interact with the polypeptide from no observation of an induced CD band and no difference of absorption spectra of TPPS between in the presence of the polypeptide and in the absence (date not shown). These results indicate that sulfonate groups cannot simply electrostatically interact with ε-ammonium groups of lysine residues, and positive charges in the center of TPPS may require TPPS to bind to the polypeptide. The positive charges of a TPPS diacid species may interact with γ-carboxylic groups of glutamic acid of the polypeptide for formation of the TPPS-poly(Glu-Leu-Lys-Leu) complex.

Interaction of TMPyP with poly(Glu-Leu-Lys-Leu)

Absorption spectra of TMPyP in the absence of poly(Glu-Leu-Lys-Leu) are the same as those of in the presence of the polypeptide at various [P]/[D] ratios, NaCl concentrations and pHs (data not shown). In addition, induced CD bands are not observed under any conditions (data not shown). These results indicate that TMPyP cannot interact with poly(Glu-Leu-Lys-Leu). Because the lysine residue has a longer side chain than the glutamic acid residue, TMPyP has tendency to interact with the lysine residue rather than the glutamic acid residue. Accordingly, it is likely that TMPyP cannot interact with poly(Glu-Leu-Lys-Leu) due to electrostatic repulsion between the *N*-methylpyridinium groups and ε-ammonium groups of lysine residues in preference to electrostatic attraction between the *N*-methylpyridinium groups and the γ-carboxylic groups.

In conclusions, poly(Glu-Leu-Lys-Leu) is a β-sheet structure of a zwitterionic polypeptide, which interacted with the TPPS diacid species which has positive charge in the porphyrin center. On the other hand, no interaction of TMPyP or the TPPS free species with the polypeptide was observed. Therefore, the zwitterionic polypeptide can interact with the zwitterionic porphyrin derivative, and positive charge in the porphyrin center for the diacid species may play an important role in the complex formation. In addition, the polypeptide can induce to arrange the binding molecule dissymmetically. The TPPS diacid species was aggregated by the addition of the polypeptide and/or NaCl; at least three kinds of TPPS aggregates were observed. I suggest that the poly(Glu-Leu-Lys-Leu)-TPPS complex may provide a generally useful model for studying the spatial arrangement and binding site of porphyrin derivatives bound to biological macromolecules.

Acknowledgments

I thank Dr. M. Hirami of Unitika Ltd. for many helpful discussions and valuable comments on this work.

References

1 Muller-Eberhard U (1978) Transport by Proteins. Walter de Gruyter and Co., Berlin
2 Calvin M (1992) Acc Chem Res 11: 869
3 Ikeda S, Nezu T, Ebert G (1991) Biopolymers 31: 1257
4 Nezu T, Ikeda S (1993) Bull Chem Soc Jpn 66: 18
5 Nezu T, Ikeda S (1993) Bull Chem Soc Jpn 66: 25
6 Fukushima Y (1996) Polym J 28: 113
7 Pasternack RF, Huber PR, Boyd P, Engasser G, Gibbs E, Francesconi L, Fasella P, Venturo GC, Hinds L (1972) J Am Chem Soc 94: 4511
8 Fleischer EB, Palmer J M, Srivastava TM, Chatterjee A (1971) J Am Chem Soc 93: 3162
9 Tinoco I (1964) J Am Chem Soc 86: 297
10 Ikeda S, Yoshida T, Imae T (1981) Biopolymers 20: 2395
11 Kadish KM, Maiya GB, Araullo C, Guilard R (1989) Inorg Chem 28: 2725