Association Behavior of Protoporphyrin IX in Water and Aqueous Poly(*N*-vinylpyrrolidone) Solutions. Interaction between Protoporphyrin IX and Poly(*N*-vinylpyrrolidone)

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Synopsis. The association behavior of protoporphyrin IX was investigated at different pH by absorption spectroscopy and gel chromatography. Protoporphyrin IX existed as monomer in acidic water, as aggregate in neutral water, and as dimer in basic water. Poly(*N*-vinylpyrrolidone) tended to suppress dimerization and aggregation of protopophyrin IX.

Protoporphyrin IX is a metal-free porphyrin of the same structure as hemin. It has both the acid and base properties due to two pyrrole nitrogens capable of accepting protons and two propionic acid residues. In addition to these ionizable groups, this molecule contains a wide hydrophobic region. Tough hemin has been widely studied by many investigators,[1,2] there are only a few reports on the association behavior of protoporphyrin IX. Savitski et al.[3] reported that protoporphyrin IX was solubilized as monomer in the micelles of Triton X-100. Gallagher et al.[4] observed spectral changes of hematoporphyrin and protoporphyrin IX reflecting the dimer-to-monomer conversion. Except for these works, the association behavior of protoporphyrin IX has not been elucidated.

We recently succeeded in solubilizing hemin as monomer in both neutral and acidic water by complexing with poly(*N*-vinylpyrrolidone)] (PVP).[5] In view of this, we got interested in clarifying the interaction between PVP and protoporphyrin IX, and its influence on the association behavior of protoporphyrin IX.

In the present work, we measured at different pH the absorption spectra of protoporphyrin IX both in water and in aqueous PVP solutions. Some of the solution were examined by gel chromatography. Three types of absorption spectra were observed, and assigned to monomer, dimer, and aggregate of protoporphyrin IX. The effects of pH and PVP on the association behavior of protopophyrin IX are discussed on the basis of the structure of protoporphyrin IX.

Experimental

Materials. Protoporphyrin IX disodium salt was purchased from Nacalai Tesque, Inc. PVP (molecular weight 40000) was from Kishida Chemical Co., Ltd, and was purified by reprecipitation in water–acetone.

Preparation of Aqueous Protoporphyrin IX/PVP Solution. Protoporphyrin IX disodium salt (2 mg) and PVP (1.32 g) were dissolved in water to obtain 80 ml of an aqueous solution (pH 4.8). The pH of the solution was adjusted with HCl or NaOH. Aqueous protoporphyrin IX solutions were prepared in a similar way.

Gel Chromatography. Gel chromatography of aqueous protoporphyrin IX and protoporphyrin IX/PVP solutions was performed as follows. Two milliliters of the sample (pH 4.8 or 10) was applied onto a column (0.8×40 cm) of Toyopearl HW-55 equilibrated with water adjusted to pH of the sample. Then it was eluted with water (pH 4.8 or 10) at a flow rate of 0.13 ml min^{-1}. Concentrations of samples applied to the column were 2.5×10^{-4} mol dm^{-3} for protoporphyrin IX, and 10% wt/vol-solution for PVP. The content of protoporphyrln IX in the eluted fraction (4 ml) was monitored by measuring the maximum absorbance in a wavelength range of 350 nm to 800 nm. The amount of PVP was monitored by weighing the fraction after evaporation, which usually contained a negligible content of protoporphyrin IX.

Analytical Method. Absorption spectra at room temperature were measured with a double beam spectrophotometer UVIDEC-510 (Japan Spectroscopic Co., Ltd.).

Results

Figure 1 shows the absorption spectra of protoporphyrin IX in different states. At pH 1.0, protopor-

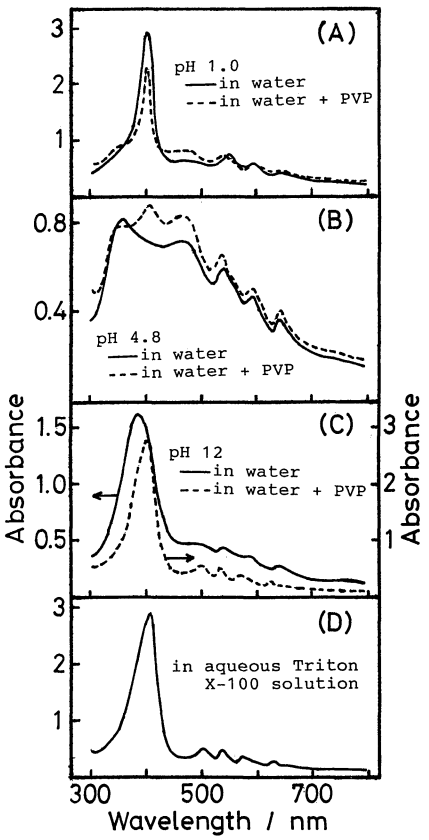

Fig. 1. Absorption spectra of protoporphyrin IX in different states.
Protoporphyrin IX in water (——) and in aqueous PVP solution (------) at pH 1.0 (A), 4.8 (B) and 12 (C). Protoporphyrin IX in aqueous Triton X-100 (1.5%) solution at pH 5.4 (D). Concentrations of protoporphyrin IX and PVP are 4.12×10^{-5} mol dm^{-3} and 1.65×10^{-2} g cm^{-3}, respectively.

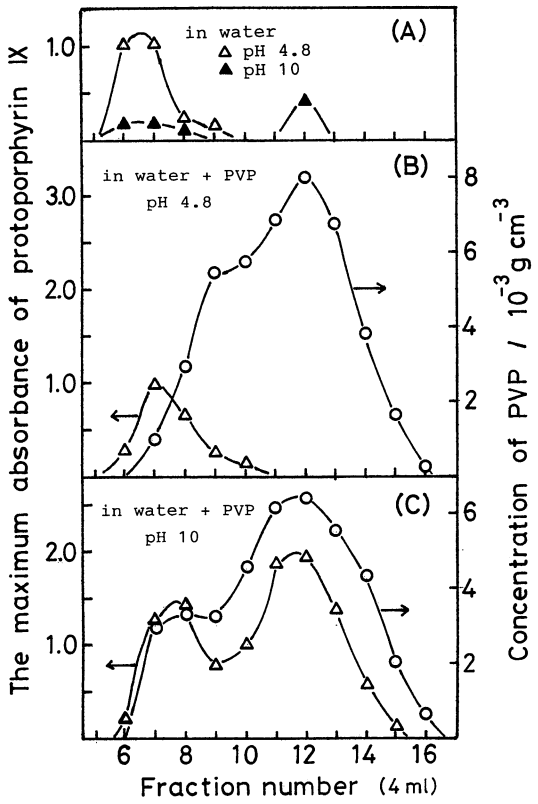

Fig. 2. Elution patterns of gel chromatography.
(A) aqueous protoporphyrin IX solution at pH 4.8 and 10, (B) aqueous protoporphyrin IX/PVP solution at pH 4.8, (C) aqueous protoporphyrin IX/PVP solution at pH 10.

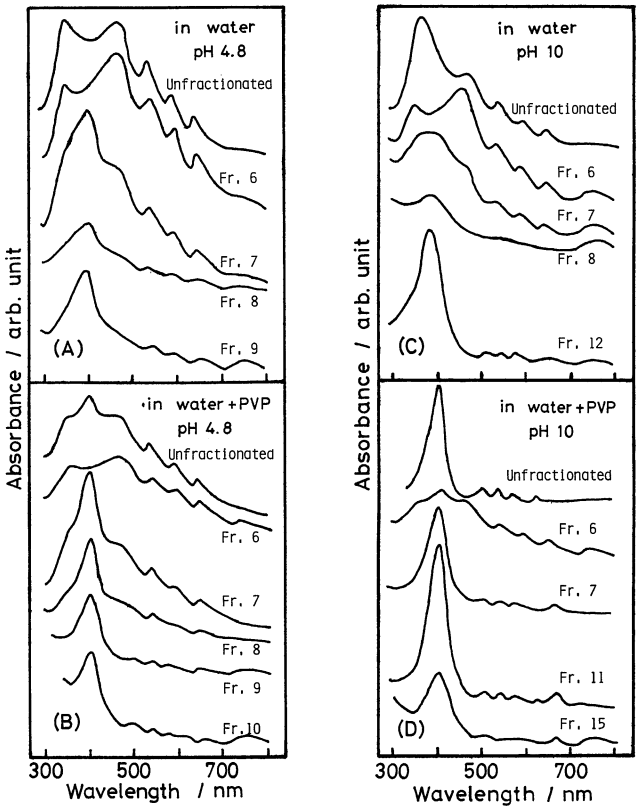

Fig. 3. Absorption spectra of protoporphyrin IX in unfractionated and fractionated solutions in gel chromatography.
(A) protoporphyrin IX in water at pH 4.8, (B) protoporphyrin IX in aqueous PVP solution at pH 4.8, (C) protoporphyrin IX in water at pH 10, (D) protoporphyrin IX in aqueous PVP solution at pH 10. Fraction numbers are the same as those in Fig. 2. The absorption spectra of fractions 8—10 and 12—14 for sample (D) are not shown, because they changed successively as the fraction number grew.

phyrin IX in water and in aqueous PVP solution had a sharp Soret band at 405 nm and four weak Q-bands. Protoporphyrin IX in water at pH 4.8 exhibited a weak Soret band at about 350 nm and four weak Q-bands; on interacting with PVP, a new Soret band appeared at 406 nm. Protoporphyrin IX in water at pH 12 showed a sharp Soret band at 388 nm and four weak Q-bands; on interacting with PVP, the Soret band shifted to 404 nm and its intensity increased about 1.7 times, resulting in the same intensity as the Soret band at pH 1.0.

Thus, the following three types of absorption spectra were observed for protoporphyrin IX in water and aqueous PVP solutions. Type 1—a weak Soret band at about 350 nm and four weak Q-bands, Type 2—a sharp Soret band at 388 nm and four weak Q-bands, Type 3—a sharp Soret band at about 405 nm and four weak Q-bands.

Figure 2 shows the elution patterns of gel chromatography. Each plot in this figure gives the amount of protoporphyrin IX or PVP contained in each fraction. The elution curves of protoporphyrin IX and PVP were remarkably dependent on pH.

Figure 3 shows the absorption spectra of protoporphyrin IX in unfractionated (sample applied onto the column) and fractionated solutions. For each sample, Fr. 6 showed the Type 1 absorption spectrum. As the fraction number increased, the Soret bands shifted from about 350 nm to 398 nm (Fig. 3-A), 403 nm (Fig. 3-B), 381 nm (Fig. 3-C), or 405 nm (Fig. 3-D), and their intensities became much higher as compared with Q-bands. These absorption spectra were classified into Types 2 and 3 as follows. The absorption spectra of later fractions in water at pH 4.8 (Fig. 3-A), and in aqueous PVP solutions at pH 4.8 (Fig. 3-B) and at pH 10 (Fig. 3-D) belonged to Type 3, since the Soret bands were at 398—405 nm. On the other hand, the absorption spectrum of a later fraction, Fr. 12, in water at pH 10 (Fig. 3-C) belonged to Type 2 with the Soret maximum at 381 nm.

Discussion

Effect of pH. Since protoporphyrin IX is an amphoteric electrolyte, it is considered to dissociate into ions in acidic and basic water. This explains the result that the intensity of the Soret band of protoporphyrin IX in water increased in the order at pH 4.8< at pH 12< at pH 1.0 (Fig. 1). At pH 4.8, the solubility of protoporphyrin IX was substantially small due to the low degree of electrolytic dissociation.

Assignment of Three Types of Absorption Spectra. In

gel chromatography, it is expected that as the fraction number increased, the molecular weight was lower. Therefore, Type 1 observed in the earliest fraction, Fr. 6, may represent a bulky species of protoporphyrin IX. On the other hand, Types 2 and 3 observed in later fractions may be due to a small species. Based on these, we assigned the three types of absorption spectra of protoporphyrin IX as follows.

Type 1 was supposed to arize from a large species. The molecular weight of this species was inferred to be larger than 700000, because as specified by the manufacturer, Toyopearl HW-55 excludes proteins of molecular weight larger than 700000. A most probable candidate for Type 1 is thus an aggregate of protoporphyrin IX. The lowest intensity of the Soret band of Type 1 in water at pH 4.8 (Fig. 1-B) was consistent with this assignment. Savitski et al.[3] reported a similar absorption spectrum for protoporphyrin IX in water at pH 8.0.

Type 2 was observed in basic water of pH 10 and 12 (Figs. 1-C and 3-C), and the gel chromatographic elution time suggested that it was a small species. The spectral feature of Type 2 was similar to that of a hematoporphyrin dimer in 0.02 M NaOH (1 M=mol dm^{-3}), reported by Gallagher et al.[4] The dimer of hematopophyrin had a Soret band at about 375 nm and four weak Q-bands, and on dilution the Soret band shifted to 395 nm and its intensity increased about 1.5 times, due to disintegration of dimer to monomer. They also described that a similar spectral change was observed for protoporphyrin IX under the same conditions.[4] Therefore, Type 2 was assigned to a dimer of protoporphyrin IX.

Type 3 was also suggested to reflect a small species from gel chromatography. It should be noted that this type was also observed in an aqueous Triton X-100 solution (Fig. 1-D). Savitski et al.[3] reported that protoporphyrin IX was solubilized as monomer in Triton X-100 micelles, on the basis of fluorescence spectra. Therefore, Type 3 was assigned to the monomer of protoporphyrin IX. The highest intensity of the Soret band of type 3 (Fig. 1) supports this assignment.

From the assignments of three types of absorption spectra, it can be concluded that protoporphyrin IX was dissolved as monomer in acidic water, as aggregate in neutral water, and as dimer in basic water, though a small amount of other species was involved in some cases.

Model for Dimerization and Aggregation. It is interesting to note that protoporphyrin IX is dissolved as monomer in acidic water whereas as dimer in basic water, though the ionization occurs in both media. This fact seems to suggest that ionization of two pyrrole nitrogens at nearly the center of the porphyrin ring under acidic conditions, is more effective in inhibiting dimerization than that of two propionic acid residues outside the ring under basic conditions. This interpretation is in line with the "face-to-face" or "ring stacking" model[2] for dimerization and aggregation of protoporphyrin IX. Furthermore, the blue shift of the Soret band of protoporphyrin IX due to conversion of monomer to dimer (Fig. 1-C) also supports this model for dimerization.[2,6,7]

Interaction between PVP and Protoporphyrin IX. When PVP was present, protoporphyrin IX tended to show Type 3 assigned to a monomer, even in neutral and basic water (Figs. 1—3). Therefore, we could conclude that PVP tends to suppress dimerization and aggregation of protoporphyrin IX. This effect of PVP was more conspicuously observed at pH 10 and 12 than at pH 4.8. Since the protoporphyrin IX molecule has a wide hydrophobic region, PVP probably binds the molecule within its random coil chain through a hydrophobic interaction, thereby preventing dimerization and aggregation of protoporphyrin IX.

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