REDUCTION OF Mn^{3+}-TETRAKIS(4-METHYLPYRIDYL)PORPHINE WITH ALBUMIN OBSERVED IN RESONANCE RAMAN SPECTRA

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When the resonance Raman spectra of Mn^{3+}-tetrakis(4-methylpyridyl)-porphine are measured in the presence of albumins, the resonance Raman bands of Mn^{2+}-tetrakis(4-methylpyridyl)porphine are frequently observed. This reduction of $Mn^{3+}$to Mn^{2+} could be caused by an action of unfolding albumins resulting from heat and/or light.

KEYWORDS resonance Raman spectra; porphine; albumin; reduction; manganese

Previously, we investigated the resonance Raman (RR) spectra of manganese-porphines (MnP), which play a main role in the enzyme-like activities of the MnP-modified resins formed through the interactions between MnP and an ion-exchange resin.[1,2] In the present study, our interest is focused on the behavior of MnP in the presence of vital materials, and we mainly examined interactions between albumins and Mn^{3+}-tetrakis(4-methylpyridyl)porphine (Mn^{3+}TM, see Fig.1) by means of RR spectroscopy. Consequently it has been observed in RR spectra that Mn^{3+}TM is reduced to Mn^{2+}TM with albumins.

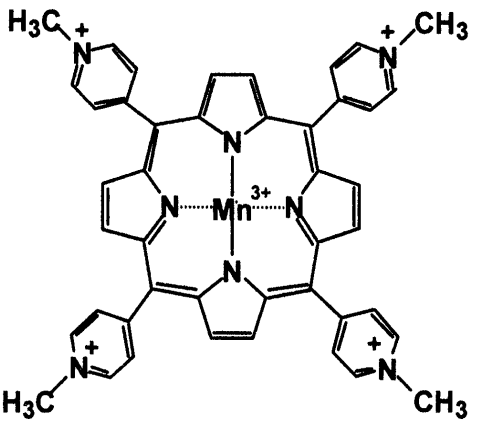

Fig. 1 Structure of Mn^{3+}TM.

EXPERIMENTAL PROCEDURES

Mn^{3+}TM prepared from H_2TM and $MnCl_2$ was purified several times by reprecipitation. Human (1× recryst., HSA) and bovine (1×recryst., BSA) serum albumins were purchased from Sigma Chemical Co., (St. Louis, USA) and chicken egg albumin (5×recryst., EA) from Seikagaku Co. (Tokyo, Japan). Other chemicals were of analytical or reagent grade. RR spectra were recorded on a Jasco (Tokyo, Japan) NR-1000 laser Raman spectrophotometer, using the 476.5-nm excitation line of an NEC (Tokyo, Japan) Ar^+ laser (GLS3480, 4W). UV spectra were measured on a Shimadzu (Kyoto, Japan) UV-180 spectrophotometer with 1.0 mm glass cells. A projector with a halogen lamp (200 W/110 V, Pj-lamp) and a Shimadzu RF-500 spectrofluorophotometer with a xenon short arc lamp (UXL-155-0-LCA, Ushio Inc. Tokyo, Japan, Xe-lamp) were used as the continuous and monochrome (band width = 20 nm) light sources, respectively.

RESULTS AND DISCUSSION

Resonance Raman Spectra When the measurements of RR spectra were carried out using a rotational cell, 4×10^{-5} mol/l Mn^{3+}TM in 0.2 g/ml BSA solution (pH *ca.* 8.0) gives essentially the same RR spectrum as that of Mn^{3+}TM aqueous solution (pH 8.0), as shown in Fig. 2 A. However, the measurements made using a stationary cell give additional RR bands to those of Mn^{3+}TM, as shown in Fig. 2 B-D, irrespective of the kind of albumin. The additional two bands at 1545 and 1345 cm^{-1} can be attributed to

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$Mn^{2+}TM$ as indicated by the comparison with the RR spectrum of $Mn^{2+}TM$ (Fig. 2 E). The longer irradiation of the laser beam causes an increase in the additional bands in Raman intensity and, finally, the RR spectrum of the mixture agrees with that of $Mn^{2+}TM$. Naturally, no new RR band is observed for the aqueous solution of $Mn^{3+}TM$. These results indicate that $Mn^{3+}TM$, when coexisting with albumins, is reduced to $Mn^{2+}TM$ by irradiation the laser beam (476.5 nm) for a few minutes. The reduction of $Mn^{3+}TM$ could be due to adsorption of laser light and/or heat caused by the laser beam. To determine the cause of the reduction, we investigated the UV spectra of $Mn^{3+}TM$ in the presence of BSA, because it is possible to control the light and heat independently in UV spectroscopy.

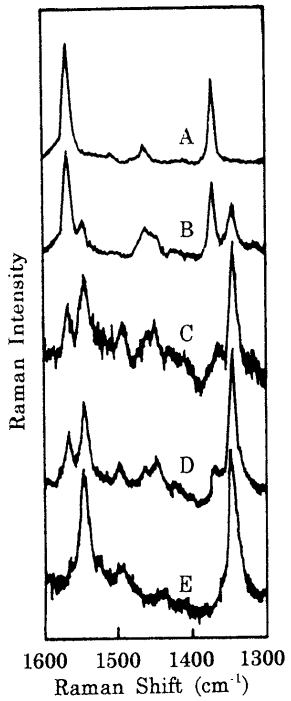

Fig. 2. Resonance Raman Spectra of $Mn^{3+}TM$

A: 4×10^{-5} mol/l $Mn^{3+}TM$ in buffer solution (pH 8), **B**: 4×10^{-5} mol/l $Mn^{3+}TM$ in 0.2 g/ml EA solution (pH *ca.* 8.0), **C**: 4×10^{-5} mol/l $Mn^{3+}TM$ in 0.2 g/ml HSA solution (pH *ca.* 8.0), **D**: 4×10^{-5} mol/l $Mn^{3+}TM$ in 0.2 g/ml BSA solution (pH *ca.* 8.0), **E**: 4×10^{-5} mol/l $Mn^{2+}TM$ reduced with sodium dithionate solution (pH *ca.* 8.0).

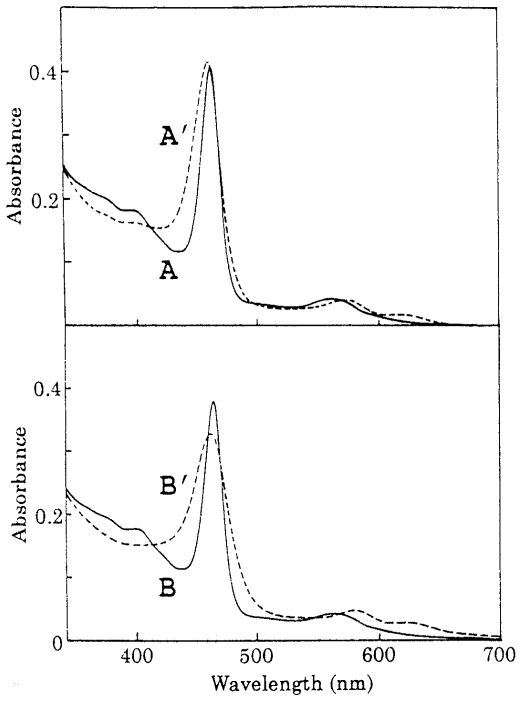

Fig. 3. Effects of Irradiation with the Pj-Lamp and Heat on UV Spectra of $Mn^{3+}TM$ in the Presence of BSA

A: 4×10^{-5} mol/l $Mn^{3+}TM$ in 0.2 g/ml BSA solution (pH *ca.* 8.0, no irradiation with Pj-lamp), **A'**: After irradiation with Pj-lamp for 30 min, **B**: 4×10^{-5} mol/l $Mn^{3+}TM$ in 0.2 g/ml BSA solution (pH *ca.* 8.0, no heat), **B'**: After warming at 50℃ for 30 min.

Effect of Irradiation on UV Spectra The formation of $Mn^{2+}TM$ can be easily confirmed by UV spectroscopy, since the UV spectrum of $Mn^{3+}TM$ (λ_{max} = 462 nm) is different from that of $Mn^{2+}TM$ (λ_{max} = 455 nm). When the Pj-lamp is irradiated for 30 min to 4×10^{-5} mol/l $Mn^{3+}TM$ in 0.2 g/ml BSA solution, the λ_{max} of the initial solution shifts to shorter wave-lengths by about 10 nm, where λ_{max} of $Mn^{2+}TM$ is observed (see Fig. 3). This result indicates that $Mn^{3+}TM$ is reduced to $Mn^{2+}TM$ by irradiation with the Pj-lamp. In this case, the temperature of the solution is not increased as much and is maintained below 30℃. However, irradiation with monochrome lights from the Xe-lamp for 12 h at 570 or 462 nm, corresponding to the Q and the Soret bands, does not result in any shift of λ_{max} but a small decrease in absorbance of less than 5% is seen. This result supports the assumption that the reduction of $Mn^{3+}TM$ is not concerned with a photo reaction but a photo unfolding of BSA. In addition, in the presence of a small amount of BSA (20 mg/ml), the reduction of $Mn^{3+}TM$ is scarcely observed by irradiation with the Pj-lamp but a decrease in the absorbance in the Soret

band is observed, suggesting that the reduction requires a large amount of BSA.

Effect of Heat on UV Spectra The unfolding of BSA is also caused by heat.[3] We therefore examined the effect of heat on the UV spectra. As shown in Fig. 3 B, essentially similar shifts of λ_{max} as in the case of irradiation with the Pj-lamp were observed for a solution of 4×10^{-5} mol/l Mn^{3+}TM in 0.2 g/ml BSA solution, when the solution was allowed to warm at 50°C for 30 min. Thus the reduction of Mn^{3+}TM observed in the RR spectrum could be related to the unfolding of the albumins by heat. In addition, similar to the case of the irradiation with the Pj-lamp, a small amount of BSA causes only a decrease in the absorbance at 463 nm, suggesting that Mn^{3+}TM is decomposed under this condition. Moreover, when the 476.5-nm laser beam broadened by a concave mirror is irradiated for 1 h, no shift in λ_{max} was observed for 4×10^{-5} mol/l Mn^{3+}TM in 0.2 g/ml BSA solution. Accordingly, only the focused strong laser beam causes the reduction of Mn^{3+}TM to Mn^{2+}TM. Therefore the reduction observed in the RR spectrum is mainly attributed to the heat caused by the extremely strong laser beam in the presence of a large amount of albumins.

Proposal Reaction Profile In a basic solution, Mn^{3+}TM may be close to BSA, attracting the partially negative charge of the BSA surface. In this situation, heat (less than 50°C) [3] and that from continuous strong light cause the reversible unfolding of BSA, and the –S-S- bond was cleaved to give $-S^-$ or $-S\text{-}S^-$ [4] (see Chart 1). The plus charges of TM in Mn^{3+}TM are attracted by the negative charge of the $-S^-$ or the $-S\text{-}S^-$ group, and Mn^{3+}TM is reduced to Mn^{2+}TM. The resulting Mn^{2+}TM reduces oxygen in the solution to active oxygen, and is oxidized to original Mn^{3+}TM, as shown in Chart 1. Probably the redox cycle, Mn^{3+}TM $\Leftrightarrow$ Mn^{2+}TM, was repeated, and consequently, excess active oxygens were formed. If a solution contains a large amount of BSA, it produces $-S^-$ or $-S\text{-}S^-$ groups sufficient to scavenge the resulting active oxygen,[5] and the Mn^{2+}TM should be retained in the solution. However, when the solution does not contain sufficient $-S^-$ or $-S\text{-}S^-$ groups to scavenge the active oxygens, the oxygens attack both Mn^{3+}TM and Mn^{2+}TM and the decompose.[6] Thus in the presence of a small amount of BSA, the absorbance of MnTM decreases, and in the presence of a large amount of BSA, the RR bands of Mn^{2+}TM appear.

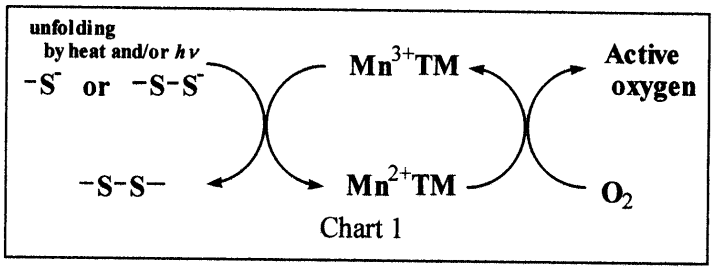

Chart 1

In conclusion, the reduction of Mn^{3+}TM observed in the RR spectrum results from an interaction with albumins *via* oxygen, which is caused by the heat generated by the extremely strong laser beam.

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