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 dithionite 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°C 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 ($\lambda_{\text{max}} = 462$ nm) is different from that of Mn^{2+}TM ($\lambda_{\text{max}} = 455$ 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°C . 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 $\lambda_{\max}$ 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 $\lambda_{\max}$ 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 $-\text{S}-\text{S}-$ bond was cleaved to give $-\text{S}^-$ or $-\text{S}-\text{S}^-$ ⁴⁾ (see Chart 1). The plus charges of TM in Mn^{3+}TM are attracted by the negative charge of the $-\text{S}^-$ or the $-\text{S}-\text{S}^-$ 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, $\text{Mn}^{3+}\text{TM} \rightleftharpoons \text{Mn}^{2+}\text{TM}$, was repeated, and consequently, excess active oxygens were formed. If a solution contains a large amount of BSA, it produces $-\text{S}^-$ or $-\text{S}-\text{S}^-$ 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 $-\text{S}^-$ or $-\text{S}-\text{S}^-$ 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.

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.

ACKNOWLEDGEMENTS

The authors thank Junichi Odo for his discussion in initial stage of this study. This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture, Japan.

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(Received January 14, 1998; accepted January 31, 1998)