Solubilization of Hemin in Neutral and Acidic Aqueous Solutions by Forming Complexes with Water-Soluble Macromolecules

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Synopsis. Hemin was solubilized in neutral and acidic aqueous solutions by complexing with poly(*N*-vinylpyrrolidone) (PVP), polyethylene glycol (PEG), poly(vinyl alcohol) (PVA) and dextran. In neutral aqueous solutions, hemin in the PVP, PEG, and PVA complexes was monomeric and that in the dextran complex was dimeric. The effects of pH and ultraviolet (UV) irradiation on the spectroscopic properties of the complexes were examined.

It is well-known that hemin is soluble in organic solvents, alkaline aqueous solutions, and aqueous detergent micelles, but insoluble in neutral and acidic aqueous solutions.[1-3] Nishida et al.[4] reported that in alkaline aqueous solutions the aggregated ferroheme (ferroprotoporphyrin IX) and ferroheme·pyridine complex dissociated into the monomers upon the addition of water-soluble macromolecules.

We have recently succeeded in solubilizing hemin in neutral and acidic aqueous solutions by means of water-soluble linear polymers of PVP, PEG, PVA, and dextran. The properties of hemin in aqueous macromolecular solutions were investigated by comparing its absorption spectra with those of hemin in dimethyl sulfoxide (DMSO), an aqueous NaOH solution, and an aqueous Triton X-100 solution.

Experimental

Materials. Hemin (chlorohemin) manufactured by Eastman Kodak Co. was used without further purification. PVP (molecular weight (mol wt), 40000) and PEG (mol wt, 20000) were purchased from Kishida Chemical Co., Ltd. PVA (PVA-117: mol wt, 77000, degree of saponification, 98.5 mol%) was kindly donated by Kuraray Co., Ltd. Dextran (mol wt, 75000) was purchased from Wako Pure Chemical Industries, Ltd. PVP, PEG, PVA, and dextran were purified by reprecipitation of them in solvent-nonsolvent, namely in water-acetone, benzene-petroleum ether, water-methanol, and water-ethanol, respectively.

Method for Solubilization of Hemin in Neutral and Acidic Aqueous Solutions. Solid hemin (0.5 mg) was added to an aqueous macromolecular paste (PVP, PEG, and dextran, 33%; PVA, 15%) containing 1.0 g polymer and gently stirred. The mixture was added by 0.3 ml of 28% aqueous ammonia and stirred gently, resulting in hemin being dissolved. It was then evaporated to a hemin-polymer film under reduced pressure at room temperature. A small amount of water was added onto the film, and stirred gently at room temperature (PVP, PEG, and dextran films) or 100 °C (PVA film) until a paste was formed. The paste was diluted with water to a given concentration, resulting in a neutral aqueous macromolecular solution of hemin.

Acidic aqueous macromolecular solutions of hemin were obtained by the addition of concentrated hydrochloric acid to the neutral aqueous solutions.

Hemin was also dissolved in an aqueous Triton X-100 solution in a similar way by using aqueous ammonia.

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

Photobleaching of Hemin. Photobleaching experiments were carried out under ultraviolet (UV) light. UV irradiation was performed with a fluorescence lamp (Mitsubishi GL-15) placed 10 cm from a quartz cuvette containing the hemin solution. The photodegradation (%) of hemin was calculated by dividing the absorbance after UV irradiation by that before irradiation. The wavelengths used to monitor the photodegradation were those of the absorption maxima of Soret bands.

Results and Discussion

There have been several suggestions that a sharp

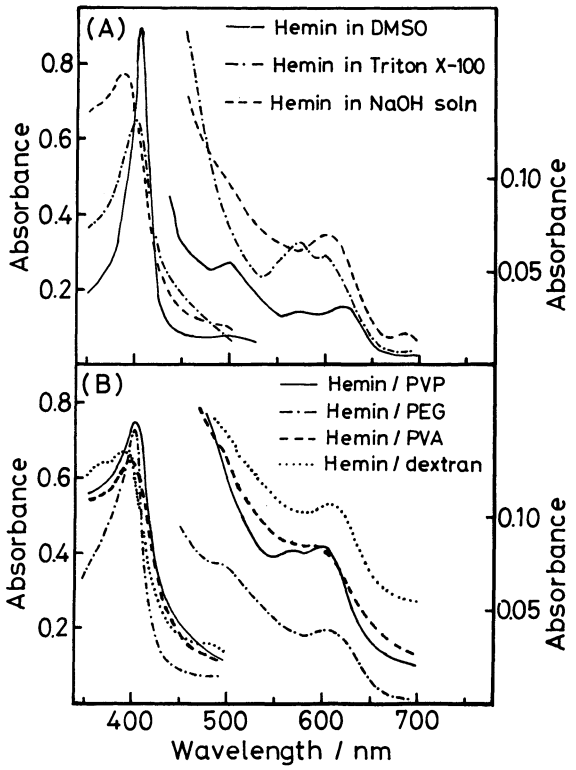

Fig. 1. Absorption spectra of hemin in different states. (A) hemin dissolved in various solvents: ——, hemin in DMSO (hemin, 5.1×10^{-6} mol dm^{-3}); —·—·—, hemin in an aqueous Triton X-100 solution (hemin, 1.0×10^{-5} mol dm^{-3}; Triton X-100, 1.0%, pH 5.7); ---, hemin in an aqueous NaOH solution (hemin, 1.5×10^{-5} mol dm^{-3}; pH 10.4). (B) hemin-macromolecular complexes in water: ——, hemin-PVP complex (hemin, 1.53×10^{-5} mol dm^{-3}; pH 7.5); —·—·—, hemin-PEG complex (hemin, 1.0×10^{-5} mol dm^{-3}; pH 7.1); ---, hemin-PVA complex (hemin, 1.6×10^{-5} mol dm^{-3}; pH 6.3); ·····, hemin-dextran complex (hemin, 1.3×10^{-5} mol dm^{-3}; pH 7.5).

single Soret band corresponds to monomeric ferriheme (ferriprotoporphyrin IX), whereas a doublet shows the presence of dimers.[1,5] Further, the absorption spectra of ferriheme are considered to depend on the species bound to iron at the fifth and sixth positions.[1]

Figure 1-(A) shows the absorption spectra of hemin dissolved in various solvents. The absorption maxima were as follows: 405, 498, 575, and 623 nm for hemin in DMSO; 401, 573, and 600 nm for hemin in an aqueous Triton X-100 solution; 360, 388, 500, and 603 nm for hemin in an aqueous NaOH solution. It is generally considered that hemin is monomeric in DMSO[1] and aqueous Triton X-100 solutions,[2,3] and dimeric[6] in aqueous NaOH solutions.[2]

Hemin was dissolved in neutral aqueous solutions by means of water-soluble linear polymers of PVP, PEG, PVA, and dextran. The resulting solutions gave the absorption spectra of hemin shown in Fig. 1-(B). Since hemin itself is insoluble in neutral aqueous solutions,[1-3] it can be inferred that the linear polymers complexed with hemin, resulting in the solubilization of hemin in neutral aqueous solutions. The polymers probably bound hemin within a random coil of the chain.

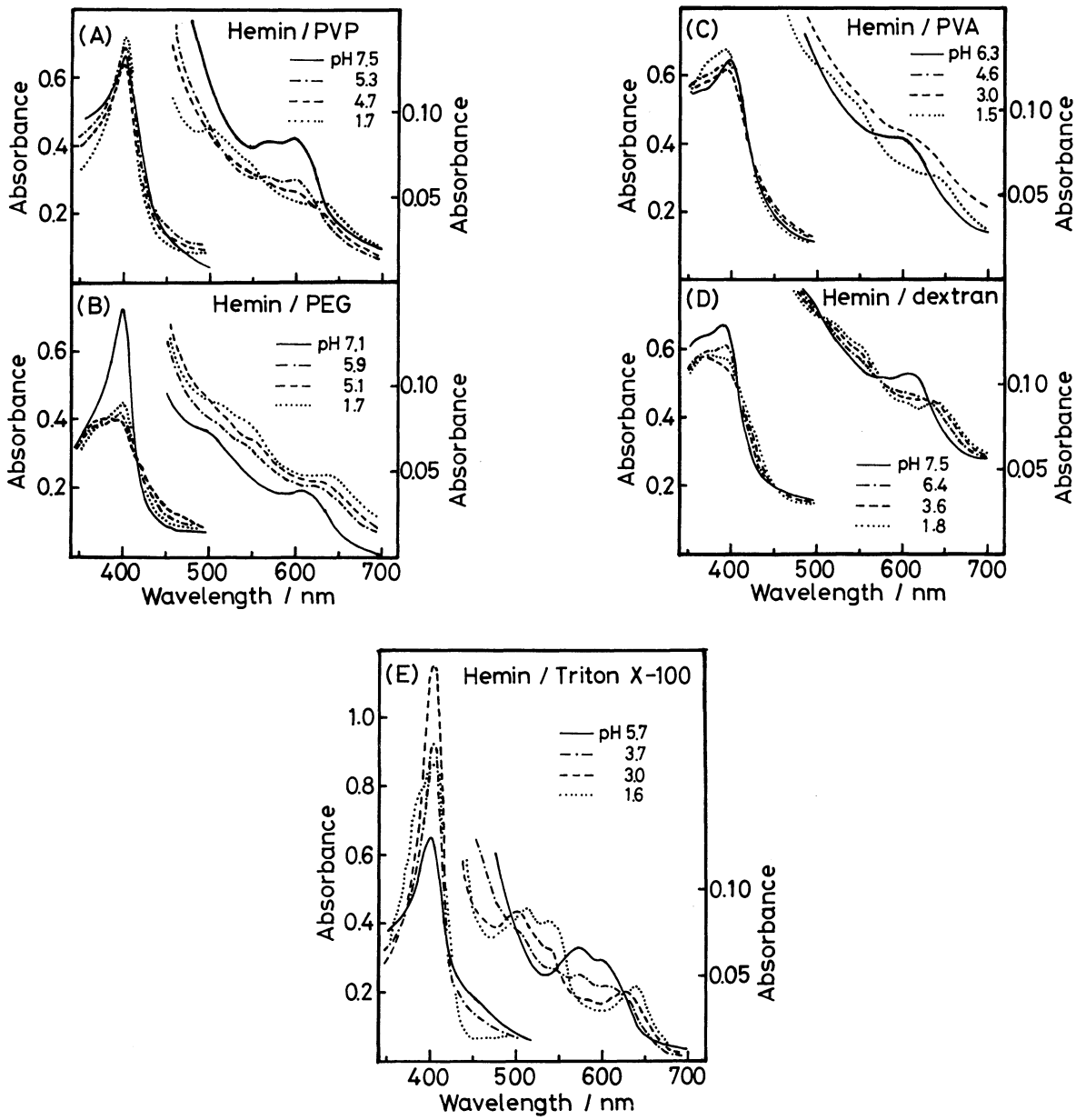

Fig. 2. Dependence on pH of absorption spectra for hemin in different states. The concentrations of hemin were the same as in Fig. 1. (A) hemin-PVP complex in water, (B) hemin-PEG complex in water, (C) hemin-PVA complex in water, (D) hemin-dextran complex in water, (E) hemin in an aqueous Triton X-100 (1.0%) solution.

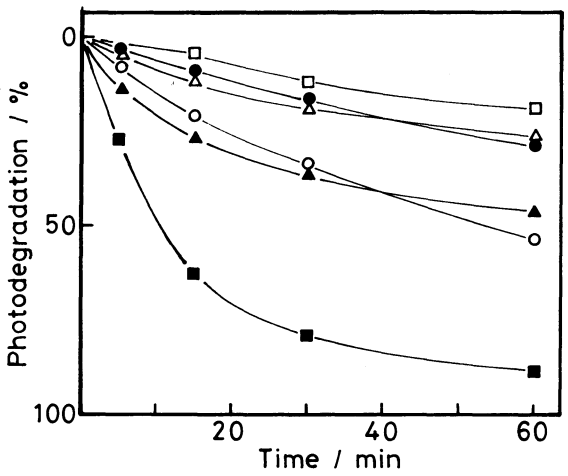

Fig. 3. Photodegradation of hemin in different states by irradiation of UV light. The wavelengths monitoring the photodegradation were those of absorption maxima of Soret bands. The concentrations of hemin were the same as in Fig. 1. ○, hemin-PVP complex in water (pH 7.5); ▲, hemin-PEG complex in water (pH 6.9); ●, hemin-PVA complex in water (pH 7.1); △, hemin-dextran complex in water (pH 7.5); □, hemin in aqueous Triton X-100 (1.3%) solution (pH 6.5); ■, hemin in DMSO.

The absorption maxima for the hemin-macromolecular complexes in neutral aqueous solutions (Fig. 1-(B)) were as follows: 401, 568, and 600 nm for PVP complex; 400, 490, and 608 nm for PEG complex; 398 and 600 nm for PVA complex; 365, 389, and 608 nm for dextran complex. The PVP, PEG, and PVA complexes have a sharp single Soret band which resembled, in both shape and position, that of hemin in DMSO and the aqueous Triton X-100 solution. However, the dextran complex had a double Soret band similar to that of hemin in an aqueous NaOH solution. On the other hand, the Q-bands of the PVP and PEG complexes resembled those of hemin in the aqueous Triton X-100 solution and DMSO, respectively. The Q-bands of the dextran complex were similar to those of hemin in the aqueous NaOH solution. From these results, it can be inferred that hemin in the PVP, PEG, and PVA complexes is monomeric and that in the dextran complex it is dimeric.

Figure 2 shows the dependence on pH of the absorption spectra of hemin in different states. The pH-dependence of the Soret bands of the hemin-macromolecular complexes markedly depended on the polymer species. The Soret bands of the PVP complex became narrower as the pH decreased, as well as those of hemin in the aqueous Triton X-100 solution. The Soret band of the PEG and PVA complexes became broader with decreasing pH, and the Soret band of the PEG complex was largely decreased in intensity, even at pH 5.9. In the case of the dextran complex, as the pH decreased the intensity of the Soret band at 365 nm became larger than that at 389 nm. From these results it is considered that the monomeric hemin in the PVP complex was stable even in acidic aqueous solutions, but that in the PEG and PVA complexes was unstable and probably changed into a dimer or aggregates under acidic conditions.

The pH-dependence of the Q-bands for the hemin-macromolecular complexes hardly depended on the polymer species, as shown in Fig. 2. At the lowest pH, three new peaks were observed at similar positions for each complex: that is, at 500, 540, and 628 nm for PVP complex; at 500, 548, and 640 nm for PEG complex; at 510, 545, and 640 nm for PVA complex; at 515, 550, and 638 nm for dextran complex. For hemin in the aqueous Triton X-100 solution, the pH-dependence of the Q-bands were clearly observed, showing it to be similar to that for the hemin-macromolecular complexes. That is, at the lowest pH (1.6), three new peaks appeared at 510, 541, and 640 nm. The appearance of three new peaks for all of the samples implies that upon adding hydrochloric acid to neutral aqueous solutions of hemin to lower the pH (see Experimental section), the chloro ligand bound to iron gradually increases in the presence of excess of Cl^- ions.[1)]

Figure 3 shows the photodegradation of hemin in different states upon irradiation of UV light. It was found that hemin in macromolecular complexes in neutral aqueous solutions was more stable against UV irradiation than that in DMSO. The rates of photodegradation of hemin in macromolecular complexes depended on the polymer species. That is, hemin in the PVA and dextran complexes was photodegraded more slowly than that in the PVP and PEG complexes. Hemin in the aqueous Triton X-100 solution was more stable against UV irradiation than that in macromolecular complexes.

From the above results it can be inferred that in neutral aqueous solutions the carbonyl group of PVP and ether oxygen of PEG have a stronger coordinating power to iron of hemin than the hydroxyl group of PVA and dextran. Ether oxygen of PEG, however, probably loses its strong coordinating power in acidic aqueous solutions.

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