Resonance Raman Spectra of Manganese-Porphyrins on Ion-Exchange Resins Exhibiting Uricase-Like Catalytic Activity¹

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Ion-exchange resins modified with some manganese(III)-porphyrins ($Mn^{3+}-P_r$) show a uricase-like catalytic activity, which is applicable to the determination of uric acid. The resonance Raman spectra of $Mn^{3+}-P_r$ indicate that Mn^{3+} -porphyrins on modified resins are reduced to Mn^{2+} -porphyrins with oxidizing uric acid; Mn^{2+} -porphyrins then return to Mn^{3+} -porphyrins by oxidation with O_2 in the reaction system. It was concluded on the basis of these results that the modified resins show an uricase-like activity by repeating this redox cycle of manganese-porphyrins on the modified resins. $Mn^{3+}-P_r$ exhibited no oxidative catalytic activity for 7-methylated derivatives of uric acid, indicating that the nitrogen atom at the 7-position of uric acid plays an important role in the activity of $Mn^{3+}-P_r$.

Keywords Modified resin, uricase-like activity, uric acid, resonance Raman spectrum, porphyrin-core vibration, manganese-porphyrin

In clinical assays, enzyme reactions catalyzed by uricase, peroxidase and catalase are frequently used as important analytical reactions. However, these enzymes have some disadvantages regarding stability, handling, purity and storage. As a study of improvement of these disadvantages, it was previously indicated that some resins modified with metallo-porphyrins exhibit such enzyme-like activities as peroxidase, catalase and uricase, and are available for clinical analyses as good mimics of these enzymes.²⁻⁵

In the present study resins modified with Mn^{3+} -tetrakis(4-sulfophenyl)porphine and Mn^{3+} -tetrakis(1-methylpyridinium-4-yl)porphine, exhibiting a uricase-like catalytic activity, were dealt with by means of resonance Raman spectroscopy. In order to know how these modified resins act as a catalyst, the resonance Raman spectra were measured in the course of oxidizing uric acid. As the result, the uricase-like activity of the modified resins was found to be caused by a redox cycle of manganese ion.

purchased from Tokyo Kasei Kogyo Co. Ltd. and used without further purification. Their manganese(III)-complexes, Mn^{3+} -TPPS and Mn^{3+} -TMPyP (Fig. 1), were prepared by methods similar to those given in the literature.⁶ Anion-exchange (Amberlite IRA 900) and a cation-exchange (Dowex MSC-1) resins were regenerated in their chloride and sodium forms, respectively. The resins modified with manganese-porphyrins (25 μ mol/g), Mn^{3+} -TPPS, and Mn^{3+} -TMPyP, were prepared as described before.⁶ All other chemicals were of reagent grade and used without further purification.

Stock solutions of the samples were prepared by dissolving uric acid (UA) or its derivatives (0.1 g, Sigma Chemical Co.) with lithium carbonate (0.1 g) in 100 ml water. A buffer solution (pH 11.0) was prepared by mixing a 0.2 M sodium carbonate solution with a 0.2 M boric acid–0.2 M potassium chloride solution.

Experimental

Compounds

Tetrakis(4-sulfophenyl)porphine (TPPS) and tetrakis(1-methylpyridinium-4-yl)porphine (TMPyP) were

Fig. 1 Structures of manganese-porphyrins.

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Fig. 2 Measurement method of RRS of manganese-porphyrin on modified resins.

Spectral measurements

The resonance Raman spectra (RRS) were recorded on a JASCO NR-1000 Raman spectrophotometer with a double monochromator by using 457.9 nm excitation from a NEC 3300 Ar⁺-ion laser. The RRS of the Mn³⁺-porphyrin on the modified resins were measured by using a rotating cell in which the resins were kept in a sample solution (Fig. 2). The frequencies were calibrated using the standard bands of indene.

Results and Discussion

Uricase-like catalytic activity

When a mixture of UA solution, buffer solution (pH 11.0) and Mn-TPPS_r is incubated at 35°C for 60 min, UA is oxidized to allantoin with hydrogen peroxide and carbon dioxide by the following reaction catalyzed by Mn-TPPS_r:⁷

The amount of allantoin produced was equivalent to that of UA added.⁸ However, the aqueous solution of Mn³⁺-TPPS and Mn³⁺-TMPyP did not catalyze reaction (1), and no amount of UA was oxidized, suggesting that only Mn³⁺-porphyrins on the ion-exchange resins act as catalysis in reaction (1). In order to investigate any change of Mn³⁺-porphyrins on the resins, resonance Raman spectroscopy is available; ordinary Raman, IR and UV spectroscopies can not be used.

Resonance Raman spectra

The RRS of Mn-TPPS_r in a buffer solution (pH 11) are shown in Fig. 3. All of the bands observed are due to Mn³⁺-porphyrin on the modified resin; no band of the mother ion-exchange resin was observed. As described in the proceeding⁹, RRS is essentially the same as that of an aqueous solution of Mn³⁺-TPPS, indicating that the structure of Mn³⁺-TPPS on the resin is not so different from that in an aqueous solution. Most of the resonance Raman band can be assigned to porphyrin-

Fig. 3 RRS of Mn-TPPS_r in the course of the oxidation of UA. (A): before the addition of UA; (B) and (C): 5 and 20 min after the addition of UA, respectively; (D): treated with sodium dithionite.

core vibrations by referring to the assignment for related compounds^{10,11} (Table 1). After the addition of UA, new bands at 993, 1342 and 1540 cm⁻¹ were especially observed in the RRS, even after 5 min; the relative intensities of these bands increased with the incubation time. On the contrary, the intensities of the bands at 1564, 1370 and 1006 cm⁻¹ decreased relatively with progress of the reaction. This result indicates that the band of Mn³⁺-TPPS at 1564, 1370 and 1006 cm⁻¹ are replaced by the bands at 1540, 1342 and 993 cm⁻¹, respectively, with the oxidation of UA; also, the structure of Mn³⁺-TPPS on the resin changes to a compound which is characterized by resonance Raman bands at 1540, 1342 and 993 cm⁻¹. In addition, the existence of these resonance Raman bands suggests that the compound has an absorption band to resonance with an excitation laser line around that of Mn³⁺-TPPS; that is, the structure of the compound is essentially similar to Mn³⁺-TPPS. By assignments of metal-tetraphenylporphyrin^{10,12}, the bands of physiological Hemes and Fe³⁺-porphyrin around 1560, 1370 and 1010 cm⁻¹ have been assigned to the core vibrations for the porphyrin ring; the band around 1370 cm⁻¹ is attributed to a well-known as "oxidation state marker band", shifting to the lower frequency

Table 1 Resonance Raman frequencies and band assignments for Mn-TPPS_r and Mn-TMPyP_r

Mn ³⁺ -TPPS _r	Mn-TPPS _r +UA	Mn ²⁺ -TPPS _r	Mn ³⁺ -TMPyP _r	Mn-TMPyP _r +UA	Mn ²⁺ -TMPyP _r	Assignment ^a
— ^b	— ^b	— ^b	908	907	907	δ _s (por)
— ^b	— ^b	— ^b	975	972	— ^b	
— ^c	993 ^d	993	— ^c	1000 ^d	1000	ν _s (C _α -C _m)
1006	1005 ^e	— ^c	1014	1014 ^c	— ^c	ν _s (C _α -C _m)
1085	1085	1079	1100	1097	1097	δ _s (C _β -H)
1125	1123	1123	1187	1190	1191	δ(phenyl)
— ^b	— ^b	— ^b	1220	1220	1219	δ(phenyl)
1240	1236	1236	1255	1257	1255	δ(C _m -phenyl)
— ^b	— ^b	— ^b	1302	1300	— ^b	ν _{as} (C _α -C _β)
— ^c	1342 ^d	1343	1345	1345 ^d	1344	ν _s (C _α -N)
1370	1365 ^e	— ^c	1370	1370 ^e	— ^c	ν _s (C _α -N)
— ^b	— ^b	— ^b	1410	1417	— ^b	
1463	1450	1444	1465	1465	1445	ν _s (C _α -C _β)
1502	1492	1492	— ^b	— ^b	— ^b	ν _{as} (C _β -C _β)
— ^c	1540 ^d	1540	— ^c	1548 ^d	1547	ν _s (C _β -C _β)
1564	1562 ^e	— ^c	1570	1570 ^e	— ^c	ν _s (C _β -C _β)
1600	1602	1600	1646	1645	1644	phenyl

a. δ, deformation; ν, stretching; s, symmetric; as, asymmetric; phenyl, pyridinium ring for Mn-TMPyP_r and phenyl ring for Mn-TPPS_r. b. Not observed. c. Not expected. d. Characteristic bands for Mn²⁺-P_r. e. Characteristic bands for Mn³⁺-P_r.

region by about 20 cm⁻¹ with the reduction of Fe³⁺-porphyrins to Fe²⁺-porphyrins.¹³ By analogy with these points, the shift of the 1370 cm⁻¹ band to 1342 cm⁻¹ suggests that an oxidation state of Mn³⁺ atom of Mn-TPPS_r may reduce to Mn²⁺ state in the course of the oxidation of UA. In order to confirm the reduction of the Mn³⁺ ion, the RRS of Mn²⁺-TPPS_r reduced chemically with sodium dithionite was measured. As can be seen in Fig. 3, it is obvious that the bands at 993, 1342 and 1540 cm⁻¹ (assigned to the core vibrations, as summarized in Table 1) are characteristic for Mn²⁺-TPPS_r, indicating that the resulting compound on the resin with oxidation of UA should be Mn²⁺-TPPS. It is of interest that the oxidation marker band at 1342 cm⁻¹ is lower by about 20 cm⁻¹ than that of Mn³⁺-TPPS similar to the case of Fe-porphyrins. It has thus been apparent that Mn³⁺-TPPS on the modified resin was reduced to Mn²⁺-TPPS along with the oxidation of UA.

After completing the oxidation of UA, the characteristic bands at 993, 1342 and 1540 cm⁻¹ for Mn²⁺-TPPS gradually decrease and the Mn³⁺-TPPS bands at 1000, 1370 and 1564 cm⁻¹ increase, replacing the Mn²⁺-TPPS band. Finally, the spectra perfectly returned to that of Mn³⁺-TPPS_r. Accordingly, it is clear that Mn²⁺-TPPS_r reduced by UA was then oxidized to Mn³⁺-TPPS_r with oxygen in the reaction system. These results indicate that the uricase-like activity of Mn-TPPS_r is based on the cycle of from Mn³⁺-TPPS_r to first Mn²⁺-TPPS_r, and then to Mn³⁺-TPPS_r. Since the oxidation reaction of Mn²⁺ on the resin is rapid, Mn³⁺ always exists in the reaction system. This is a reason why the RRS of Mn³⁺-TPPS_r in the presence of UA could not change completely to that of Mn²⁺-TPPS_r.

The spectra obtained for Mn³⁺-TMPyP_r are shown in Fig. 4. New bands at 1000, 1344 and 1547 cm⁻¹ were

Fig. 4 RRS of Mn-TMPyP_r in the course of the oxidation of UA. (A) and (B): before and after the addition of UA, respectively; (C): treated with sodium dithionite.

observed after the addition of UA (in analogy with the case of Mn-TPPS_r); they have been assigned to the porphyrin-core vibrations (Table 1). Especially, the band of Mn³⁺-TMPyP_r at 1370 cm⁻¹, assignable to the oxidation state marker band, showed a large shift of

26 cm⁻¹ upon the oxidation of UA, suggesting that Mn³⁺ is reduced by UA. Comparing these spectra with that of Mn²⁺-TMPyP, obtained by a chemical reduction of Mn³⁺-TMPyP, with sodium dithionite, the newly observed bands are characteristic for Mn²⁺-TMPyP. Considering these spectral changes, it is clear that Mn-TMPyP also exhibits catalytic activity by a similar redox cycle as that in the case of Mn-TPPS.

As described above, the uricase-like activity of Mn³⁺-TPPS, is due to a redox reaction of manganese ion, as summarized in the following reaction:

Resonance Raman spectra of Mn-P, with axial ligands

The interference of foreign substances on the uricase-like activity was examined after incubation for 10 min at 35°C by measuring any decrease in the absorbances at 293 nm of mixtures of a UA solution (110 µg), 0.1 ml; a buffer solution (pH 11), 4 ml; and Mn-TMPyP, 20 mg. As shown in Table 2, citrate and EDTA ions inhibited the activity. The RRS of Mn-TPPS, and Mn-TMPyP, were measured in the presence of either citrate or EDTA ion. However, these substances do not show any apparent influence on the RRS of Mn-TPPS, and Mn-TMPyP; no evidence for the coordination of these substances was observed in the RRS.

Catalytic activity for UA derivatives

In order to examine the oxidative catalytic activity of Mn-P, for UA derivatives, the RRS of Mn-TPPS, and Mn-TMPyP, were measured in the presence of UA derivatives. The catalytic activity was estimated by measuring especially the porphyrin-core vibration bands,

Table 2 Effects of axial ligands

Substance	Final concentration/ 10 ⁻⁴ M	Changed uric acid ^a , %
(None)	100	100
Pyridine	100	100
Imidazol	100	96
Na azide	100	99
Na cyanide	100	98
Na oxalate	100	94
Na acetate	100	100
Na lactate	100	96
Na citrate	100	59
	50	74
	10	83
Na EDTA ^b	100	27
	10	45
	1	61

a. Concentration of uric acid: 1.1×10⁻⁴ M.

b. Sodium ethylenediamine tetraacetate.

Table 3 Uricase-like activity for uric acid and its derivatives

Uric acid & its derivatives	UV method ^a		RRS method ^b	
	A ^c	B ^c	A ^c	B ^c
(None)	—	—	0	0.2 ^d
Uric acid (UA)	0	0	3.2	1.6
1-Methyl-UA	0	0	3.1	1.5
3-Methyl-UA	11	26	2.6	1.0
9-Methyl-UA	65	42	1.9	0.9
7-Methyl-UA	99	93	0	0.2
1,7-Dimethyl-UA	100	98	0	0.2
3,7-Dimethyl-UA	100	95	0	0.2
1,3,7-Trimethyl-UA	100	94	0	0.2

a. Ratio (%) of the amount of the unchanged UA derivatives to that of the UA derivatives added.

b. Ratio of the intensity of the band at 1342 cm⁻¹ for Mn-TPPS, (1344 cm⁻¹ for Mn-TMPyP) to the intensity of the band at 1370 cm⁻¹.

c. Resin: A, Mn-TPPS; B, Mn-TMPyP.

d. A weak band is observed at 1344 cm⁻¹ for Mn³⁺-TMPyP.

which are characteristic for Mn²⁺-TPPS. In the case of 9-methyl-UA, these characteristic bands for Mn²⁺-P, were obtained, but not in the case of 7-methyl-UA. This result indicates that Mn³⁺-TPPS, shows catalytic activity for 9-methyl-UA but not for 7-methyl-UA. However, in both the cases, no difference in RRS from those in the case of UA was observed. The results obtained for other UA derivatives with Mn³⁺-TPPS, and Mn³⁺-TMPyP, are summarized in Table 3. As can be seen, the characteristic bands for Mn²⁺-TPPS, and Mn²⁺-TMPyP, were measured for UA and 1-, 3- and 9-methyl-UA, indicating that Mn³⁺-TPPS, and Mn³⁺-TMPyP, shows catalytic activity for these UA derivatives. In order to confirm these oxidative activities for the UA derivatives, the decreases of the UA derivatives after incubation with Mn³⁺-P, were measured by the UV method, similarly to an examination of uricase-like activity.¹⁴ These results are shown in Table 3. In agreement with the result of RRS, 1-, 3- and 9-methyl-UA were oxidized but not 7-methylated UA derivatives, 7-methyl-, 1,7-dimethyl-, 3,7-dimethyl- and 1,3,7-trimethyl-UA. This result corresponds to that obtained by the Raman method.

As discussed above, it is clear that Mn³⁺-TPPS, and Mn³⁺-TMPyP, show no catalytic oxidative activity for the 7-methylated derivatives of UA. This result indicates that the nitrogen atom at the 7-position of UA plays an important role in the oxidation of UA with modified resins.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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(Received January 28, 1991)

(Accepted April 15, 1991)