Photoreaction Generating Active Oxygens of In^{3+}-Tetrakis(4-methylpyridyl)-porphine in the Presence of Albumins

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The complex formation of In^{3+}-tetrakis(4-*N*-methylpyridyl)-porphine (In-TMPyP) with albumin was studied by resonance Raman spectroscopy. Albumin coordinated to In^{3+} through the $-S^-$ group(s). The photoreaction was investigated using the visible spectral change and In-TMPyP-thiourea complex was used as a model. It was demonstrated that the complex in a weak basic solution (pH 8.5) is excited by light and the excited complex converts oxygen to superoxide anion, which finally cleavages the porphine ring of In-TMPyP.

Key words In-; Ga-; porphyrin; photo reaction; superoxide; active oxygen

To date, photoreactions of metal-porphyrins have attracted many interests in photodynamic therapy for tumor,[1,2] care of porphyrias[3] and activities of biomimetics.[4,5] The investigators frequently discussed that the vital activities of metal-porphyrins are concerned with active oxygens. $^{111}In^{3+}$-tetrakis(4-*N*-methylpyridyl)-porphine has been reported to be a potential tumor imaging agent[6] or a prototype for a radiopharmaceuticals.[7] In order to get a fundamental information about In^{3+}-tetrakis(4-*N*-methylpyridyl)-porphine (In-TMPyP, see Fig. 1) in vital materials, we studied the photoreaction in the presence of albumins by resonance Raman (RR) and visible spectroscopies.

Water soluble In- and Ga-TMPyP ([In- and Ga-TMPyP-$(H_2O)_3Cl]Cl_4$) were prepared from commercially available H_2-TMPyP and $InCl_3$ ($GaCl_3$) according as the literature method.[8—10] Human and bovine serum albumins (1×crystallized, HSA and BSA) were purchased from Sigma Chemical Co. Ltd., and chicken egg albumin (5×crystallized, EA) from Seikagaku Co. Ltd. Other chemicals were of analytical or of reagent grade. The RR spectra were measured on a JASCO NR-1000 (476.5 nm excitation line of an Ar^+-laser) equipped with a spinning cell. The visible spectra were recorded on a Shimadzu UV-180 spectrophotometer with 1.0 mm glass cells. A projector with a halogen lamp (300 w/100V, Pj lamp) and a Shimadzu RF-500 spectrofluorophotometer with a xenon arc lamp were used as the continuous and monochrome (bandwidth=20 nm) light (430 or 565 nm) sources, respectively.

Figure 2 shows the changes of irradiation-induced visible spectra of In-TMPyP solution ($4\times10^-$ mol/l) in the absence (A) and the presence (B) of BSA (200 mg/ml). When BSA was added to In-TMPyP solution, the wavelength of absorption maximum (λ_{MAX}=430 nm) scarcely changed, whereas the molecular absorption coefficient (ε) decreased (Figs. 2A and B, 0 min). When the In-TMPyP solution with BSA was irradiated with the Pj-lamp, the absorbance decreased less than half in 30 min (Fig. 2B, 30 min). On the other hand, when In-TMPyP alone was irradiated with the Pj-lamp for 120 min, the visible spectrum slightly changed (Fig. 2A, 120 min). Similar decreases of the absorbance were caused by the addition of yellowish HSA and colorless EA (data not shown). These results show that albumins are involved in the decrease of the absorbance, caused by decomposition of In-TMPyP. In addition, In-TMPyP with BSA was irradiated at 430 or 565 nm and the visible spectra were measured. The decrease of absorbance was observed only on the irradiation at 430 nm (Soret band of In-TMPyP). Similar RR and visible spectral changes were observed when Ga-TMPyP solution

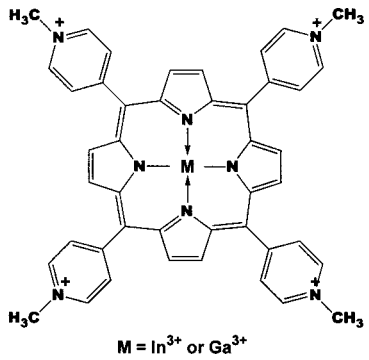

Fig. 1. Structures of In- and Ga-TMPyP

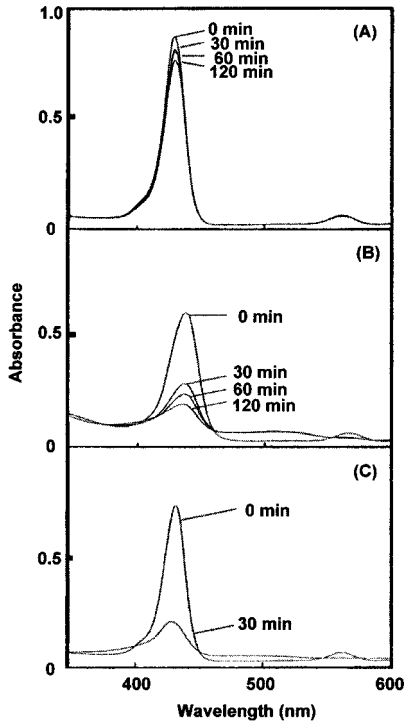

Fig. 2. Visible Spectra of In-TMPyP

(A) Without BSA (4×10^{-5} mol/l In-TMPyP in pH 8.5 buffer), (B) with BSA (4×10^{-5} mol/l In-TMPyP and 200 mg/ml BSA in pH 8.5 buffer), (C) with thiourea (4×10^{-5} mol/l In-TMPyP and 4×10^{-3} mol/l thiourea in pH 8.5 buffer).

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was used instead of In-TMPyP (data not shown).

Above results indicate that the albumin complex of In-TMPyP plays a role in the photoreaction of In-TMPyP. However, no evidences for the complex formation were shown in the visible-spectra. To make clear the conformational and structural changes of In-TMPyP under the presence of albumin, we measured RR spectra of In-TMPyP. BSA caused a change of the RR spectrum of In-TMPyP (Fig. 3B), indicating the conformational and/or structural changes through the complex formation. One of the possible mechanisms is an axial-ligand exchange between Cl^- and $-S^-$ group that has a strong affinity to In^{3+} ion. Therefore, we measured the RR spectra of In-TMPyP in the presence of a ligand(s) containing $-S^-$ group such as thiourea (TU), cysteine and glutathione (4×10^{-3} mol/l), at pH 8.5. The presence of the ligand changed RR spectrum (Fig. 3C) and decreased the absorbance of visible spectrum of In-TMPyP (Fig. 2C), as is the case of BSA. RR spectrum by the addition of glutathione was similar to Fig. 3C. Thus, we concluded that the In-TMPyP complex coordinated by the $-S^-$ group(s) of albumin plays a main role in the photoreaction observed in this study. It is of interest that In-TMPyP forms a complex with albumin although Mn-TMPyP does not.[11)]

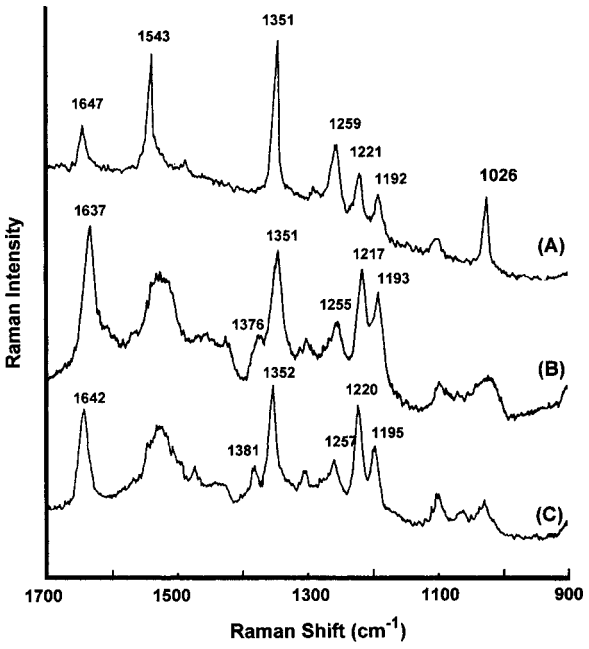

Fig. 3. Resonance Raman Spectra of In-TMPyP

(A) Without BSA (5×10^{-4} mol/l In-TMPyP in pH 8.5 buffer), (B) with BSA (5×10^{-4} mol/l In-TMPyP and 100 mg/ml BSA in pH 8.5 buffer), (C) with thiourea (5×10^{-4} mol/l In-TMPyP and 5×10^{-2} mol/l thiourea in pH 8.5 buffer).

As discussed above, TU at pH 8.5 (the anionic form) is one of good model compounds, which behave like albumin in the visible and RR spectra (see Figs. 2 and 3). Thus, we selected TU as a model of albumin to examine the mechanism of photoreaction of In-TMPyP. Figure 4 shows the proposed pathway from the results of the following examinations by the visible spectroscopy; (1) in the absence of O_2, (2) in the presence of scavengers for $\cdot$OH, mannitol and dimethylsulfoxide, (3) in the presence of scavenger for $^1O_2$, NaN_3, (4) in the presence of $^1O_2$ forming reagents, H_2O_2 with $NaClO_4$, in the dark, (5) in the presence of a reagent prolonging the life of $^1O_2$, a mixture of acetone-d_6 and D_2O, (6) in the presence of scavengers for O_2^-, epinephrine and superoxide dismutase (SOD), (7) in the presence of O_2^- generating reagents, xanthine with xanthine oxidase, in the dark.

To investigate the requirement of O_2 for the photoreaction, a solution of In-TMPyP (4×10^{-5} mol/l) with TU (4×10^{-3} mol/l) (In-TMPyP-TU solution) was bubbled with N_2 gas for 60 min. When the In-TMPyP-TU solution was irradiated with the Pj-lamp for 30 min under the condition without O_2, the decrease of the absorbance at 430 nm was about a half comparing with the case without N_2 babbling and under the condition in air. During the irradiation at 430 nm for 240 min in the presence of an O_2^- scavenger, epinephrine (1×10^{-3} mol/l), the UV spectrum of the complex solution scarcely changed. In addition of SOD, decomposition of In-TMPyP was about 1/4 comparing with the case without SOD. In the presence of O_2^- generating reagents, xanthine with xanthine oxidase, the absorbance at 430 nm, on the contrary, was almost vanished within 60 min even in the dark. These results indicate that O_2^- cleavages the porphine ring of In-TMPyP-TU and decomposes the complex. In addition, an $\cdot$OH scavenger, mannitol, did not block the decomposition of In-TMPyP-TU at all, suggesting that $\cdot$OH is not concerned with the photoreaction.

The experiments (3)—(5), which can make clear the role of $^1O_2$, gave significant information as follows. An $^1O_2$ scavenger, NaN_3, completely inhibited the decomposition of In-TMPyP-TU induced by the irradiation with a Pj-Lamp. In the presence of a mixture of acetone-d_6 and D_2O (Fig. 5B), in which the life span of $^1O_2$ lengthens,[12)] the absorbance of In-TMPyP-TU irradiated decreased more rapidly than that of the standard solution (Fig. 5A), but that of unirradiated com-

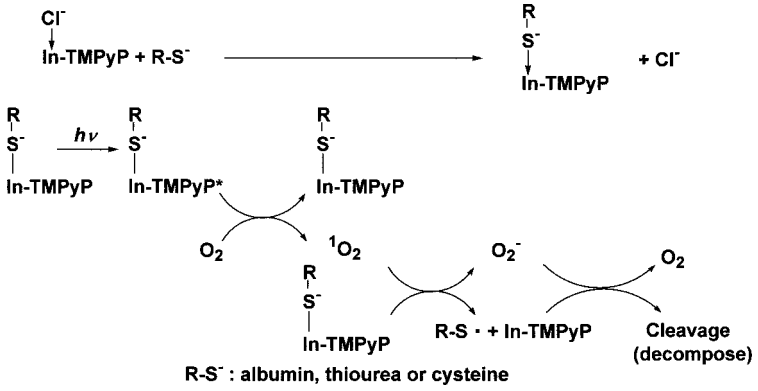

Fig. 4. Proposed Pathway

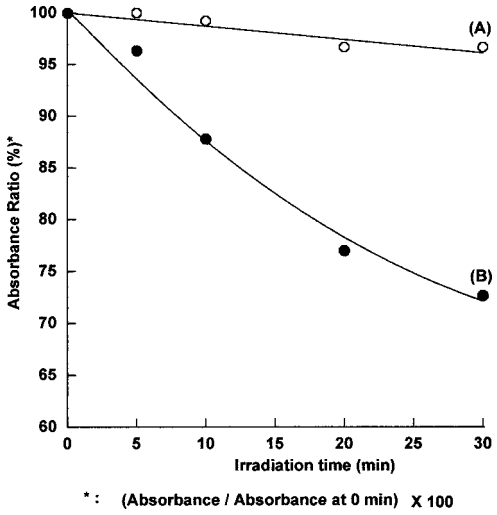

Fig. 5. Effect of Deuterated Solvent

A: ○ 4×10^{-5} mol/l In-TMPyP and 4×10^{-3} mol/l thiourea in 1 : 1 mixture of H_2O and acetone. B: ● 4×10^{-5} mol/l In-TMPyP and 4×10^{-3} mol/l thiourea in 1 : 1 mixture of D_2O and acetone-d_6.

plex did not. These results show that $^1O_2$ generated through irradiation of In-TMPyP-TU decomposes the In-TMPyP part. However, no visible spectral changes of In-TMPyP-TU was observed in the presence of $^1O_2$ generating reagents, H_2O_2 (5×10^{-2} mol/l) and $NaClO_4$ (1×10^{-1} mol/l). Accordingly, the results demonstrated that the decomposition of In-TMPyP-TU is due to not $^1O_2$ itself but the co-existence of $h\nu$: the In-TMPyP part is decomposed in cooperation of $^1O_2$ and In-TMPyP-TU excited by $h\nu$ (In-TMPyP*-TU). It was proposed in Fig. 4 that $^1O_2$ is essential in the photoreaction of In-TMPyP–S^--R, and the excited In-TMPyP*–S^--R converts $^1O_2$ to O_2^- by transforming charge and energy.

In conclusion, the proposed pathway shown in Fig. 4 is consistent with the results obtained in the examinations in this study. We hope the pathway obtained will contribute to the analysis of mechanism of vital activities of metal porphyrines and developments such as a therapy for tumor and a care of porphyrias.

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